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Mechanoregulated tissue organisation during skeletal repair: A computational study

Trinity College Dublin, August 9, 2012

A thesis submitted to the University of Dublin in partial fulfilment of the requirements for the degree of

Doctor in Philosophy

Supervisor: Dr. Daniel J. Kelly
Internal Examiner: Dr. Bruce Murphy
External Examiner: Prof. José Manuel García Aznar
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"You can't really know anything if you just remember isolated facts and try and bang 'em back. If the facts don't hang together on a latticework of theory, you don't have them in a usable form. You've got to have models in your head. And you've got to array your experience - both vicarious and direct - on this latticework of models."

Charlie Munger
Summary

Articular cartilage is a highly specialised bearing material that provides low friction, wear resistant contact between the ends of articulating bones in diarthrodial joints. In order to function in this mechanically challenging environment over an individual's lifetime the tissue is equipped with a unique internal structure – in particular the so-called Benninghoff collagen architecture. The unique composition and anatomy of the tissue lead, however, to a very limited potential for self repair and degenerative joint diseases like osteoarthritis constitute an ongoing and increasing problem in healthcare. Great research effort is therefore being directed at engineering cartilage tissue. To date, a tissue engineered cartilage construct with a native-like architecture has not been presented, yet, and it has been realised that this deficiency might be central to the mid and long term failure of engineered tissues in vivo. This thesis set out to further investigate a) the role of the collagen architecture in determining the mechanical properties of engineered and native cartilage and b) the reverse, i.e. the role of the mechanical environment in regulating the collagen architecture during in vivo regeneration and in tissue engineering.

In order to isolate the effects of the collagen architecture on the apparent mechanical properties of cartilage tissue, a theoretical framework was established and implemented using the finite element method. A finite strain biphasic material model was developed that can capture the nonlinear, anisotropic and viscoelastic behaviour of charged and neutral hydrated soft tissues or biomaterials.

This model was then used to elucidate the distinctly different influence of collagen fibre orientation on the apparent mechanical properties of charged vs. neutral tissues. As a consequence, the functional significance of the Benninghoff architecture can only be explained in the context of cartilage swelling. In the absence of swelling, the Benninghoff architecture would not contribute to cartilage mechanics throughout most of the tissue's thickness.

The framework was further extended by a novel collagen remodelling algorithm to investigate the possibility and significance of a mechanoregulated collagen architecture. The framework allows the consideration of both discrete and continuous fibre architectures and combines the features collagen orientation and stress-free configuration in a finite strain formulation. It successfully captured the compaction and alignment observed in collagen and fibrin gels as well as the adaptation of periosteum to sustained length changes.

Improved mechanical properties of mechanically loaded tissue engineered cartilage compared to free-swelling controls have been observed experimentally. The remodelling framework developed in this thesis was used to test the hypothesis that these observations could be explained by altered collagen network features due to loading. It could be shown that collagen (re)orientation alone could not explain the experimental observations. However, a simultaneous consideration of both fibre orientation and stress-free configuration allowed the prediction of the
effect of loading on the mechanical and geometrical properties of tissue engineered cartilage. It was further highlighted that collagen remodelling can lead to opposite trends in the evolution of material properties than would be expected based on composition alone.

The implantation of tissue engineered cartilage of varying composition into a chondral defect in a tibial plateau was then simulated in order to determine the influence of implant maturity on the prospects of recapitulating a Benninghoff architecture. The foundation for this study was the ability of the simulation framework to predict the Benninghoff architecture in tibial plateau cartilage based on internal swelling pressures and joint loading. It was predicted that the environment created by a mature construct is more conducive to the recapitulation and maintenance of a Benninghoff architecture than an immature implant. The dependence of tissue adaptability on tissue maturity was shown to be another factor in facilitating successful remodelling.

Natural skeletal regeneration was studied drawing on the example of neoarthrosis formation due to cyclic bending imposed on a femoral fracture in a rat model. For this purpose, the computational framework above was extended by a tissue differentiation theory. Based on fluid flow and shear strain the non-union of the fracture could be predicted, namely the formation of fibrous and cartilaginous tissues. The collagen remodelling algorithm was further able to predict the fibre angles measured experimentally. This provides evidence that the collagen architecture is mechanoregulated during skeletal regeneration in a way similar to that observed in other soft tissues.

It was concluded that, similar to e.g. cardiovascular tissues, the collagen architecture in regenerating skeletal tissues and tissue engineered cartilage can be modulated via the mechanical environment. It was further shown that the traditionally considered feature of collagen orientation may be insufficient to assess the functionality of fibre reinforced tissues but that the stress-free configuration of the collagen network deserves equal attention. In cartilaginous tissues, the functional significance of the cartilage architecture can only be interpreted in the context of swelling and models considering cartilage as a neutral material may therefore be inappropriate.
Acknowledgements

The number of people that deserve to be acknowledged here is huge. I will try to make the list as complete as possible – should I nevertheless forget somebody please don’t take offense.

It seems impossible to say something specific about my parents. Because they were always there to help. I simply thank you for your invaluable, continuous and complete support for the last almost 29 years! And the next, in advance. I extend my thanks to the rest of my family, in particular my grandmother who luckily was able to visit me in Ireland and to whom I owe a lot.

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Nomenclature

The nomenclature contains some abbreviations as well as the definition of some operators used throughout the thesis. It is not a full list of all symbols used – they will be explained in the text whenever used.

\( (\bullet)'_\alpha \) \hspace{1cm} Material time derivative of \((\bullet)\) following the motion of constituent \(\alpha\)

\( \text{det}(\bullet) \) \hspace{1cm} Determinant of \((\bullet)\).

\( \cdot \) \hspace{1cm} Partial time derivative of \((\bullet)\).

\( \text{grad}(\bullet) \) \hspace{1cm} Partial derivative of \((\bullet)\) with respect to the current position \(x\).

\( \text{Grad}_\alpha(\bullet) \) \hspace{1cm} Partial derivative of \((\bullet)\) with respect to the reference position \(X_\alpha\) of constituent \(\alpha\).

\( d\Gamma \) \hspace{1cm} Area element

\( d\Omega \) \hspace{1cm} Volume element

\( A : B \) \hspace{1cm} Double contraction of two tensors \(A\) and \(B\), e.g. \(A_{ij}B_{ij}\).

\( a \otimes b \) \hspace{1cm} Tensor product between the vectors \(a\) and \(b\) defining the second order tensor \(a \otimes b\) such that \((a \otimes b)c = (b \cdot c)a\).

\( \text{div}(\bullet) \) \hspace{1cm} The divergence of \((\bullet)\) defined in spatial coordinates \(x\).

\( \text{Div}_\alpha(\bullet) \) \hspace{1cm} The divergence of \((\bullet)\) defined with respect to the material coordinates \(X_\alpha\) of constituent \(\alpha\).

\( \%\text{d/ww} \) \hspace{1cm} weight per dry weight [%]

\( \%\text{w/ww} \) \hspace{1cm} weight per wet weight [%]

COL \hspace{1cm} Collagen

DL \hspace{1cm} Dynamic loading
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<tr>
<td>ECM</td>
<td>Extracellular matrix</td>
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<tr>
<td>FCD</td>
<td>Fixed charge density</td>
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<td>FS</td>
<td>Free swelling</td>
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<td>PG</td>
<td>Proteoglycans</td>
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<td>SED</td>
<td>Strain energy density function</td>
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Publications

This is a list of publications that originated from work presented in this thesis.

Journal articles


Bookchapters

Conference proceedings


1 Introduction

1.1. Mechanobiology

Cells are regulated by genetic, epigenetic and environmental factors (Petronis, 2006; Davies, 2012; Rothstein et al., 2009). With each somatic cell in the body containing the same genetic information, epigenetic and environmental factors play a key role in determining which genes are expressed and are therefore critical determinants of cell phenotype and behaviour. The regulating mechanisms of cellular behaviour are tremendously complex. Mechanics has been applied to the study of human body structure and locomotion for hundreds of years\(^1\) and specific links that even today seem modern have been established a long time ago. For example, around 1870 Wolff used findings by Culmann and von Meyer that demonstrated a qualitative similarity between the trabecular architecture in the femur and principal stress trajectories in a similarly shaped crane to hypothesise a relationship between mechanics and bone growth and remodelling (Cowin and Doty (2007); Skedros and Baucom (2007) and references therein). Today, similar ideas have formed the field of *mechanobiology* that involves studies from the tissue and organ level to the cell-matrix and eventually subcellular as well as genetic level (Ateshian and Friedman, 2009). Mechanobiology therefore investigates whether and how function determines form (van der Meulen and Huiskes, 2002) or, more generally, what role mechanical forces play in biology across all length scales.

Understanding the mechanobiology of cells will be crucial for the regeneration of damaged or diseased tissues and organs. Many tissues exhibit quite complex functional and compositional gradients, structural hierarchies, temporal changes and interfaces. Such complexity is magnified at the organ level where multiple tissues are combined into functional entities. In order to recreate tissues or even

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\(^1\)A historical review can be found in chapter 1 of Mow and Huiskes (2005).
1. Introduction

organs *ex vivo* (the field of *tissue engineering*) or regenerate them *in vivo* (the field of *regenerative medicine*) (Butler et al., 2009), one first has to understand their growth, remodelling and morphogenesis during normal development and maturation. This is typically approached in three ways: *in vivo* studies, and specifically transgenic models in more recent times, that allow *in situ* investigations in the intact organism; *in vitro* experiments allow the targeted isolation of certain aspects of the complex and interacting biomechano-chemical processes outside of the organism; and finally *in silico* experiments: computational models and simulations that can alleviate the need for physical experiments, aid in the development and testing of hypotheses or theories and provide insight as well as data beyond those gained through experimentation alone.

Therefore, *in silico* models offer the opportunity to systematically investigate hypotheses in a give-and-take interplay with physical experiments. Medical device developers can use mechanobiological models to fulfil design criteria like "compliance matching" to, for example, avoid stress shielding in vascular grafts or bone implants, which can lead to bone resorption and subsequent implant loosening in the case of orthopaedic implants and stenosis in the case of vascular grafts. In tissue engineering, knowledge in mechanobiology will help to identify and prioritise design criteria and requirement specifications, shorten development times, standardise quality assurance and implement suitable bioreactor protocols (Butler et al., 2000). As outlined in the next section, mechanobiology is crucial for engineering load bearing tissues with the structure and functionality to remain viable under the challenging conditions *in vivo*.

1.2. Articular cartilage repair

Osteoarthritis (OA) and cartilage repair remain great challenges and financial burdens – over 40% of the population aged over 65 years are estimated to have symptomatic osteoarthritis of the hip or knee. With ageing populations in the industrialised countries the prevalence of OA is likely to increase. Young, physically active persons more likely present with acute or overuse joint injuries (Adirim and Cheng, 2003) which can predispose them to the development of OA later in life.

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Articular cartilage especially has a poor capacity for repair due to its avascular nature and low cellularity. No satisfactory treatments for cartilage damage exist to date despite continuous medical interest. In the middle of the 18th century, Hunter wrote “from Hippocrates down to the present Age, we shall find, that an ulcerated Cartilage is universally allowed to be a very troublesome Disease [..] and that, when destroyed, it is never recovered.” (Hunter, 1742, p. 520). Tissue engineering has been recognised as a possible strategy to aid these insufficient repair processes (Guilak et al., 2001). While articular cartilage has been conceived “next on the list” of successfully engineered materials (Rawe, 2000) this goal remains elusive and significant challenges persist. One such challenge in creating cell based therapies for cartilage repair is understanding and quantifying how the cells respond to external stimuli and change their biosynthetic activity. Additionally, articular cartilage’s complex biomechanical behaviour is still not fully understood but forms the basis of the tissue’s functionality which has not been paralleled by an engineered substitute hitherto. Differences in the mechanical properties between engineered and native cartilage cannot be explained based on the different concentrations of the main extracellular matrix constituents alone and may involve aspects of matrix assembly (Vunjak-Novakovic et al., 1999). Similar observations have been made between mechanically loaded and free-swelling cartilaginous tissues (Yan et al., 2009; Hoenig et al., 2011; Bian et al., 2010). This leads to the question of mechanisms aside from composition that contribute to the mechanical properties of engineered and native articular cartilage. A possible candidate mechanism that will be investigated specifically in this thesis is the collagen architecture of articular cartilage and its engineered equivalents.

Collagen is often the primary load bearing constituent in soft tissues. Type II collagen is also the major solid component of the cartilage extracellular matrix. Its distinct architecture has been well documented by Benninghoff (after whom it is often named) for a long time (Benninghoff, 1925b) and even linked to tissue mechanics (Benninghoff, 1925a,b), but its biomechanical contributions are manifold and uncertainties remain. From an engineering point of view the architecture of articular cartilage and other load bearing tissues seems to be optimised for their mechanical role. Alterations to this structure therefore diminish the mechanical integrity and can contribute to the aetiology of degenerative diseases like osteoarthritis or even tissue failure. Conversely, to restore the functionality of an injured tissue its internal structure must be restored as well. During natural heal-
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ing processes such as spontaneous osteochondral defect repair that is often not the case.

A general question in tissue engineering of musculoskeletal and cardiovascular collagenous soft tissues therefore is: How does the local mechanical environment regulate the collagen architecture? Answering this question will determine the ability to restore not only the desired tissue phenotype but also the native tissue structure. Without this ability the engineered substitute is likely to fail and degenerate under in vivo conditions. This integrated approach has moved into the focus of tissue engineering only recently (Klein et al., 2009). An engineered cartilaginous tissue with a native-like architecture has not been presented, yet, and it is poorly understood how mechanical cues might regulate the organisation of engineered cartilage in vitro or, perhaps more importantly, in vivo following implantation into a load bearing environment. The influence of mechanics on tissue architecture has immediate relevance for addressing further open questions in tissue engineering that will be addressed in this thesis: How does bioreactor culture affect the development of the collagen architecture in engineered cartilage? How do altered features of the collagen architecture in response to loading further affect the mechanical properties of engineered cartilaginous tissues and hence its functionality? There is reason to believe that mechanical cues can guide articular cartilage collagen architecture as its collagen structure once again seems to be optimised for the local mechanical environment (Wilson et al., 2006a; Owen and Wayne, 2006; Shirazi and Shirazi-Adl, 2008) and mechanical loading can be used to aid chondrogenesis in tissue engineering (Guilak et al., 2001; Zhang et al., 2009). The zonal collagen architecture only develops during maturation from an initially rather homogeneous tissue (Julkunen et al., 2010a,b; Rieppo et al., 2009; van Turnhout et al., 2010b), a process which is believed to be strongly affected by mechanical loading (Brama et al., 2000, 2002; Brommer et al., 2005; Brama et al., 2009). However, remodelling of adult cartilage in response to loading and of cartilage during maturation may involve different mechanisms and proceed at different rates (Hyttinen et al., 2001; Julkunen et al., 2010a). This opens up another important question in tissue engineering to be addressed in this work: How will the maturity of an engineered tissue affect remodelling events in this construct once it is implanted into a defect in vivo.
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1.3. Objectives and outline

To direct efforts towards engineering a functional cartilage replacement, the mechanical foundations of that functionality have to be understood. This work therefore investigated the role of the collagen architecture in determining the biomechanics of native and engineered cartilage and vice versa – akin to the circular concept of “function follows form follows function” outlined in van der Meulen and Huiskes (2002). The overarching hypothesis under investigation in this thesis is as follows: Tissue architecture is a fundamental contributor to the biomechanical fitness of native and engineered cartilage and can be regulated both in vitro and in vivo by a combination of extrinsic loading and internal swelling pressures.

As the influence of tissue architecture is difficult to isolate experimentally, a theoretical approach based on continuum mechanical principles was chosen. The main objective was to develop a computational framework to explore the role of the local mechanical environment on the evolving collagen architecture in cartilage engineered in vitro as well as during regeneration in vivo. In tissue engineering, biomaterial scaffolds are often used to support the initial cell population, while natural regenerative processes are characterised by a sequence of tissue phenotypes with distinct mechanical behaviours. A versatile constitutive model that can capture aspects of all these material classes is therefore desirable. Since the mechanoregulation of the collagen architecture will be investigated, the representation of tissue structure must not be static in this model but able to evolve as the tissues develop and adapt.

Several objectives were defined and will be addressed in the subsequent chapters following a literature review in chapter 2. They were:

1. To develop a thermodynamically consistent constitutive model that can capture salient material effects of hydrated soft biological tissues and biomaterials, in particular articular cartilage, at finite strains (chapter 3).

2. Investigate how the collagen architecture affects the mechanical behaviour of cartilaginous, i.e. charged, tissues vis-à-vis neutral (uncharged) materials (chapter 4).

3. To develop a general framework that can capture collagen remodelling in response to external stimuli independent of the particular representation of the fibre architecture (discrete, continuous) such that it can be applied to a large number of bioengineering problems (chapter 5).
4. Investigate how mechanically induced remodelling events of the collagen network might influence structure-function relationships in tissue engineered cartilage during bioreactor culture (chapter 6).

5. Investigate how the composition of tissue engineered cartilaginous constructs at the time of implantation affects the possibility of recapitulating a native architecture within a chondral defect in the knee based on the mechanical environment inside the tissue (chapter 7).

6. To test the hypothesis that both the cell phenotype and the collagen architecture in a regenerating skeletal tissue are mechanoregulated (chapter 8).

Chapter 9 summarises the obtained results and ends with a general discussion and some perspectives for future work. More detailed theoretical aspects as well as model verification are presented separately in the Appendix to this work.
2 Literature Review

This literature review will begin by providing an overview of articular cartilage composition and its relation to the biomechanical functionality of the tissue. Next, general aspects of remodelling in soft biological tissues are reviewed. More specific references will also be presented within subsequent chapters of the thesis where they are most relevant.

2.1. Cartilage biomechanics and composition

There are three types of cartilage – hyaline, elastic and fibrocartilage. This thesis focussed on the articular hyaline cartilage and the other two kinds are mentioned only briefly. The glassy bluish-white hyaline cartilage covers the articulating ends of bones in synovial joints and also forms the basis for endochondral ossification in the growth plate. Elastic fibres (fibrillin + elastin) give the yellowish elastic cartilage its elasticity and therefore name. It can be found in the ear and the epiglottis, among others, and is very cell rich. Fibrocartilage appears rough in contrast to the smooth hyaline cartilage due to its thick collagen bundles. It can be found where tendons insert into bones, the annulus fibrosus and the meniscus of the knee (Mow and Huiskes, 2005; Cowin and Doty, 2007).

Healthy hyaline cartilage can withstand very high joint loads and constitutes an almost frictionless bearing material in synovial joints (Ateshian and Hung, 2006). Lubrication mechanisms are complex and involve fluid pressurisation, which is a general theme in cartilage load bearing (Ateshian, 2009). Structure-function relationships like this are of enormous interest for the study of osteoarthritis, tissue engineering and tissue metabolism. Cartilage is avascular, aneural, alymphatic and nutrition of chondrocytes occurs via diffusion and convection.

Articular cartilage is a multiphasic material with 68–85% water containing dissolved electrolytes (Na+, Ca2+, Cl−, K+, ...). The solid phase contains mostly
collagen (10–20% wet weight) and proteoglycans (5–10% wet weight), as well as the only cell type in the tissue – the chondrocytes – and other proteins. These other proteins can serve important biological regulating functions, but collagen and proteoglycan aggregates form the structurally most relevant basis of the tissue (Cowin and Doty, 2007; Mow and Huiskes, 2005).

2.1.1. Interstitial water

The water content is influenced by the fixed charge density due to glycosaminoglycans (see section 2.1.3), the ion concentration in the fluid and the integrity, organisation and strength of the collagen network. If this network is damaged, as happens in osteoarthritis, water content increases by 10% and mechanical properties diminish significantly (Maroudas, 1976; Akizuki et al., 1986). About 30% of the interstitial water is bound in collagen fibres (intrafibrillar water content). This has important implications for the fixed charge density, raising the Donnan osmotic pressure, and the mechanics of the tissue, as the intrafibrillar water is trapped and not free to evade during compression (Maroudas and Bannon, 1981; Torzilli, 1988; Wilson et al., 2006b). High pressure gradients are required to move the extrafibrillar fluid through the pores of the solid matrix due to significant frictional drag between the two phases (Mow et al., 1992). Due to the intrinsically incompressible nature of the solid and fluid constituents a local volume change can only be achieved by the exudation (or imbibition) of fluid out of the pore space. The frictional drag associated with this motion gives rise to significant flow dependent viscoelastic phenomena (Mow et al., 1992). A measure of the ease of fluid flow through the matrix is the permeability. The low permeability (1 – 10·10^{-15} \text{m}^4/\text{Ns}) is further reduced when the tissue is compacted (Lai and Mow, 1980; Lai et al., 1981; Holmes and Mow, 1990). This nonlinear (deformation dependent) permeability aids in maintaining a high degree of fluid pressurisation. Upon loading the fluid load support therefore can be well above 90% of the applied load which not only shields the solid matrix (and therefore the cells) from excessive stresses and strains (Mow et al., 1992) but also constitutes an important mechanism for the maintenance of the extraordinarily low coefficient of friction, e.g. during joint loading (Ateshian, 2009).
2.1.2. Collagen

Collagen is the main structural protein in many tissues. There are more than 20 types of collagen known today. Often its primary function is to resist tensile loading. The fibril-forming collagen II is the main type of collagen in articular cartilage with traces of type IX and XI. Fibril diameters ranging from 10 to 300 nm can be found in articular cartilage (Cowin and Doty, 2007; Mow and Huiskes, 2005; Mow et al., 1992). The type I collagen in tendons, ligaments and the menisci forms fibres with much larger diameters. Small fibril networks, as seen in cartilage, can still provide significant resistance to tensile loading, which is increased by intermolecular cross-linking (Mow et al., 1992). This network balances the swelling pressures exerted by the proteoglycan gel and, upon compression, will assist fluid pressurisation by counteracting matrix dilatation, increasing flow-dependent viscoelastic effects as well (Akizuki et al., 1986, 1987; Li et al., 2002a). A review of the various collagen types present in articular cartilage can be found in Eyre (2002).

(a) Aggregate PG

(b) PG-COL interaction

Figure 2.1.: (a) Aggrecan monomers where keratan and chondroitin sulfate are attached to a protein core are connected to hyaluronic acid via link proteins to form large aggregate PGs. Adapted from Mansour (2003). (b) The PGs are entangled with the collagen network which counteracts the swelling pressure originating from the negatively charged GAGs. Adapted from Mow et al. (1992).
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2.1.3. Proteoglycans

Proteoglycans (PGs) are macromolecules where many glycosaminoglycan (GAG) side chains covalently attach to a protein core molecule. There are numerous combinations of different PGs with GAGs. About 80 to 90% of the PGs in articular cartilage are aggrecans: keratan sulfate and chondroitin sulfate GAG chains attached to a protein core. These aggrecan monomers aggregate and attach to hyaluronic acid (HA) via link proteins to form a large proteoglycan aggregate (Fig. 2.1a). These large aggregates are confined to about 1/5 of their volume in free solution and immobilised within the collagen network and the negatively charged sulfate and carboxyl groups on the GAG side chains establish the fixed charge density (FCD, 0.05–0.3 mEq/ml) which determines electrolyte transport (Mow et al. (1992) and references therein). The fixed negative charges cause an osmotic pressure and a chemical expansion stress due to electrostatic repulsion which in addition to an entropic contribution to swelling by the proteoglycans explain a large proportion of the compressive stiffness of articular cartilage (Mow et al., 1992; Cowin and Doty, 2007; Kovach, 1995; Ateshian et al., 2009). Furthermore, chondrocytes have a hyaluronan cell surface receptor (CD44) by which they are directly linked to the HA of the extracellular matrix (Knudson and Knud-
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The glycosaminoglycans, namely chondroitin sulfate, are thought to be largely responsible for the resistance the solid imposes on fluid flow as well as for the regulation of tissue hydration (Mow et al., 1992). The swelling pressure produced by the PGs is counteracted by the collagen network (Fig. 2.1b). Generally, the collagen network is therefore inflated in the unloaded state, i.e. pre-stressed, which is considered the main cause for the shear stiffness of the tissue (on the order of 0.2–0.4 MPa at equilibrium, Mow et al. (1992)). This shear stiffness seems to be increased significantly by cross-linking of the collagen network (Spirt et al., 1989; Zhu et al., 1993). Another biomechanical interaction between PGs and the collagen network is of a frictional type. In addition to PG-PG interactions this PG-COL interaction seems to be responsible for much of the flow-independent or intrinsic viscoelasticity (Schmidt et al., 1990; Huang et al., 2001) that becomes directly apparent under isochoric deformations without pressure gradients (e.g. pure shear) where the cartilage again exhibits significant rate dependent behaviour (Mow et al., 1992).

2.1.4. Heterogeneity and anisotropy

A significant body of work has been undertaken to characterise the depth-dependent properties of articular cartilage (e.g. Schinagl et al., 1997; Jurvelin et al., 1997; Chen et al., 2001a; Mow and Guo, 2002; Chahine et al., 2004; Huang et al., 2005; Klein et al., 2007; Xia et al., 2007). The ratio of its constituents and their organisation as well as chondrocyte morphology vary with depth (Fig. 2.2). The
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Figure 2.4.: Split line pattern of a human femoral condyle and tibial plateau. Adapted from Benninghoff (1925b)

heterogeneity of the biochemical composition of articular cartilage gives rise to a similar depth dependency of its apparent mechanical properties. While the exact relationships between its composition, architecture and function still remain elusive, some key principles have emerged. The highest contents of collagen and water can be found in the uppermost 10% to 20% of the cartilage layer, the superficial zone, while GAG content is lowest. Chondrocytes have a flattened shape. Collagen alignment in this zone is mainly parallel to the articulating surface. In the local plane parallel to the surface the fibres align in a local direction that is termed split line (Fig. 2.4). The superficial zone exhibits the highest tensile properties (in the split line direction) and plays an important role both biochemically and biomechanically (Roth and Mow, 1980; Ateshian, 2009). The stress-strain curves in tension and compression do not transition in a continuous fashion – the tissue is tension-compression nonlinear. Tensile moduli of above 40 MPa in the split line direction have been reported (Akizuki et al., 1986; Roth and Mow, 1980) while compressive Young’s moduli are in the range of 0.3–1.5 MPa (Mow et al., 1992). Both stress-strain relationships are nonlinear and their transition region is characterised by an especially complex behaviour (Chahine et al., 2004). The following 40% to 60% of the tissue, the middle zone, show a more random alignment of thicker and less densely packed collagen fibres and decreasing water
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content. The final 30% – 50% of the tissue form the deep or radial zone (Cowin and Doty, 2007). Collagen fibres form bundles perpendicular to the surface and anchor the cartilage to the calcified zone and subchondral bone. FCD and compressive stiffness are highest in this zone. Roundish chondrocytes are arranged in columns. Towards the calcified cartilage zone chondrocytes increasingly become hypertrophic and the cartilage calcifies until a transition to the subchondral bone is made. The distinct collagen architecture with its variation in fibre alignment (Fig. 2.3) causes anisotropy both during direct mechanical loading and during swelling induced deformation (Chahine et al., 2004; Cowin and Doty, 2007). While tensile moduli have consistently been reported as being higher in the fibre direction than perpendicular to it, the influence of fibre orientation on the compressive properties is less understood. The collagen network can further contribute to fluid pressurisation by resisting tissue bulging under load (Li and Herzog, 2004) and determines anisotropies also in tissue permeability (Federico and Herzog, 2008; Reynaud and Quinn, 2006). Superficial collagen fibres parallel to the articular surface take up tensile stresses applied during compressive loading (Owen and Wayne, 2006) while deeper fibres are considered to resist shearing against the subchondral bone (Shirazi and Shirazi-Adl, 2008).

In summary, the collagen architecture contributes significantly to the load bearing properties of articular cartilage though its precise interaction with the charged components of the solid matrix remains to be established. Finally, it is important to note that the notion of distinct zones is conceptually useful but the transition is continuous in reality (Xia, 2008) as well as species dependent (Kääb et al., 1998). Other estimates for the zonal demarcations therefore differ from the above values, e.g. 8% of the total thickness for the superficial and 74% for the deep zone (Xia, 2008).

2.2. Modelling cartilage mechanics

It is well-established that mechanical loading of cartilaginous tissue stimulates biochemical activities resulting in tissue generation or degeneration. Numerical (in silico) experiments based on the knowledge of biomechanical and mechanobiological processes are of increasing importance and bear the opportunity of making a substantial contribution to clinical diagnostics and therapy (e.g. cartilage repair, tissue engineering) in connection with experimental in vivo data acquisition.

According to its function as a low friction and wear-resistant connective tissue
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with exceptional load-bearing properties under a wide variety of loading conditions, articular cartilage tissue exhibits a complex hydrated composite structure, see Maroudas (1979); Mow et al. (1980); Mow and Ratcliffe (1997) and others. It consists of a highly heterogeneous and anisotropic porous, permeable and deformable solid skeleton which is completely filled with the interstitial fluid.

There is a large number of publications dealing with the material behaviour of hyaline articular cartilage, and its appropriate modelling. Early biomechanical approaches were based on the description of the mechanical behaviour of cartilaginous tissue as an isotropic linear-elastic material. The tissue was then modelled as a complex continuum consisting of one or more phases characterised by nonlinear stress-strain relations. The linear biphasic models used in early studies (Mow et al., 1980; Mak et al., 1987; Mow et al., 1989) were often not capable of describing the material behaviour under varying loading scenarios (DiSilvestro et al., 2001a,b; Suh and Spilker, 1994). The models therefore quickly evolved to a higher degree of complexity in order to capture cartilage material properties more accurately. The biphasic theory was extended to include an ionic phase to the triphasic theory (Lai et al., 1991) for small strains and solid phase material isotropy. Electro-chemically induced swelling and its contribution to load-bearing have been of particular interest in many studies (Eisenberg and Grodzinsky, 1987; Myers et al., 1984; Sun et al., 1999; Olsen and Oloyede, 2002; Olsen et al., 2004). Based on Lanir’s hypothesis (Lanir, 1987a,b) that electrolyte flux can be neglected in articular cartilage mechanical studies, computationally less expensive simpler swelling models were also proposed (Wilson et al., 2005b,c). In Huyghe and Janssen (1997), the quadriphasic theory was formulated for finite deformations with individual kinematic paths for four phases (solid, fluid, cations, anions). Some authors also distinguish between extra- and intra-fibrillar fluid phases (Loret and Simões, 2004, 2005; Huyghe et al., 2003) or allow n ion species (Gu et al., 1998). The anisotropy of cartilaginous tissues due to collagen fibres has been captured mostly using (locally) transverse isotropy (Cohen et al., 1998; Donzelli et al., 1999; Bursac et al., 1999; DiSilvestro et al., 2001a,b) or fibril reinforced models (García et al., 1998; Li et al., 1999; Soulihat et al., 1999; Li et al., 2000; Korhonen et al., 2003; Wilson et al., 2004, 2005c; García and Cortés, 2007). Tension-compression nonlinearities have been included in the fibril reinforced models by allowing the fibres to be active in tension only, e.g. Li et al. (1999); Wilson et al. (2004). In another study the conewise linear elasticity theory from Curnier et al. (1995) was adopted to model the tension-compression nonlinearity (Soltz and Ateshian, 2000;
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Huang et al., 2001). After nonlinear strain-dependent permeability was studied in Lai et al. (1981) it was included in many of the different modelling approaches because of its impact on the material behaviour, e.g. Prendergast et al. (1996); Almeida and Spilker (1997, 1998); Ehlers and Eipper (1999); Ehlers and Markert (2001); Chen et al. (2001b); Li et al. (2001); Gu et al. (2003); Olsen et al. (2004). Especially at high strain rates the intrinsic viscoelasticity of the solid matrix was found necessary to explain cartilage material behaviour (Mak, 1986; DiSilvestro et al., 2001a). Many authors then incorporated various types of flow independent viscoelasticity in their models (Ehlers and Markert, 2001; Huang et al., 2001; García and Cortés, 2006; Ehlers et al., 2009). While in some studies viscoelasticity has an effect on the total deformation (Suh and Bai, 1998; Suh and DiSilvestro, 1999) it is active in others only in shear deformation and the contribution of the hydrostatic strain is assumed negligible (DiSilvestro et al., 2001b,a; DiSilvestro and Suh, 2001). In fibril reinforced models the viscoelasticity is often only attributed to the fibres (Li and Herzog, 2004; Wilson et al., 2004). As soft biological tissues undergo large deformations models making small strain assumptions are not valid under certain circumstances. Many authors therefore proposed finite strain models, often using hyperelastic relations based on Helmholtz free energy formulations (Holmes and Mow, 1990; Huyghe and Janssen, 1997; Ehlers and Eipper, 1999; Wang et al., 2001; Acartürk et al., 2003; Sun et al., 2004; Loret and Simões, 2005; Garcia and Cortés, 2006; Ehlers et al., 2009). Finally, the properties described above vary through the thickness of the tissue, e.g. Chen et al. (2001a). This heterogeneity of the tissue affects its material response and has been included in several models when the purpose of the study made it necessary (Li et al., 2000; Wang et al., 2001; Li et al., 2002b; Wilson et al., 2007).

Very few of these models combine the versatile aspects of cartilage material behaviour in one model and most are currently used in mainly academic and very specific contexts. The constitutive model presented in this thesis combines the above effects of material nonlinearity, anisotropy, heterogeneity, swelling and viscoelasticity in a biphasic formulation valid for finite strains. It has been developed with the intention to be applicable in a very variable context ranging from tissue engineering to skeletal regeneration. Current simulations for the study of skeletal regeneration mostly apply linear poroelastic models (Lacroix et al., 2002; Kelly and Prendergast, 2005; Isaksson et al., 2007) and therefore neglect both the geometric and material nonlinearities of the tissues involved as well as their anisotropies. This assumption implicitly prevents the development of simulations that simulta-
neously consider changes in cell or tissue phenotype and tissue structure in response to environmental cues.

2.3. Cartilage repair

Cartilage has a very limited ability for self repair. Its metabolic-catabolic equilibrium offers only little room for manoeuvre and excessive alterations or injury therefore often lead to continued degeneration until osteoarthritis (Muldrew, 2002; Schinhan et al., 2012) and eventually a complete loss of joint function occur, at which point total joint replacement becomes necessary. Focal defects therefore have to be treated to stop or at least delay degenerative events in the surrounding tissue. Common clinical treatments include microfracture, mosaicplasty and allogenic osteochondral grafting, biomaterial substitutes and tissue engineering approaches (for an extensive review and criticism see Hunziker (2002)). Tissue engineering offers the opportunity to create a biological substitute that does not suffer from the limited availability associated with usual transplants. In order to endure in vivo, a tissue engineered substitute has to be functional in the sense that it can sustain the joint loads while maintaining cell viability and its structural integrity as well as integrate well with the surrounding tissue. Clearly, the ideal construct would be one that mimics the native tissue that is has to replace in certain key aspects like stiffness and tribological properties. This would ensure not only functionality of the replacement itself but also provide a compliance match (and hence limited stress gradients) with the surrounding tissue as well as superior contact interactions with the opposing joint surface. It is therefore of great interest to tissue engineers to recapitulate the depth dependent properties of the tissue. Several approaches exist (reviewed in Klein et al. (2009)) but the goal remains elusive to date. Current tissue engineered cartilage lacks both the native composition and the architecture (Klein et al., 2009; Temenoff and Mikos, 2000; Hunziker, 2002; Koga et al., 2009). Understanding the role and relative contributions of tissue composition, architecture, heterogeneity as well as the interplay between ex vivo culture and in situ adaptation in determining the functionality and viability of tissue engineered constructs is crucial for directing research efforts. While both are likely to contribute to the short term viability, their effects on long term graft survival are especially unknown. The principal mechanical behaviour and the evolution of associated internal remodelling stimuli might serve as indicators of graft performance.
Finally, the collagen network also plays a crucial role in the aetiology of osteoarthritis (Mow et al., 1992). An early event in osteoarthritis is damage and remodelling of the collagen network, usually superficially (Guilak et al., 1994). This decreases the tensile stiffness of the collagen network such that the tissue can swell more, i.e. it becomes more hydrated. Additionally, the proteoglycans are now less restrained and can get lost from the solid matrix. Overall this leads to a weakening of the cartilage and an increase in deformation which can have further detrimental consequences. The collagen network architecture and its changes are therefore not only important for proper joint biomechanics but also relevant for the progression of degeneration as well as for engineering a functional cartilage substitute.

2.4. Tissue remodelling

The following definitions were proposed by Taber (1995):

- **Growth** means a change in mass. Positive growth adds mass and can happen through hyperplasia (cell division), hypertrophy (cell enlargement) and extracellular matrix secretion. Negative growth removes mass via cell death, shrinkage or extracellular matrix resorption and is called atrophy.

- **Remodelling** occurs through changes in microstructure and is often captured through altered macroscopic material properties like moduli and material symmetry. Although it often occurs simultaneously with growth, remodelling is uncoupled from growth for modelling purposes and thus shall exclude mass change.

- **Morphogenesis** refers to a change in shape and usually involves both growth and remodelling.

A number of theories have been proposed for the remodelling and/or growth of biological tissues (Humphrey and Rajagopal, 2003; Garikipati et al., 2006, 2004; Menzel, 2005, 2007). The role of collagen architecture and remodelling has been most extensively studied in cardiovascular mechanics. Its omnipresence in these tissues has been described by Humphrey (2008). The importance of remodelling can be extended to many other tissues including bone (Cowin and Hegedus, 1976; Carter, 1984; Prendergast and Taylor, 1994; Jacobs et al., 1997; Turner, 1998; Schaffler and Jepsen, 2000; Currey, 2003; Taylor et al., 2003; Lee et al., 2006) and
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Articular cartilage (Grodzinsky et al., 2000; Kääb et al., 2000; Wilson et al., 2006a; Stokes et al., 2006; de Visser et al., 2008; Rieppo et al., 2009). Articular cartilage has a very low cell density and the chondrocytes are supplied with nutrients via diffusive processes through the articular surface. It has been hypothesised that the particular structure of cartilage that results in its incapacity for self-repair is an evolutionary compromise to achieve adequate biomechanical function while retaining the ability to maintain the tissue for a life time (Muldrew, 2002). Despite the low cellular activity cartilage has been shown to adapt to altered mechanical stimuli and common experimental models include anterior cruciate ligament transection to induce osteoarthritic changes (Adams and Brandt, 1991; Brandt, 1991), change in activity levels (Palmoski and Brandt, 1981) and immobilisation that leads to an increase in catabolic activities and tissue degradation due to a lack of stimulation (Finsterbush and Friedman, 1975; Palmoski et al., 1980). Joint loading and motion are however required for the maintenance of a healthy cartilage tissue (Lu and Mow, 2008). The (at least partial) reversibility of changes due to joint immobilisation after remobilisation is further testimony to the tissues ability to remodel to some degree (Setton et al., 1997).

The constant adaptation of biological tissues to altered conditions is happening on all length scales from the genetic level all the way up to the organ level. Some processes seem to point toward the preservation of a “mechanical homeostasis” (Humphrey, 2008). Blood vessels, for example, seem to adapt in such a way as to maintain constant wall shear and circumferential wall stresses. A possible interesting implication of this is that the normal loading conditions would be somehow encoded in tissue structure. If, due to injury (e.g. ligament rupture in a joint), that loading environment changes, a possible treatment to prevent degenerative changes would be to restore that normal loading. These loading conditions could be “read out” of the tissue structure, if the relationship is known (Muldrew, 2002).

ECM remodelling can occur as the net result of a variety of mechanisms – cells can produce matrix components, secrete matrix enhancing or degrading products as well as their inhibitors, or even apply traction forces to existing ECM fibres (Baaijens et al., 2010). Furthermore, deformation can directly modulate enzymatic degradation of collagen fibres (Huang and Yannas, 1977; Ruberti and Hallab, 2005). How collagen remodelling might influence the load bearing properties of tissue engineered cartilage has not been studied. It is further considered unlikely that the stress-free configuration of cartilage is unaffected by the tissue configuration during matrix turnover (Baaijens et al., 2010) but the consequences
of such a modulation have not been highlighted. Suitable implantation conditions that may aid the recapitulation of the native architecture in the replacement tissue in a chondral defect have not been investigated. And finally it remains to be elucidated whether during skeletal regeneration the collagen architecture is guided by similar stimuli as have been established in cardiovascular mechanics.
3 Constitutive Model

Articular cartilage might appear a comparatively simple tissue from an anatomical perspective – it is aneural, avascular and only has one main cell type. Its ECM constituents are, however, distributed very heterogeneously and interact in a manner on the microscopic level that gives rise to a complex macroscopic mechanical behaviour. Constitutive models aimed at capturing cartilage behaviour therefore need to reflect this complexity. The material model developed here is also intended to represent other tissues that occur during skeletal healing such as fibrous tissue and bone as well as biomaterials like agarose. The material effects of interest vary between these materials as does the availability of experimental data. Therefore, a macroscopic thermodynamically consistent approach based on an overlay concept (Olsen and Oloyede, 2002) was chosen. This chapter begins with reviewing the fundamental balance principles and some basics regarding the modelling of porous media at large strains followed by an introduction to the overlay concept. The constitutive relations for the isotropic, anisotropic, swelling and viscoelastic behaviour will be described subsequently.

3.1. Balance principles

Independent of the specific material law applied certain balance principles have to be satisfied. Together with constitutive relations they form the basis of continuum thermomechanics. They have axiomatic character and are often written in global and local forms. In principle, the balance laws can be written for any part $\Omega$ of the body $\mathcal{S}$ ($\Omega \subset \mathcal{S}$). The effects of the remaining part $^1 \mathcal{S}_r$ on $\Omega$ have to be accounted for as well, for example via surface traction and heat flux. The balance principles will be presented for closed systems, i.e. systems of constant mass. For more details and additional aspects of open systems see e.g. Kuhl and Steinmann

$^1\mathcal{S}_r \cup \Omega = \mathcal{S}$ and $\mathcal{S}_r \cap \Omega = \emptyset$
3. Constitutive Model


3.1.1. Balance of mass

In a closed system the global relation

\[ \frac{\text{d}m}{\text{d}t} = \frac{\text{d}}{\text{d}t} \int_{\Omega} \rho(x, t) \text{d}\Omega = 0 \]  

(3.1)

holds where \( \rho(x, t) \) is the current local mass density. The local form can be derived by reformulating the integral using Reynold’s transport theorem (appendix A.1) and \( v = \dot{x} \)

\[ 0 = \int_{\Omega} \left( \frac{\text{d}\rho}{\text{d}t} + \rho \text{div} v \right) \text{d}\Omega 
\]

The localisation argument (the integral statement has to be valid for any sub-volume) leads to the local balance

\[ \frac{\text{d}\rho}{\text{d}t} + \rho \text{div} v = 0 \]  

(3.2)

with the material time derivative

\[ \frac{\text{d}\rho}{\text{d}t} = \frac{\partial \rho}{\partial t} + \text{grad} \rho \cdot v \]  

(3.3)

By using the chain rule of differentiation one finds

\[ \frac{\partial \rho}{\partial t} + \text{div} (\rho v) = 0 \]  

(3.4)

3.1.2. Balance of momentum

A change in linear momentum is caused by surface tractions \( t(x, t, n) \) and body forces per unit mass \( b(x, t) \). The global form of the spatial momentum balance thus reads

\[ \frac{\text{d}}{\text{d}t} \int_{\Omega} \rho v \text{d}\Omega = \int_{\partial \Omega} t \text{d}\Gamma + \int_{\Omega} b \text{d}\Omega \]  

(3.5)

By employing Cauchy’s formula and the divergence theorem (appendix A.1) to the first term of the right hand side as well as Reynold’s transport theorem (appendix
3. Constitutive Model

A.1) and the balance of mass to the left hand side of the above equation one arrives at

\[ \mathbf{0} = \int_{\Omega} (\nabla \sigma + \rho \mathbf{b} - \rho \mathbf{\dot{v}}) \, d\Omega \]  

(3.6)

Localisation finally implies (and using \( \mathbf{a} = \mathbf{\dot{v}} \))

\[ \nabla \sigma + \rho \mathbf{b} = \rho \mathbf{a} \]  

(3.7)

Conservation of angular momentum leads to the symmetry of the Cauchy stress tensor (Holzapfel, 2008):

\[ \sigma = \sigma^T \]  

(3.8)

### 3.1.3. Balance of energy

The balance of mechanical energy states that the external mechanical power \( P_{\text{ext}} \) is the sum of the stress power (rate of internal mechanical work) \( P_{\text{int}} \) and the change in kinetic energy \( E_{\text{kin}} \) (Holzapfel, 2008):

\[
\frac{d}{dt} \int_{\Omega} \frac{1}{2} \rho \mathbf{v}^2 \, d\Omega + \int_{\Omega} \mathbf{\sigma} : \mathbf{d} \, d\Omega = \int_{\partial \Omega} \mathbf{t} \cdot \mathbf{v} \, d\Gamma + \int_{\Omega} \rho \mathbf{b} \cdot \mathbf{v} \, d\Omega
\]  

(3.9)

where \( \mathbf{d} = \text{sym} \left( \nabla \mathbf{v} \right) \) is the rate of deformation tensor. Defining the internal stress power per unit reference volume \( w_{\text{int}} \) such that

\[ P_{\text{int}} = \int_{\Omega_0} w_{\text{int}} \, d\Omega_0 \]  

(3.10)

and using various pull-back operations allows the definition of work conjugate stress and strain measures\(^2\), e.g.

\[ w_{\text{int}} = \mathbf{\tau} : \mathbf{d} = \mathbf{P} : \dot{\mathbf{F}} = \mathbf{T} : \dot{\mathbf{E}} \]  

(3.11)

The invariance of the stress power will be of significance for the derivation of constitutive relations later in this chapter. The first law of thermodynamics states the equivalence between a change in the total energy of a system (sum of kinetic energy \( E_{\text{kin}} \) and internal energy \( U \)) an the sum of the external mechanical and

\(^2\)With the Kirchhoff stress \( \mathbf{\tau} = J \mathbf{\sigma} \), the first and second Piola-Kirchhoff stress tensors \( \mathbf{P} \) and \( \mathbf{T} \), respectively, as well as the Green-Lagrange strain tensor \( 2 \mathbf{E} = \mathbf{F}^T \mathbf{F} - \mathbf{I} \). \( \mathbf{F} \) is the deformation gradient, \( J = \det \mathbf{F} \) and \( \mathbf{I} \) the second order identity tensor.
3. Constitutive Model

thermal work done on the system (i.e. the power terms \( P_{\text{ext}} \) and \( Q \)), which reads in terms of the time rates:

\[
\frac{d}{dt}E_{\text{kin}} + \frac{d}{dt}U = P_{\text{ext}} + Q \tag{3.12}
\]

With Eq. 3.9 one can write

\[
\frac{d}{dt}U = P_{\text{int}} + Q \tag{3.13}
\]

Introducing the heat flux vector \( q \), heat sources (or sinks) per unit mass and time \( r \) and the specific internal energy \( u \) leads to the integral equation

\[
\frac{d}{dt} \int_{\Omega} \rho u d\Omega = \int_{\Omega} \sigma : d\Omega + \int_{\Omega} \rho r d\Omega - \int_{\partial\Omega} q \cdot n d\Gamma \tag{3.14}
\]

Applying Reynold’s transport theorem (appendix A.1) and the balance of mass to the left hand side of the equation, as well as the divergence theorem (appendix A.1) to the last term on the right hand side, and subsequently employing the localisation argument yields the local form of the first law of thermodynamics in spatial description

\[
\rho \frac{du}{dt} = \rho r - \text{div} q + \sigma : d \tag{3.15}
\]

3.1.4. Entropy inequality

The second law of thermodynamics clarifies the direction of energy transfer and makes a statement on the reversibility of processes. With the specific entropy \( s \) the total entropy of a system is given as

\[
S = \int_{\Omega} \rho s d\Omega \tag{3.16}
\]

The second law of thermodynamics now demands that the time rate of change of entropy is greater or equal to the thermal power with respect to the absolute temperature \( \theta(\mathbf{x}, t) \geq 0 \):

\[
\frac{d}{dt} \int_{\Omega} \rho s d\Omega - \int_{\Omega} \rho \frac{r}{\theta} d\Omega + \int_{\partial\Omega} \frac{q}{\theta} \cdot n d\Gamma \geq 0 \tag{3.17}
\]

where the last two terms correspond to the entropy supply and flux through heat, respectively, related to thermal quantities. An alternative formulation of this statement is that the internal entropy production (\( \gamma \) is the specific internal entropy
3. Constitutive Model

production) is non-negative:
\[
\frac{d}{dt} \int_{\Omega} \rho \dot{\Omega} = \int_{\Omega} \rho \dot{\theta} d\Omega - \int_{\partial \Omega} \frac{\partial q}{\partial n} \cdot \mathbf{n} d\Gamma + \int_{\Omega} \rho \gamma d\Omega \geq 0
\] (3.18)

Here, we proceed from Eq. 3.17. Applying Reynold's transport theorem (appendix A.1) and the balance of mass to the first term, as well as the divergence theorem to the last term followed by the application of the localisation argument yields
\[
\rho \frac{d}{dt} + \frac{1}{\theta} \left[ \text{div} \mathbf{q} - \frac{1}{\theta} \text{grad} \theta \cdot \mathbf{q} - \rho r \right] \geq 0
\] (3.19)

Substituting \( \text{div} \mathbf{q} - \rho r \) using the balance of energy in Eq. 3.15 and introducing the specific free Helmholtz energy \( \psi = u - \theta s \) one arrives at the Clausius-Duhem inequality that is the basis for the derivation of thermodynamically consistent constitutive models:
\[
\sigma : \mathbf{d} - \rho s \frac{d\theta}{dt} - \rho \frac{d\psi}{dt} - \frac{1}{\theta} \text{grad} \theta \cdot \mathbf{q} \geq 0
\] (3.20)

The system of equations needs to be completed by constitutive relationships between the deformation and stress measures as well as their rates. Due to the introduction of a biphasic material model in the following section the above balance equations will be extended to their biphasic counterparts where needed.

3.2. Theory of porous media at large strains

The presented theory is limited to isothermal quasistatic processes with no mass exchange between the immiscible and intrinsically incompressible constituents. The discussion is further restricted to biphasic materials where the porous solid matrix is fully saturated with a liquid pore fluid. More general derivations can be found in de Boer (2005) and the references therein.

One fundamental assumption is that all constituents are in ideal disarrangement and can be represented using smeared substitute continua with reduced partial mass densities. Local macroscopic quantities therefore represent the physical properties of the individual constituents merely in the sense of a local average (Ehlers,

\[3^{3}\text{The incompressibility assumption is often valid as most liquid pore fluids are incompressible in a wide range of hydrostatic pressures. At the same time the compressibility of the solid phase material is negligible compared to the compressibility of the solid matrix.}\]
2002; Görke et al., 2012). As a measure of the local portions of the constituents volume fractions $\phi$ can be introduced in the initial and current configurations

$$\phi_{S(0)} = \frac{d\Omega_{S(0)}}{d\Omega_{(0)}}, \quad \phi_{F(0)} = \frac{d\Omega_{F(0)}}{d\Omega_{(0)}}$$  \hspace{1cm} (3.21)

where $d\Omega_\bullet$ denotes a volume element. The indices $F$ and $S$ refer to the fluid and solid component, respectively, and the index 0 identifies a quantity in the initial configuration. The important quantity $\phi_F$ is also known as “porosity”.

The saturation condition introduces the constraint

$$\phi_S + \phi_F = 1$$  \hspace{1cm} (3.22)

and the assumption of intrinsic incompressibility of all constituents implies that the true densities

$$\rho_{SR} = \frac{dm_S}{d\Omega_S} \quad \text{and} \quad \rho_{FR} = \frac{dm_F}{d\Omega_F}$$  \hspace{1cm} (3.23)

are constant. The partial densities of the smeared substitute continua follow from

$$\rho_S = \frac{dm_S}{d\Omega} \quad \text{and} \quad \rho_F = \frac{dm_F}{d\Omega}$$  \hspace{1cm} (3.24)

The two density measures are related via

$$\rho_S = \phi_S \rho_{SR} \quad \text{and} \quad \rho_F = \phi_F \rho_{FR}$$  \hspace{1cm} (3.25)

Thus the intrinsically incompressible constituents behave compressible with respect to the continuum. The mean density of the homogenised porous material is

$$\rho = \rho_S + \rho_F$$  \hspace{1cm} (3.26)

3.2.1. Kinematics and volume balance

The kinematics of multi-phase media is based on two fundamental assumptions

1. Every particle at position $x$ at the current time point $t$ is composed simultaneously out of all constituents.

2. All constituents are distinct in the individual motions of their particles $X$, independent of the motion of the other constituents (see fig. 3.1).
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The laws of motion and their inverse expressions are thus given as

\[ x = \varphi_S(X_S, t) \quad \text{and} \quad x = \varphi_F(X_F, t) \]  
\[ X_S = \varphi_S^{-1}(x, t) \quad \text{and} \quad X_F = \varphi_F^{-1}(x, t) \]

The basis of deducing constitutive laws at large strains is the deformation gradient. Its definition with respect to the solid skeleton represents the fundamental kinematic quantity for biphasic materials:

\[ F_S = \text{Grad}_S x, \quad J_S = \det F_S = \frac{d\Omega}{d\Omega_0} \]

The velocities of material particles of both constituents at time \( t \) are given as

\[ \mathbf{v}_S = \mathbf{x}'_S = \frac{\partial \varphi_S(X_S, t)}{\partial t} = (\mathbf{u}_S)'_S \]
\[ \mathbf{v}_F = \mathbf{x}'_F = \frac{\partial \varphi_F(X_F, t)}{\partial t} \]

For spatial functions different material time derivatives can be defined following the individual motions introduced in Eq. 3.27. Considering an arbitrary spatial scalar function \( \xi(x, t) \) we can write (with \( \alpha \in F, S \)):

\[ \xi' = \frac{d\alpha}{dt} \xi = \frac{\partial \xi}{\partial t} + \text{grad} \cdot \mathbf{v}_\alpha \]

The seepage velocity \( \mathbf{w}_F \), which is the velocity of the fluid relative to the deforming solid skeleton,

\[ \mathbf{w}_F = \mathbf{v}_F - \mathbf{v}_S \]
3. Constitutive Model

can be used to connect both derivatives:

\[ \xi'_F = \xi'_S + \nabla \cdot w_F \quad (3.34) \]

Some restrictions need to be imposed on the motions of the constituents for a saturated mixture of intrinsically incompressible materials. Evaluating the material time derivative of the saturation condition with respect to one of the constituents yields (using relation 3.34).

\[ (\phi_S)'_S + (\phi_F)'_S = (\phi_S)'_S + (\phi_F)'_F - \nabla \cdot w_F \quad (3.35) \]

Using Eqs. 3.25 leads to

\[ (\phi_S)'_S = \left( \frac{\rho_S}{\rho_{SR}} \right)'_S \phi_S - \frac{\phi_S}{\rho_{SR}} (\rho_{SR})'_S \quad (3.36) \]

\[ (\phi_F)'_F = \left( \frac{\rho_F}{\rho_{FR}} \right)'_F \phi_F - \frac{\phi_F}{\rho_{FR}} (\rho_{FR})'_F \quad (3.37) \]

where the last terms in both equations vanish due to the intrinsic incompressibility assumption. Neglecting mass exchange between the constituents introduces the mass balance for each constituent

\[ 0 = (\rho_S)'_S + \rho_S \text{div} x'_S \quad (3.38) \]

\[ 0 = (\rho_F)'_F + \rho_F \text{div} x'_F \quad (3.39) \]

Substituting these equations into Eq. 3.35 produces (with the use of the zero-addition of the term \( \phi_F \text{div} x'_S \))

\[ (\phi_S)'_S + (\phi_F)'_S = -(\phi_S + \phi_F) \text{div} x'_S - \text{div} (\phi_F w_F) \quad (3.40) \]

With the saturation condition we get the volume balance of a biphasic fluid-saturated porous medium in spatial description:

\[ \text{div} [x'_S + \phi_F w_F] = 0 \quad (3.41) \]

Finally, the evolution equation for the porosity shall be mentioned:

\[ \phi_F = 1 - \frac{1 - \phi_{F0}}{\Omega_S} \quad (3.42) \]

where use was made of the fact that the control volume is defined on the boundaries of the solid skeleton and hence \( d\Omega_S = d\Omega_{S0} \).
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3.2.2. Stress tensors in the Theory of Porous Media

The stress state in every point in space can be decomposed into several partial stresses. One part equally acts in the present material particles of both fluid and solid (hydrostatic state) and is called pore pressure $p$. It describes the interaction between flowing fluid and deforming solid skeleton. The total stress will therefore be determined by further parts depending on the history of the fluid flow and/or the deformation of the solid skeleton. These stresses, for which constitutive equations have to be formulated, are called effective stresses, denoted by $\sigma^E$ (Ehlers, 2002; Görke et al., 2012).

Based on the concept of effective stresses the partial stresses for solid and fluid phase can be written in the following way

$$
\sigma_S = -\phi_S p I + \sigma^E_S
$$

(3.43)

$$
\sigma_F = -\phi_F p I + \sigma^E_F
$$

(3.44)

where the common sign convention is used:

$$
p > 0 \text{ for pressure, } \quad \sigma^E_S > 0 \text{ for tension}
$$

(3.45)

As the partial substitute continuum is assumed to completely fill out the space of the total continuum, a compressible material law is needed for $\sigma^E_S$ despite material incompressibility. Furthermore, we assume that dissipative stresses in the fluid are negligible compared to fluid-solid interaction, and thus $\sigma^E_F = 0$.

Pulling back these stress relations into a material description (Görke et al., 2012) yields the second Piola-Kirchhoff stresses

$$
T_S = -J_S \phi_S p C_S^{-1} + T^E_S
$$

(3.46)

$$
T_F = -J_S \phi_F p C_S^{-1}
$$

(3.47)

Using the saturation condition we get the total stress in the continuum by adding the partial stresses:

$$
T = T^E_S - p J_S C_S^{-1}
$$

(3.48)

For $T^E_S$ the constitutive relations are developed in the following sections. Equation 3.48 shows that for a vanishing pore pressure, which is the case for the equilibrium state of free draining porous media, the material response is equivalent to that of a single phase material with the constitutive behaviour of the solid matrix.
3. Constitutive Model

3.2.3. Momentum balance and entropy inequality

The linear momentum balance for each constituent with momentum production terms resulting from the interaction with the other species and under neglection of mass exchange between them yields

\[
\text{div } \sigma_S + \rho_S b_S + \dot{p}_S = 0
\] (3.49)

\[
\text{div } \sigma_F + \rho_F b_F + \dot{p}_F = 0
\] (3.50)

The constituent balance equations have to add up in such a way that the equations for the mixture correspond to those of a single phase continuum. Using \( \rho b = \rho_S b_S + \rho_F b_F \) and \( b = b_S = b_F \) we get the local spatial linear momentum balance as

\[
\text{div } \sigma + \rho b = 0
\] (3.51)

and find \( \dot{p}_S = -\dot{p}_F \). The entropy inequality under the assumptions made so far (for a derivation see Appendix A.2) is given as

\[
\sigma_S^E : d_S - \rho_S (\dot{\psi}_S)'_S - \dot{p}_F^E w_F \geq 0
\] (3.52)

The inequality \(-\dot{p}_F^E w_F \geq 0\) can be satisfied by the definition

\[
\dot{p}_F^E = -\phi_F^2 \mu_{FR} k_S^{-1} w_F
\] (3.53)

where the fluid viscosity \( \mu_{FR} \) is a positive material parameter and the second order intrinsic permeability tensor \( k_S \) is positive definite. In other words, the frictional drag between solid and fluid is proportional to the relative velocity between the phases. Incorporating this definition (in combination with Eq. A.8) into the fluid momentum balance for \( \sigma_F = -\phi_F p I \) yields Darcy's law

\[
\phi_F w_F = -\frac{k_S}{\mu_{FR}} (\text{grad } p - \rho_{FR} b_F) = -k (\text{grad } p - \rho_{FR} b_F)
\] (3.54)

where the product \( \phi_F w_F \) is the filter velocity and \( k \) the hydraulic permeability tensor, that will be simply termed “permeability” in the sequel. While this definition allows for anisotropic permeabilities, isotropy will be assumed in the sequel: \( k = k I \). The permeability decreases with decreasing pore volume and becomes zero when all pores are closed. In Ehlers and Eipper (1999), the permeability is described by the power law

\[
k = k_0 \left( \frac{\phi_F}{\phi_{F0}} \right)^M
\] (3.55)
For biological tissues the formulations of Gu et al. (2003) and Lai and Mow (1980) can be combined using the formulation of Holmes and Mow (1990):

\[ k = k_0 \left[ \frac{(1 - \phi F_0)\phi_F}{(1 - \phi_F)\phi_{F0}} \right]^{-M_1} e^{-\frac{M_2(J_2^0 - 1)}{2}} \quad (3.56) \]

With the last term in relation 3.52 now fulfilling the inequality, it was deemed advantageous to pull back the remaining dissipation relation into a material description (Appendix A.2)

\[ -\rho s_0 \dot{\psi}_S + \frac{1}{2} \mathbf{T}_S^F : \dot{\mathbf{C}}_S \geq 0 \quad (3.57) \]

The dependence of the free energy functionals on deformation measures and further internal variables will be determined by the material properties of the solid skeleton. The solid matrix material behaviour will be derived in the following sections.
3. Constitutive Model

3.3. Constitutive model for the solid phase

The constitutive model to calculate the solid extra stresses will be derived in this section. As became evident in the previous section, the notion of smeared substitute continua requires the derivation of a compressible material law. However, since the constituents are intrinsically incompressible, the solid matrix can be compressed to the so-called point of compaction at which all pore fluid has been squeezed out and the pores are closed. The material will therefore behave as an (one-sided) incompressible solid thereafter. This phenomenon can be incorporated into the strain energy functions, see e.g. Ehlers and Eipper (1999). The point of compaction is reached at \( J_S = \phi_{S_0} \), where the solidity for soft biological tissues and biomaterials typically is below \( 30\% \). In the following simulations compressive strains were never large enough to approach volume ratios in this low range. A point of compaction treatment is therefore not presented. The derivation of constitutive relations for the solid extra stress of the biphasic material follows the same rules as that of single phasic materials. The index \( S \) designating the solid phase will therefore be omitted in the sequel.

The material model has been implemented into both MSC Marc (version 2008r1, MSC Software Corp., Santa Anna, CA, USA) and Abaqus (version 6.8, Simulia, Providence, RI, USA) and was partially made available online (Nagel and Kelly, 2012a). For implementational issues see appendix A.3.

3.3.1. The overlay concept

The overlay concept was developed in the 1970s (Owen et al., 1974; Pande et al., 1977) and has been applied to the study of biological tissues in the past (Olsen and Oloyede, 2002). The idea behind this concept is to fictitiously decompose the material into \( n \) layers that each undergo the same deformation. However, as each of the layers has unique material properties or constitutive relations, each layer also contributes with its own stress response. The total stress then follows from

\[
T = \sum_{k=1}^{n} T_k
\]  

(3.58)

The overlay concept is well suited to individually represent structural components of the cartilage matrix, such as proteoglycans and collagen fibre reinforcement, as well as distinct features of the material response, such as time dependent and independent behaviour of the solid matrix.
3. Constitutive Model

In the following sections we will introduce four main layers: An isotropic ground phase \( T_{iso} \), an anisotropic overlay \( T_{aniso} \) with discrete as well as continuously distributed families of fibres, intended to represent the collagen network, and a swelling pressure contribution \( T_{swell} \). Finally, the fourth overlay captures the viscoelastic response of the solid matrix via an overstress concept \( T_{ov} \).

In summary:

\[
T_E^S = T_{iso} + T_{aniso} + T_{swell} + T_{ov}
\]  

(3.59)

3.3.2. Isotropic hyperelasticity

Proceeding from the Clausius-Duhem inequality

\[
-\rho_0 \frac{d\bar{\psi}}{dt} + \frac{1}{2} T : \dot{\mathbf{C}} \geq 0
\]

(3.60)

with a fully elastic isotropic ground phase, the equality in above equation holds. Therefore, the 2nd Piola-Kirchoff stresses are derived from

\[
T = 2\rho_0 \frac{\partial \bar{\psi}}{\partial \mathbf{C}} = 2 \frac{\partial \psi}{\partial \mathbf{C}}
\]

(3.61)

where \( \bar{\psi} \) and \( \psi \) are the specific free Helmholtz energy (per unit mass) and the free Helmholtz energy density (per unit volume), respectively. Any isotropic tensor function can be expressed in terms of the principal invariants of its argument. The free Helmholtz energy density function can therefore be written as

\[
\psi = \psi(I_1(C), I_2(C), I_3(C))
\]

(3.62)

The principal invariants of the right Cauchy Green tensor are given as

\[
I_1(C) = \mathbf{C} : I
\]

\[
I_2(C) = \frac{1}{2} [(\mathbf{C} : I)^2 - \mathbf{C} \cdot \mathbf{C} : I]
\]

(3.63)

\[
I_3(C) = \text{det} \mathbf{C} = J^2
\]

Applying the chain rule of partial differentiation to 3.61 yields:

\[
T = 2 \sum_{\alpha=1}^{3} \frac{\partial \psi}{\partial I_\alpha} \frac{\partial I_\alpha}{\partial \mathbf{C}}
\]

(3.64)

To reduce the number of material parameters and limit model complexity a formulation independent of the second invariant was chosen. The partial derivatives of \( I_1 \) and \( I_3 \) with respect to \( \mathbf{C} \) are given as:

\[
\frac{\partial I_1}{\partial \mathbf{C}} = I \quad \text{and} \quad \frac{\partial I_3}{\partial \mathbf{C}} = (\text{det} \mathbf{C}) \mathbf{C}^{-1}
\]

(3.65)
3. Constitutive Model

so that

\[ T = 2 \frac{\partial \psi}{\partial I_1} I + 2 \frac{\partial \psi}{\partial I_3} I_3 C^{-1} \] (3.66)

To include the possibility of stiffening as seen in many biological materials, a Fung-like formulation was implemented:

\[ \psi_{\text{iso}} = \frac{C_1}{\alpha} \left[ e^{\alpha(I_1 - \ln I_3 - 3)} - 1 \right] + D_2 (\ln I_3)^2 \] (3.67)

For small strains the parameters \( C_1 \) and \( D_2 \) are related to Young’s modulus and Poisson’s ratio via the relations

\[ C_1 = \frac{E}{4(1 + \nu)} \quad \text{and} \quad D_2 = \frac{C_1 \nu}{2(1 - 2\nu)} \] (3.68)

In addition to the stress tensors the material elasticity tensor has to be determined as a result of the consistent linearisation performed in the context of iterative Newton-Raphson type solution strategies. The fourth order elasticity tensor in material description follows from

\[ C = 2 \frac{\partial T}{\partial C} \] (3.69)

Because \( T \) and \( C \) are symmetric, it possesses the minor symmetries

\[ C_{ABCD} = C_{BACD} = C_{ABDC} \] (3.70)

and thus 36 independent components. For hyperelastic materials the following additionally holds:

\[ C = 2 \frac{\partial^2 \psi}{\partial C^2} \] (3.71)

and now the major symmetries

\[ C_{ABCD} = C_{CDAB} \quad \text{or} \quad C = C^T \] (3.72)

reduce the number of independent components to 21. The repeated use of the chain rule of partial derivatives yields the general form of the material elasticity tensor (see appendix A.4).

\(^4\)With \( \alpha = 0 \) the stress-strain relationship of a Neo-Hookean material is recovered with

\[ \psi = C_1 (I_1 - \ln I_3 - 3) + D_2 (\ln I_3)^2 \].
3. Constitutive Model

3.3.3. Collagen fibre reinforcement – anisotropy

Fibres contribute to cartilage load-bearing in a complex manner. They not only counteract lateral expansion and thus stiffen the overall tissue response in unconstrained compression and complex loading scenarios but due to their stiffening with strain induce additional rate dependency in cartilage (Li and Herzog, 2004). Their confining effect supports fluid pressurisation so that during fast loading a great portion of the load is born by the fluid phase. If the fibrous network is pre-stressed due to internal swelling pressures the material behaviour becomes very complex (Nagel and Kelly, 2010a, 2012a); see also chapter 4.

Discrete families of fibres

A model with \( n_{\text{fib}} \) families of fibres is introduced where usually \( n_{\text{fib}} \leq 4 \). The fibre directions are defined in the undeformed configuration with the unit vector fields\(^5\) \( \mathbf{a}_0^i(X, t) \). During deformation the vector \( \mathbf{a}_0^i \) is stretched and rotated into its representation \( \tilde{\mathbf{a}}^i \) in the current configuration. Defining the vector \( \mathbf{a}^i \) as the current fibre direction with \( |\mathbf{a}^i| = 1 \) (eliminating stretch \( \lambda \)) the relations

\[
\tilde{\mathbf{a}}^i = \mathbf{F} \mathbf{a}_0^i = \lambda_i \mathbf{a}^i
\]

hold. Using the definition of a structural tensor \( \mathbf{M}^i \) in the initial configuration:

\[
\mathbf{M}^i = \mathbf{a}_0^i \otimes \mathbf{a}_0^i
\]

a fourth invariant \( I_4^i \) can be introduced in the following equivalent ways

\[
I_4^i = \mathbf{a}_0^i \cdot \mathbf{C} \mathbf{a}_0^i = \text{tr}(\mathbf{M}^i \mathbf{C}) = \mathbf{I} : \mathbf{M}^i \mathbf{C} = \lambda_i^2
\]

The partial derivatives of \( I_4^i \) with respect to \( \mathbf{C} \) follow as

\[
\frac{\partial I_4^i}{\partial \mathbf{C}} = \mathbf{a}_0^i \otimes \mathbf{a}_0^i \quad \text{and} \quad \frac{\partial^2 I_4^i}{\partial \mathbf{C}^2} = 0
\]

Following the overlay concept, the free Helmholtz-Energy density function can be decoupled into an isotropic and anisotropic parts

\[
\psi(I_1, I_2, I_3, I_4^i) = \psi_{\text{iso}}(I_1, I_2, I_3) + \sum_{i=1}^{n_{\text{fib}}} \psi_{\text{aniso}}(I_4^i)
\]

\(^5\)Unless explicitly indicated no summation is implied when the superscript \( i \) occurs twice in this context.
3. Constitutive Model

It follows analogously to the isotropic derivations

$$\mathbf{T} = 2 \frac{\partial \psi_{\text{iso}}}{\partial I_1} \mathbf{I} + 2 \frac{\partial \psi_{\text{iso}}}{\partial I_3} I_3 C^{-1} + 2 \sum_{i=1}^{n_{\text{fib}}} \frac{\partial \psi_{\text{aniso}}}{\partial I_4^i} a_0^i \otimes a_0^i$$

(3.78)

The anisotropic tangent moduli can be found in appendix A.5. Tension-compression nonlinearity can be introduced by letting a family of fibres only contribute to the total stress when the associated fibre stretch is tensile (ensured by the Heaviside step function):

$$\mathbf{T} = \mathbf{T}_{\text{iso}} + \sum_{k=1}^{n_{\text{fib}}} H(I_4^i - 1) \mathbf{T}_{\text{aniso}}^k$$

(3.79)

So far only the tension-compression nonlinearity has involved a constitutive assumption – namely, that collagen fibres only resist tension and not compression. The remaining relations can be implemented regardless of the constitutive model used, making the implementation of new models straight forward.

Several constitutive models were implemented each with certain advantages or applications. The first relation is again based on an exponential model and has been used in the modelling of collagen in the cardiovascular system (Holzapfel et al., 2000). The second strain energy function is motivated from works in rubber elasticity (Gent, 1996). This Gent-like model captures a limited fibre extensibility and has been applied to arterial wall mechanics as well (Horgan and Saccomandi, 2003). The third formulation, finally, is a power law and has been used in cartilage mechanics (Ateshian et al., 2009; Nagel and Kelly, 2012b). The formulations for the free Helmholtz energy density functions are

$$\psi_{\text{aniso}}^\text{Fung} = \frac{C_4^i}{2 \beta^i} \left[ e^{\beta^i (I_4^i - 1)^2} - 1 \right]$$

(3.80)

$$\psi_{\text{aniso}}^\text{Gent} = -C_6^i J_a^i \ln \left[ 1 - \frac{(I_4^i - 1)^2}{J_a^i} \right]$$

with \( J_a^i \geq (I_4^i - 1)^2 \)

(3.81)

$$\psi_{\text{aniso}}^\text{Ateshian} = C_4^i \left[ I_4^i - 1 \right]^{\beta^i}$$

with \( \beta^i \geq 2 \)

(3.82)

(3.83)

The Gent SED-function approaches infinity when the limiting extensibility of the fibre \( \lambda_{\text{max}}^i = \sqrt[4]{J_a^i} + 1 \) is reached.
3. Constitutive Model

The necessary partial derivatives are listed in appendix A.5. The verification of
the discrete fibre model using transverse isotropy can be found in appendix B.1

Continuous fibre distributions

When the collagen network of articular cartilage is viewed under transmission
electron microscopy it appears very random despite the overlying structure (Eyre
et al., 2006; Clark, 1991). Statistical or continuous fibre distribution models can
capture this distribution of fibre angles. The implementation of the angular fi-
bre distribution in this work is based on a recent contribution (Ateshian et al.,
2009) that successfully modelled the complex behaviour of articular cartilage es-
pecially during the initial stages of compressive loading during which pre-stress
is relieved from the collagen network and stress softening phenomena as well as
other nonlinearities are observed.

Fibres are assumed to be distributed such that the fibre directions in the un-
deformed configuration form a unit sphere. The anisotropic stress contribution is
then found via integration:

\[ T_{\text{aniso}} = 2 \int_0^{2\pi} \int_0^\pi H(I_4 - 1) \frac{\partial u_{\text{aniso}}}{\partial C} \sin \phi \, d\phi \, d\theta \]  \hspace{1cm} (3.84)

where with the spherical angles with respect to the fixed Cartesian basis, \( \{e_i\} \),
\( \theta \in [0; 2\pi] \) and \( \phi \in [0; \pi] \) a direction is given as \( a_0 = \cos \theta \sin \phi \, e_1 + \sin \theta \sin \phi \, e_2 + \cos \phi \, e_3 \). The Heaviside step function ensures that individual fibres buckle under
compressive loads without stress contribution, i.e. the present model is fully ten-
sion compression nonlinear in each direction. This distinguishes it from other fibre
dispersion approaches common in cardiovascular mechanics (Gasser et al., 2006;
Cortes et al., 2010).

To incorporate anisotropy, the fibre stiffness is varied with direction, i.e. \( C_4 = f(a_0) \). In principle, any distribution function can be integrated with Eq. 3.84.
Here, an ellipsoidal distribution was chosen following Ateshian et al. (2009). For
that purpose, an anisotropy tensor is introduced\(^6\):

\[ \Xi = \sum_{i=1}^{3} \xi_i w_i \otimes w_i \]  \hspace{1cm} (3.85)

\(^6\Xi\) also forms the basis for the remodelling framework presented in chapter 5 and a variety of
fibre architectures can be derived from it aside from ellipsoidal distributions through appro-
priate definitions.
3. Constitutive Model

(a) Anisotropy ellipsoid $\Xi$

Figure 3.2.: (a) Ellipsoid representation of the anisotropy tensor $\Xi$ defined in Eq. 3.85. (b) Aligning the vector cloud with the major axis of the ellipsoid prior to integration as shown in the figure ensures that the peak value is captured in the discretisation.

where the eigenvalues $\xi_i$ are the half axes of the ellipsoid whose orientation in space is determined by its eigenvectors $\mathbf{w}_i$ (Fig. 3.2a). In other words, the $\xi_i$ determine the anisotropy or stiffness distribution of the tissue while the $\mathbf{w}_i$ characterise the principal characteristic material axes. When the components of the direction vector $\mathbf{a}_0$ are expressed in terms of the basis $\{\mathbf{w}_i\}$ as $\mathbf{a}_0 = \cos \Theta \sin \Phi \mathbf{w}_1 + \sin \Theta \sin \Phi \mathbf{w}_2 + \cos \Phi \mathbf{w}_3$ using the spherical angles $\Theta$ and $\Phi$, the material parameter $C_4$ in a fibre direction can derived via

$$C_4(\mathbf{a}_0) = \left[ \frac{(\cos \Theta \sin \Phi)^2}{\xi_1^2} + \frac{(\sin \Theta \sin \Phi)^2}{\xi_2^2} + \frac{(\cos \Phi)^2}{\xi_3^2} \right]^{-\frac{1}{2}} \tilde{C}_4$$

(3.86)

where $\tilde{C}_4$ is a baseline material parameter for fibre stiffness that can be thought of as being scaled with the ellipsoid’s radius in the direction $\mathbf{a}_0$. The advantage of the definition in Eq. 3.86 is that tissue architecture ($\xi_i$) and overall stiffness ($\tilde{C}_4$) can be defined separately.

To numerically integrate Eq. 3.84 an algorithm to pixelise the unit sphere was used to establish a quasi-isotropic fibre distribution (Tegmark, 1996). Unless otherwise stated, $N = 492$ discrete directions were used. The discretisation algorithm creates a fixed vector cloud that does not necessarily capture the peak value of the elliptic fibre stiffness distribution (Fig. 3.2b). Prior to integration, the original vector can however be rotated and aligned with the major axis of $\Xi$ such that this peak value can be captured (see appendix B.3.3). Numerically, the integration in Eq. 3.84 is performed via the summation

$$T_{\text{aniso}} = \sum_{i=1}^{N} H(I_i - 1) T_{\text{aniso}}^i \Delta A_i$$

(3.87)
Section 3, Constitutive Model

Principal model verification can be found in appendix B.3.

3.3.4. Nonlinear viscoelasticity

In Lion (1997b) uniaxial rheological models are generalised into a three dimensional thermomechanical constitutive theory that includes nonlinear rate-dependent overstresses, nonlinear elasticity and a rate-independent equilibrium hysteresis. Here, any plastic deformation will be neglected and the presentation restricted to isothermal nonlinear viscoelasticity. Isotropy will be assumed and the reader is referred to Görke et al. (2010) for additional aspects including the anisotropic extension of this framework (i.e. viscoelastic collagen fibres). A uniaxial counterpart to the derivations presented below can be illustrated with the rheological model from Fig. 3.3a.

![Rheological model](image)

(a) Uniaxial rheological model

![Vector alignment](image)

(b) Aligning the vector cloud

Figure 3.3.: (a) Uniaxial rheological model with a Maxwell element parallel to a spring. (b) The three dimensional representation of the viscoelastic theory. An incompatible intermediate configuration is introduced. Adapted from Lion (1997b).

Stress and Strain Measures

In order to generalise the basic ideas from the uniaxial rheological model (Fig. 3.3a) into a 3D finite inelastic deformation theory a reasonable strain decomposition along with the definition of associated stress tensors is needed. To that end the concept of dual variables has been developed (Haupt and Tsakmakis, 1989). A multiplicative split of the deformation gradient into an elastic and a viscous part

\[ F = F_{ee} F_v \]  

introduces a so-called intermediate configuration \( \mathcal{F} \) between the reference configuration \( \mathcal{F} \) and the current configuration \( \mathcal{C} \). Only in this intermediate configuration
can an additive split of the strain tensor into a purely elastic and inelastic part be achieved (Fig. 3.3b) (Lion, 1997b). Inserting this decomposition into the Green-Lagrange strain tensor $E$ produces:

$$E = \frac{1}{2} (F_v^T (F_v^T F_{ev}) F_v - I)$$

(3.89)

After a push-forward into $\mathcal{F}$ we obtain the strain tensor $\epsilon_{ov}$:

$$\epsilon_{ov} := F_v^{-T} E F_v^{-1} = \frac{1}{2} (F_v^T F_{ev} - I) + \frac{1}{2} (I - F_v^{-T} F_v^{-1}) =: \epsilon_{ev} + \epsilon_v$$

(3.90)

This decomposition corresponds physically to the Maxwell element in fig. 3.3a. The first term is of Green-Lagrange type, while the second term is of Almansi-Euler type. Again motivated by the rheological model in fig. 3.3a we introduce an analogue decomposition of the 2nd Piola Kirchhoff stress (Lion, 1997b):

$$T = T_{eq0} + T_{ov}$$

(3.91)

where $T_{eq0}$ is the equilibrium stress (e.g. defined by the relations in the preceding sections) and $T_{ov}$ is the rate dependent overstress. As this stress decomposition is associated to the reference configuration $\mathcal{R}$ the overstress has to be pushed to the intermediate configuration in order to be related by constitutive equations to the associated strain tensor $\epsilon_{ev}$ (Lion, 1997b). By application of the concept of dual variables (for details see Haupt and Tsakmakis (1989)) the transformation is performed via

$$\tau_{ov} = F_v T_{ov} F_v^T$$

(3.92)

To split the stress and strain rates equivalently to the stress and strain measures they are given by the following Oldroyd rates\(^7\) within the concept of dual variables:

$$\nabla \tau_{ov} := F_v \tau_{ov} F_v^T = \dot{\tau}_{ov} - L_v \tau_{ov} - \tau_{ov} L_v^T$$

(3.93)

$$\Delta \epsilon_{ov} := F_v^{-T} \dot{E} F_v^{-1} = \dot{\epsilon}_{ov} + \dot{L}_v \epsilon_{ov} + \epsilon_{ov} L_v$$

(3.94)

where

$$L_v = \dot{F}_v F_v^{-1}$$

(3.96)

To clarify the reason for the use of the concept of dual variables consider the following representation of the stress power of the overstress with respect to $\mathcal{R}$ and $\mathcal{F}$:

$$T_{ov} : \dot{E} = \tau_{ov} : \Delta \epsilon_{ov} = \tau_{ov} : (\Delta \epsilon_{ev} + \Delta \epsilon_v)$$

(3.97)

\(^7\)Objective Oldroyd rates are given by the Lie-type derivative.
3. Constitutive Model

The fact that the stress power is invariant to a configurational transformation is a physical necessity and crucial for a thermodynamical investigation of constitutive relations.

Constitutive Theory

In analogy to 3.91 we split the free energy per unit mass (Lion, 1997b):

$$\tilde{\psi} = \tilde{\psi}_0(E) + \tilde{\psi}_v(\varepsilon_{ev})$$  \hspace{1cm} (3.98)

The constitutive equations for the equilibrium part have been defined in the previous sections 3.3.2 and 3.3.3. The constitutive relation for the overstress as well as the evolution equation for the inelastic strain (internal variable) will be derived using the isothermal Clausius-Duhem inequality

$$-\rho_0 \dot{\psi} + T : \dot{E} \geq 0$$  \hspace{1cm} (3.99)

Inserting 3.91 and 3.98 into 3.99 gives

$$0 \leq \left( T_{eq0} - \rho_0 \frac{\partial \tilde{\psi}_0}{\partial E} \right) : \dot{E} + T_{ov} : \dot{E} - \rho_0 \frac{\partial \tilde{\psi}_v}{\partial \varepsilon_{ev}} : \dot{\varepsilon}_{ev}$$  \hspace{1cm} (3.100)

The first term in brackets can be set to zero due to the hyperelastic relations used in sections 3.3.2 and 3.3.3. By replacing the inelastic stress power terms in 3.100 with their Oldroyd derivatives one obtains (Lion, 1997b):

$$0 \leq \left( \tau_{ov} - \rho_0 \frac{\partial \tilde{\psi}_v}{\partial \varepsilon_{ev}} \right) : \Delta \varepsilon_{ev} + \tau_{ov} : \Delta \varepsilon_v + \rho_0 \frac{\partial \tilde{\psi}_v}{\partial \varepsilon_{ev}} : (L_v^T \varepsilon_{ev} + \varepsilon_{ev} L_v)$$  \hspace{1cm} (3.101)

Now constitutive relations can be defined for the overstress $\tau_{ov}$ in the intermediate configuration $\mathcal{F}$. The result can then be pulled back into the reference configuration $\mathcal{R}$ to obtain $T_{ov}$:

$$\tau_{ov}(\varepsilon_{ev}) = \rho_0 \frac{\partial \tilde{\psi}_v}{\partial \varepsilon_{ev}}, \quad T_{ov} = F_v^{-1} \tau_{ov} F_v^{-T}$$  \hspace{1cm} (3.102)

With the assumption that the potential $\tilde{\psi}_v$ is an isotropic function of $\varepsilon_{ev}$ the following simplification can be used (see appendix A.6.1):

$$\frac{\partial \tilde{\psi}_v}{\partial \varepsilon_{ev}} : (L_v^T \varepsilon_{ev} + \varepsilon_{ev} L_v) = 2 \varepsilon_{ev} \frac{\partial \tilde{\psi}_v}{\partial \varepsilon_{ev}} : \Delta \varepsilon_v$$  \hspace{1cm} (3.103)

This reduces the entropy inequality 3.101 further to

$$0 \leq \rho_0 (I + 2\varepsilon_{ev}) \frac{\partial \tilde{\psi}_v}{\partial \varepsilon_{ev}} : \Delta \varepsilon_v$$  \hspace{1cm} (3.104)
3. Constitutive Model

Non-negativity of 3.104 is ensured by specifying the following constitutive relation for the viscous strain (Lion, 1997b):

$$\Delta \vec{\epsilon}_v := \frac{\rho_0}{\eta_v} (I + 2\epsilon_{ev}) \frac{\partial \vec{\psi}_v}{\partial \epsilon_{ev}}, \quad \eta_v = \eta_v(\epsilon_v, \tau_{ov}) > 0$$

(3.105)

with this flow rule the Clausius-Duhem inequality for arbitrary deformation processes is satisfied

$$\frac{1}{\eta_v} \left( \rho_0 (I + 2\epsilon_{ev}) \frac{\partial \vec{\psi}_v}{\partial \epsilon_{ev}} \right)^2 \geq 0$$

(3.106)

Transformation to $\vec{\mathcal{R}}$

The equilibrium stress operates on $\vec{\mathcal{R}}$ but the inelastic stresses and the flow rules for the inelastic strains are defined on $\vec{\mathcal{F}}$. For a numerical integration of the corresponding differential equations the Oldroyd derivatives have to be expressed in terms of ordinary time rates. The constitutive equations therefore have to be transformed to $\vec{\mathcal{R}}$ (Lion, 1997b).

For Eq. 3.103 to hold $\vec{\psi}_v$ was required to be an isotropic function of $\epsilon_{ev}$. It can then be expressed in terms of the principal invariants of its argument:

$$\vec{\psi}_v(\epsilon_{ev}) = \vec{\psi}_v(I_1^{ev}, I_2^{ev}, I_3^{ev})$$

(3.107)

where $I_1^{ev}, I_2^{ev}$ and $I_3^{ev}$ are the principal invariants of the elastic Right Cauchy Green tensor $\bar{C}_{ev} = F_{ev}^T F_{ev}$.

All these invariants can be expressed in terms of the deformation tensors $C$ and $C_{ev}$:

$$I_1^{ev} = \text{tr} (C \cdot C_{ev}^{-1})$$

(3.108)

$$I_2^{ev} = \frac{[\text{tr} (C \cdot C_{ev}^{-1})]^2 - \text{tr} (C \cdot C_{ev}^{-1})^2}{2}$$

(3.109)

$$I_3^{ev} = \text{det} (C \cdot C_{ev}^{-1})$$

(3.110)

A similar constitutive relation to the one used in the isotropic equilibrium part of the solid phase material was chosen. This implies independence from the second invariant, so that the inelastic stress can now be given as

$$T_{ov} = 2\rho_0 \left( \frac{\partial \vec{\psi}_v}{\partial I_1^{ev}} C_{ev}^{-1} + I_3^{ev} \frac{\partial \vec{\psi}_v}{\partial I_3^{ev}} C_{ev}^{-1} \right)$$

(3.111)

8All following relations are based on this independence.
3. Constitutive Model

To specify the evolution equation for the inelastic Right Cauchy Green tensor we use the relations \( \dot{C}_v = 2F_v^T \Delta \epsilon_v F_v \) to pull back the flow rule for the inelastic strain (Lion, 1997b):

\[
\dot{C}_v = \frac{4\dot{\rho}_0}{\eta_v} \left( \frac{\partial \dot{\psi}_v}{\partial I_1^{ev}} C + I_3^{ev} \frac{\partial \dot{\psi}_v}{\partial I_3^{ev}} C_v \right)
\]

(3.112)

The following strain energy density function \( \psi_v \) was used for the viscoelastic overstress in analogy to the equilibrium formulation:

\[
\psi_v = \frac{C_1v}{\alpha_v} \left[ e^{\alpha_v(I_1^{ev} - \ln I_3^{ev})} - 1 \right] + D_2v(\ln I_3^{ev})^2
\]

(3.113)

The viscosity function can be defined as Lion (1997a,b)

\[
\eta_v = \eta_0 e^{-\frac{1}{s_0} \frac{\|C_v\|_\text{eq}}{\|C_v\|_v}} = \eta_0 e^{-\frac{1}{s_0} \left( \frac{\text{tr} \left( T_{ov} C_v \right)^2}{\text{tr} \left( C_v^2 \right)^{1.3}} \right)}
\]

(3.114)

The final definition of the evolution equation reads

\[
\dot{C}_v = \frac{1}{\eta_v} C_v T_{ov} C
\]

(3.115)

Its time integration is presented in appendix A.6.2.

**Series Expansion**

The implementation is set up in a modular way making it possible to extend the model in a series like fashion (Fig. 3.4). \( n \) Maxwell elements with independent material parameters can be added in parallel simply by introducing \( n \) viscous intermediate configurations \( \mathcal{F}_v^i \) thus providing the total overstress

\[
T_{ov} = \sum_{i=1}^{n} T_{ov}^i
\]

(3.116)
This may become necessary when the material under investigation exhibits distinct relaxation behaviours on different time scales. The principal verification of the viscoelastic implementation, including an example of two Maxwell elements in parallel, can be found in appendix B.2.

Validation

The model was validated by simulating an unconfined ramp-and-hold compression test of a mesenchymal stem cell seeded 2% agarose gel (5.82 mm x 4.02 mm). 10% compression was applied within 400 s and held for 1800 s. The reaction force was measured using a 5 N loadcell from a material testing machine (Zwick GmbH & Co. KG, Ulm, Germany). The nonlinear permeability was modelled according to Gu et al. (2003) with an initial permeability of \(6.61 \cdot 10^{-13} \text{m}^4\text{N}^{-1}\text{s}^{-1}\) and \(M_1 = 3.236\) in eq. 3.56 while \(M_2 = 0\). The material was regarded as isotropic. The Poisson’s ratio of agarose was set to \(\nu = 0.1\) (Muralidharan, 2006) and the Young’s modulus was determined from equilibrium data to be \(E = 6.21\text{kPa}\). The overspring potential was modelled as Neo-Hookean \((\alpha_v = 0)\) with \(D_2 = \frac{C_1}{C_i^2}D_2\). After an initial manual parameter estimation, the remaining viscoelastic parameters were fit using a differential evolution optimization scheme (Price, 1996; Storn and Price, 1997). The objective function was defined as

\[
f_{ob} = \sqrt{\sum_{t=0}^{t=t_{max}} \left( \frac{F_{\text{sim}}(t) - F_{\text{exp}}(t)}{F_{\text{exp}}(t)} \right)^2} = \text{Min}
\]  

(3.117)

to minimize the error between the computed and measured reaction force. The boundaries provided for the parameter space were

\[
C_{1v} \in [0.5, 2.0]C_1 \\
\eta_0 \in [0.2, 5.0]
\]

A constant viscosity \(\eta = \eta_v\) was assumed. The optimisation strategy employed was DE/best/2/bin, the population size per generation was chosen as 20. The determination criterion was

\[
f_{ob} < 0.1
\]

(3.118)
or 10 completed generations without meeting the optimisation criterion. Several runs were performed (hence varying starting populations were created) and all arrived at nearly identical solutions. The best parameters obtained were \(C_{1v} = 7.31\text{kPa}\) and \(\eta_0 = 0.789\text{MPa}\text{s}\). The average error of the fit \(\frac{f_{ob}}{n_{dp}}\), where the
number of datapoints was $n_{dp} = 60$) was $4\%$. The fit is shown in Fig. 3.5 where the pure biphasic model curve illustrates the need for implementing the intrinsic flow-independent viscoelasticity to capture the transient force response of agarose in an unconfined compression test.

![Figure 3.5: Comparison of the experimental force-time curve of an unconfined ramp-and-hold test of agarose with a biphasic and a biphasic hyperviscoelastic model.](image)

### 3.3.5. Electro-osmotic Swelling

The carboxyl and sulfate groups in the GAG side chains cause an overall negative charge of the solid matrix. These negative charges repel each other causing a so-called chemical expansion stress. Additionally, for the tissue to remain electro-neutral, positive counter ions are drawn in from the surrounding fluid. The ion concentration inside the tissue is therefore higher than in the bathing solution. This causes an osmotic pressure difference between both, leading to the electro-osmotic swelling pressure.

Full multi-phasic theories (Lai et al., 1991; Huyghe and Janssen, 1997) accounting for ion species with individual motions require considerable computational and implementational effort. We base our model on the assumption that the equilibration of ion fluxes is at least an order of magnitude faster than the equilibration of transient hydraulic effects (Lanir, 1987a,b). Based on the same assumption Wilson et al. (2005b,c) developed their “biphasic swelling model” and showed excellent agreement between both theories for articular cartilage behaviour. Equivalent
3. Constitutive Model

Models have been applied widely (e.g., for intervertebral discs (Ehlers et al., 2009)) due to their ease of implementation and computational efficiency. The incorporation of osmotic pressure gradients $\Delta \pi$ or chemical expansion stresses $\sigma_{\text{chem}}$ into the user material is straightforward. Both are isotropic pressures in the current configuration and can therefore simply be added to the total stresses via

$$\sigma = -p I - \Delta \pi I + \sigma_{\text{chem}} + \sigma_{\text{iso}} + \sigma_{\text{ov}} + \sigma_{\text{aniso}}$$

(3.119)

or in a total Lagrangian description

$$T = -p J C^{-1} - \Delta \pi J C^{-1} + T_{\text{chem}} + T_{\text{iso}} + T_{\text{ov}} + T_{\text{aniso}}$$

(3.120)

In the following sections the implementation is derived. The presentation is limited to the electro-osmotic pressure while chemical expansion stresses are neglected. This approach is very common in the literature (Ateshian et al., 2004; Wilson et al., 2007; Ehlers et al., 2009). Theoretical analyses using molecular chemistry models have shown that the incorporation of Donnan osmotic effects and a charge independent effect caused by the configurational entropy of terminal GAG chains are sufficient to reproduce experimental data on the swelling pressures of concentrated proteoglycan solutions (Kovach, 1995; Canal Guterl et al., 2010).

The Fixed Charge Density

The fixed charges are most commonly described via their molar concentration $c_F$ with respect to the surrounding fluid volume, the so called “fixed charge density” (FCD). Taking into account the molar mass of one fixed charge (one electron) $M_F \approx 5.486 \cdot 10^{-7} \text{kg mol}^{-1}$, the apparent mass density (per unit volume of the smeared continuum) can be found as

$$\rho_{\text{FCD}} = \phi_F c_F M_F$$

(3.121)

As the anionic groups are fixed to the solid matrix, they undergo the same deformation which is described by the solid matrix deformation gradient $F_S$. Therefore, the balance of mass for the fixed charges reads in analogy to Eq. 3.38:

$$0 = (\rho_{\text{FCD}})'_S + \rho_{\text{FCD}} \text{div} x'_S$$

(3.122)

Inserting 3.121 into the balance of mass:

$$0 = (\phi_F c_F)'_S + \phi_F c_F \text{div} x'_S$$

(3.123)
3. Constitutive Model

From this equation and the volume balance of the solid the dependence of $c_F$ on volumetric deformation can be found as (Ehlers et al., 2009):

$$c_F = \frac{c_{F0} \phi_{F0}}{J_s - \phi_{S0}} \quad \text{with} \quad J_s = \det F_s$$

(3.124)

where $c_{F0}$ is the initial FCD. One characteristic of this equation is

$$\lim_{J_s \to \phi_{S0}} c_F = \infty$$

(3.125)

i.e. the FCD tends to infinity as the fluid volume fraction in the porous medium tends to zero. This creates a one-sided incompressible material similar to a point of compaction treatment.

Osmotic Pressure

Van't Hoff’s law describes the osmotic pressure difference at a semi-permeable membrane separating two chemically active solutions:

$$\Delta \pi = RT \left[ (c^+_{int} + c^-_{int}) - (c^+_{ext} + c^-_{ext}) \right]$$

(3.126)

where $c^\pm$ are the internal and external molar concentrations of anions and cations in the respective solutions. Lanir’s hypothesis implies, that the membrane problem can be extended to a bulk tissue domain and no initial boundary-value problem has to be solved. Donnan’s law states that chemical equilibrium at the semi-permeable membrane is reached when

$$c^+_{int} c^-_{int} = c^+_{ext} c^-_{ext}$$

(3.127)

Assuming monovalent salt solutions (e.g. Na$^+$ Cl$^-$) the electro-neutrality condition for the external salt solution requires

$$c^+_{ext} = c^-_{ext} = c_{ext}$$

(3.128)

For the internal solution, however, the cations have to balance both the effect of the anions and the fixed negative charges to retain electro-neutrality:

$$c^+_{int} = c^-_{int} + c_F$$

(3.129)

Now, the internal salt concentrations at Donnan equilibrium can be obtained:

$$c^+_{int} = \sqrt{c^2_{ext} + \frac{c^2_F}{4} + \frac{c_F}{2}}$$

(3.130)

$$c^-_{int} = \sqrt{c^2_{ext} + \frac{c^2_F}{4} - \frac{c_F}{2}}$$

(3.131)
3. Constitutive Model

Substituting these results into eq. 3.126 yields the Cauchy stress due to osmotic pressures as

\[ \sigma_{\text{osm}} = -\Delta \pi I = -2RT \left[ \sqrt{2c^2 + \frac{c_F^2}{4} - c_{\text{ext}}} \right] I \]  

(3.132)

**Implementation**

An alternative approach is mentioned in Ehlers et al. (2009) that can be used to illustrate the thermodynamical admissibility. An energy potential \( \psi_{\text{osm}} \) is introduced from which the osmotic stresses \( \sigma_{\text{osm}} \) can be derived via the standard relations introduced in earlier sections:

\[ \sigma_{\text{osm}} = \frac{2}{J} F \frac{\partial \psi_{\text{osm}}}{\partial C} F^T \]  

(3.133)

The energy potential is given as

\[ \psi_{\text{osm}} = RTc_{F0}\phi_{F0} \left[ \frac{2c_{\text{ext}}}{c_F} - \sqrt{4c_{\text{ext}}^2 + c_F^2} + \text{arsinh} \left( \frac{c_F}{2c_{\text{ext}}} \right) \right] \]  

(3.134)

where \( c_F \) follows from eq. 3.124. The second Piola-Kirchhoff stress can now be found as

\[ T_{\text{osm}} = 2 \frac{\partial \psi_{\text{osm}}}{\partial I_3} \frac{\partial I_3}{\partial C} = 2 \frac{\partial \psi_{\text{osm}}}{\partial I_3} I_3 C^{-1} \]  

(3.135)

The necessary derivative is found as

\[ \frac{\partial \psi_{\text{osm}}}{\partial I_3} = \frac{\partial \psi_{\text{osm}}}{\partial C} \frac{\partial C}{\partial I_3} \]  

\[ = -RTJ_S^{-1} \left[ \sqrt{\frac{c_{\text{ext}}^2 + c_F^2}{4} - c_{\text{ext}}} \right] \]  

(3.136)

So we finally arrive at

\[ T_{\text{osm}} = -2RTJ_S \left[ \sqrt{\frac{c_{\text{ext}}^2 + c_F^2}{4} - c_{\text{ext}}} \right] C^{-1} \]  

(3.137)

and after a push-forward

\[ \sigma_{\text{osm}} = -2RT \left[ \sqrt{\frac{c_{\text{ext}}^2 + c_F^2}{4} - c_{\text{ext}}} \right] I \]  

(3.138)

which is identical to the result obtained in the previous section.

For the analytical tangent modulus the second derivative \( \frac{\partial^2 \psi_{\text{osm}}}{\partial I_3^2} \) is needed and can be found in appendix A.7.
If a charged material such as cartilage is modelled without explicitly accounting for swelling effects, apparent material parameters have to be supplied that implicitly include the contribution of swelling effects to load bearing. If swelling is, however, explicitly modelled, the parameters for the ground phase material will be intrinsic in nature. Relations between the apparent and intrinsic material parameters can be found (Mow et al., 2002; Ateshian et al., 2004; Lu et al., 2007). The derivation for this model is listed in appendix A.7. The principal model verification can be found in appendix B.3.

3.4. Discussion

A phenomenological thermodynamically consistent material model for solid matrix deformation has been developed. The large strain biphasic finite element procedure is capable to simultaneously consider non-linear stress-strain response, intrinsic viscous effects of matrix constituents, swelling and various aspects of anisotropy as well as tension-compression non-linearities. The presented approach was limited to the structural response of the tissue and did not account for other effects like transport phenomena of solvents in the pore fluid. The coupling of hydraulic and mechanical processes has been performed within the context of the Theory of Porous Media. Based on the effective stress concept, constitutive relations for the solid matrix stress were defined. One of the outstanding features of the material model is its thermodynamical consistency. A thermodynamic foundation of constitutive relations for articular cartilage is not provided in many cases in literature (García and Cortés, 2007). An essential advantage of thermodynamically consistent material models is their flexibility for the simulation of a wide range of physical effects and loading conditions, and their open character towards new constitutive effects within the established framework and its fundamental assumptions. Recently, a similar approach to the one presented in this study has been proposed for the intervertebral disc using a custom written FE code by Ehlers et al. (2009). The current model, in contrast, is implemented in a commercial code with the intention to allow widespread use.

Most soft biological tissues show a pronounced anisotropic behaviour (arterial walls, tendons, ligaments, intervertebral discs, cartilage and others) due to the presence of specially oriented fibre constituents (mostly collagen). Among these fibre-reinforced biological materials articular cartilage distinguishes itself through a highly heterogeneous distribution and structure of the collagen network. The
topology of the collagen network changes from parallel in the superficial zone to radial in the deep zone. Even in the recent literature this fact is often neglected, and the material is considered as homogeneous (García and Cortés, 2007). Implementing heterogeneous properties into the material model is easily accomplished here as it is accessed in each integration point of the discretised domain. Via this feature, depth dependent aggregate moduli, porosities, permeabilities, collagen architectures etc. can be effortlessly implemented (Chen et al., 2001b; Wilson et al., 2007). Though this type of structure is also known from engineering as a functionally graded material this concept is essential to the mechanical performance of biological tissues – as for example the just mentioned depth dependent architecture and composition of articular cartilage. It is likely that scaffolds for tissue regeneration have to be tailored to include graded properties themselves (Kelly and Prendergast, 2006) to achieve optimal results. The structural heterogeneity need not be constant: Remodelling is a commonly observed phenomenon in many tissues (Humphrey, 2008). Modelling in vivo tissue regeneration, where local phenotypic and structural changes occur, requires the ability to capture these local changes and the resulting functionally graded material domain (Nagel and Kelly, 2010b).

In conclusion, a finite deformation material model for general soft tissue applications has been developed. Its versatility will allow the study of many hydrated tissues and biomaterials under various physiological or pathological conditions as well as in discrete developmental stages.
4 Mechanical Effects of the Collagen Architecture in Charged Media

4.1. Introduction

The biphasic theory (Mow et al., 1980) is commonly used to describe hydrated soft tissues such as cartilage. In its linear isotropic form two parameters are required to characterise the solid matrix behaviour, the Young’s modulus $E$ and Poisson’s ratio $\nu$. However, understanding and accurately modelling cartilage behaviour requires the application of more complex models. Among the included material effects are anisotropy and tension-compression nonlinearity, intrinsic viscoelasticity and nonlinear permeability. Another important development has been the extension of the biphasic to triphasic (Lai et al., 1991) and quadriphasic theories (Huyghe and Janssen, 1997). Detailed reviews on cartilage models can be found elsewhere in the literature (Huang et al., 2005; Wilson et al., 2005a).

The more individual constituents and their material effects are represented in a model, the more the used material parameters can partially resemble the intrinsic material properties of a tissue. For example, a cartilage plug in unconfined compression (UC) will exhibit a certain apparent Young’s modulus and Poisson’s ratio. These apparent values will be chosen as material parameters for the linear biphasic model. They implicitly include the contribution of swelling pressures and anisotropic effects. If one explicitly includes, for example, the swelling effects in the model, one has to supply the intrinsic Young’s modulus and Poisson’s ratio as material parameters since swelling pressures will lead to an apparent stiffening and also increase Poisson’s effects. The values of the parameters called Young’s modulus and Poisson’s ratio will thus become lower and their physical interpre-
The unconfined compression test is commonly used to acquire material parameters. Computational studies have investigated the role of fibre reinforcement (Li et al., 1999, 2002a; Wilson et al., 2005c) or other aspects of tissue composition and structure (Wilson et al., 2006c; Julkunen et al., 2010b) in the tissue's response in UC. A fundamental assumption in most computational studies is that collagen fibres can only bear tensile loads (e.g. Holzapfel et al. (2000); Canal Guterl et al. (2010)). From this assumption an often made statement is that radially and circumferentially aligned fibres stiffen the material response due to the lateral expansion of the specimen while fibres parallel to the loading direction (vertically aligned) do not contribute to load bearing. Due to internal swelling pressures, however, the collagen network is in a state of tension when the tissue sample is free of external loads. As long as the applied compression is smaller than the associated swelling strain, the actual matrix strain remains tensile, although the experimentalist will measure an apparent compressive strain. This effect in conjunction with the tension-compression nonlinearity of the cartilage matrix has been used as an explanation for strain softening phenomena observed during initial compressive loading (Chahine et al., 2004; Ateshian et al., 2009).

The fibre architecture of articular cartilage is very heterogeneous. It changes from parallel to the articular surface in the superficial layers of the tissue to perpendicular in the deep zone and more randomly arranged in the middle layer. Understanding the functional importance of this fibre structure and recapitulating this arrangement are key steps towards creating viable biological tissue substitutes. Several bioreactor studies have found enhanced mechanical properties of mechanically loaded constructs compared to their free-swelling counterparts despite no differences in biochemical composition (Lima et al., 2007; Bian et al., 2010), suggesting that changes in tissue structure and organisation in response to mechanical loading may lead to improved mechanical properties. Understanding how tissue organisation influences tissue construct properties may aid the understanding of such experimental findings.

In this chapter the effect of fibre alignment on the apparent mechanical equilibrium properties of a cartilage-like tissue in UC is predicted using a computational model. The response of a charged material is compared to that of an uncharged material. Idealised homogeneous cases of fibre alignment are investigated as limiting scenarios as well as an anatomically motivated Benninghoff-like architecture for articular
4. Mechanical Effects of the Collagen Architecture in Charged Media

cartilage. The influence of including a tare strain into an experimental protocol is simulated. The predictions are relevant not only from a modelling perspective but also for the better understanding of structure-function relationships of charged soft tissues. The chapter closes with the extension to continuous fibre distributions where the choice of material parameters is not trivial. An analogy using classical mechanical engineering mechanics illustrating the behaviour of a fibre reinforced charged material under compression can be found in appendix B.6.

4.2. Materials & methods – discrete fibre model

The investigation was limited to equilibrium properties. Hence, fluid pressurisation and solid matrix viscoelasticity were not accounted for in this study. Briefly, the total stress was given as

$$\sigma = -\Delta \tau I + \sigma_E$$  \hspace{1cm} (4.1)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_1$ [MPa]</td>
<td>0.11</td>
</tr>
<tr>
<td>$\nu$ [-]</td>
<td>0.1</td>
</tr>
<tr>
<td>$\phi_{F0}$ [-]</td>
<td>0.8</td>
</tr>
<tr>
<td>$C_4'$ [MPa]</td>
<td>0.3</td>
</tr>
<tr>
<td>$\beta'$ [-]</td>
<td>10.0</td>
</tr>
<tr>
<td>$T$ [K]</td>
<td>298</td>
</tr>
<tr>
<td>$c_{ext}$ [mmol mm$^{-3}$]</td>
<td>0.00015</td>
</tr>
</tbody>
</table>

Table 4.1.: Material parameters for the charged case. $\nu$ - Poisson’s ratio, $\phi_{F0}$ - initial porosity. Parameter $D_2$ followed from Eq. 3.68. $C_1$ see 3.67; $C_4'$, $\beta'$ see 3.80.

The solid extra stresses were derived from free Helmholtz energy potentials that were split into isotropic and anisotropic parts. For the isotropic part we chose a Neo-Hookean formulation while for the anisotropic potentials we chose an exponential formulation (Eq. 3.80). Simulations where performed with two kinds of materials: charged ($c_{F0} = 0.0002$ mEq mm$^{-3}$) and uncharged ($c_{F0} = 0.0$ mEq mm$^{-3}$). The remaining parameters are listed in table 4.1 for the charged case. The material properties have been chosen to lie in the range of cartilage values reported in previous studies, e.g. (Mow and Guo, 2002; Wilson et al., 2005b; García and Cortés, 2007; Roth and Mow, 1980), yielding a material roughly ten times stiffer in tension than in compression. For the simulations of the uncharged tissue, the material parameter $D_2$ was modified to yield the same apparent compressive modulus around
the undeformed state \( J = 1 \) of the isotropic ground substance as its intrinsic version in combination with swelling (compare appendix A.7, Eq. A.59). From that relation it follows that \( D_2^{app} > D_2^{intr} \) which is consistent with saying that the fixed charges contribute to the intrinsic compressive stiffness and Poisson's ratio of the solid matrix (Ateshian et al., 2004).

### 4.2.1. Geometry and boundary conditions

Cylindrical plugs \((\varnothing 4 \times 3 \text{ mm})\) were modelled and subjected to the following two load steps:

1. \(0 \leq t \leq t_1\): free swelling (FS) step

2. \(t_1 < t \leq t_2\): unconfined compression (UC) step, by 1 mm displacement from FS height

The sample geometry in the FS configuration was quantified by sample height, radius and volume. Simulations were performed with the following fibre orientations:

<table>
<thead>
<tr>
<th>name of simulation</th>
<th>fibre directions</th>
</tr>
</thead>
<tbody>
<tr>
<td>isotropic</td>
<td>(a_{0i} = 0, \forall i = 1, \ldots, 4)</td>
</tr>
<tr>
<td>vertical</td>
<td>(a_{0i} = e_z, \forall i = 1, \ldots, 4)</td>
</tr>
<tr>
<td>horizontal</td>
<td>(a_{01} = a_{02} = e_r)</td>
</tr>
<tr>
<td></td>
<td>(a_{03} = a_{04} = e_\varphi)</td>
</tr>
<tr>
<td>45°</td>
<td>(a_{01} = \frac{1}{\sqrt{3}}(e_r + e_\varphi + e_z))</td>
</tr>
<tr>
<td></td>
<td>(a_{02} = \frac{1}{\sqrt{3}}(e_r - e_\varphi + e_z))</td>
</tr>
<tr>
<td></td>
<td>(a_{03} = \frac{1}{\sqrt{3}}(e_r + e_\varphi - e_z))</td>
</tr>
<tr>
<td></td>
<td>(a_{04} = \frac{1}{\sqrt{3}}(e_r - e_\varphi - e_z))</td>
</tr>
</tbody>
</table>

where \(e_r, e_\varphi\) and \(e_z\) are orthonormal basis vectors of the cylindrical coordinate system aligned with the specimens.

To illustrate the behaviour of a physiologically relevant architecture, an additional unconfined compression test using a Benninghoff-type collagen architecture (Benninghoff, 1925b) but otherwise homogeneous tissue properties was simulated and evaluated in terms of its average equilibrium properties. The architecture was estimated from results obtained in extensive multi-disciplinary imaging studies on
4. Mechanical Effects of the Collagen Architecture in Charged Media

Figure 4.1.: (a) Fibre angle throughout the depth of the tissue defining the Benninghoff architecture (Eq. 4.2); (b) Vector plot of two families of fibres arranged in the mid-plane of the cylindrical cartilage plug.

canine articular cartilage (Xia, 2008). Introducing a relative depth coordinate $z \in [0; 1]$ with $z = 0$ at the articular surface, we modelled the fibre angle with respect to the plug axis using the following equation:

$$\phi(z) = \frac{\pi}{2} e^{az^b} \text{ with } a = -400 \text{ and } b = 3.38$$

The thickness of the SZ and MZ was defined as 7.7% and 15.3% of the total cartilage thickness, respectively, which is in the range of values reported by other authors (Canal et al., 2008; Xia, 2008). The transition between superficial (SZ) and middle zone (MZ) was therefore at $z_{SZMZ} = 0.077$ and between middle and deep zone (DZ) at $z_{MZDZ} = 0.23$. The parameters $a$ and $b$ in Eq. 4.2 were found by assuming $\alpha(z = z_{SZMZ}) \approx 85^\circ$ and $\alpha(z = z_{MZDZ}) \approx 5^\circ$. The fibres were arranged in two families ($a_{01} = a_{02}$ and $a_{03} = a_{04}$). The vertical orientation was given by equation 4.2 as $\phi(z)$ for the first family of fibres and $-\phi(z)$ for the second family. The depth dependence of the angle is shown in Fig. 4.1a, a visual impression of the fibre arrangement can be gained from Fig. 4.1b. Both families were arranged in the same vertical plane parallel to the split line direction. This idealisation implied that no fibres showed into the direction perpendicular to the split line.

As the free swelling state (after load step 1 in these simulations) is usually the configuration found in a solution of physiological salt concentration, this state will be the reference geometry for experiments. The results are therefore presented
4. Mechanical Effects of the Collagen Architecture in Charged Media

depending on a linear strain measure calculated with respect to this configuration, the apparent or applied axial strain:

\[ \varepsilon_{zz}^{FS} = \frac{du_z}{dz} \quad \text{with} \quad u_z(x_{FS}, t = t_1) = 0 \quad (4.3) \]

where \( x_{FS} \) are particle locations in the free swelling configuration at time \( t_1 \). For reasons of comparison some results are also presented with respect to the initial unswollen configuration in which material particles are identified by their material coordinates \( X \), so that the axial reference strain follows from

\[ \varepsilon_{zz}^0 = \frac{du_z}{dz} \quad \text{with} \quad u_z(X, t = 0) = 0 \quad (4.4) \]

### 4.2.2. Equilibrium properties

There is not just one uniquely defined stiffness measure – depending on the stress or strain measures chosen to calculate stiffness, the obtained values can be significantly different. In this study, three ways to calculate the Young’s modulus of a tissue sample were compared:

1. An *incremental* Young’s modulus \( E_{\text{inc}} \), calculated using increments of nominal stress over increments of the linear strain measure \( \varepsilon_{zz}^{FS} \):

\[ E_{\text{inc}} = \frac{\Delta F}{A_1 \Delta \varepsilon_{zz}^{FS}} \quad (4.5) \]

2. A *nominal* Young’s modulus \( E_{\text{nom}} \), calculated by dividing the current nominal compressive stress by the associated strain:

\[ E_{\text{nom}} = \frac{F}{A_1 \varepsilon_{zz}^{FS}} \quad (4.6) \]

3. A tare strain \( \varepsilon_{zz}^{FS} = \bar{\varepsilon} \) can be applied to the samples prior to the actual compression test and the force displacement readings zeroed. Then, the Young’s modulus was calculated as a nominal modulus:

\[ E_{\text{tare}} = \frac{F}{A_1 \varepsilon_{zz}^{t}} \quad \text{with} \quad \varepsilon_{zz}^{t} = \frac{\varepsilon_{zz}^{FS} - \bar{\varepsilon}}{\bar{\varepsilon} + 1} \quad \text{and} \quad F(\varepsilon_{zz}^{t} = 0) = 0 \quad (4.7) \]

A compressive tare strain of 2% was applied in this study.
4. Mechanical Effects of the Collagen Architecture in Charged Media

In these equations, $F$ is the compressive force and $A_1$ is the cross-section of the plug in the FS state prior to compression (i.e. $A_1$ represents the initial cross-sectional area).

The apparent Poisson’s ratio was calculated using

$$\nu = -\frac{\epsilon^{FS}_{rr}}{\epsilon^{FS}_{zz}}$$  \hspace{1cm} (4.8)

The apparent Poisson’s ratio is the result of several material effects, including the intrinsic Poisson’s ratio of the base matrix, fibre orientation and stiffness as well as osmotic pressures.

The simulations with the Benninghoff architecture yielded heterogeneous results. Hence, in the results section, average values will be presented to approximate the tissue response and compare it to the four homogeneous cases in terms of the above measurements.

4.3. Results – discrete fibre model

4.3.1. Tension-compression nonlinearity

To illustrate the tension compression nonlinear behaviour and the two different strain measures defined in Eqs. 4.3 and 4.4, a simulation of unconfined compression with a subsequent uniaxial tensile test was performed on the “vertical” specimen for both a charged or an uncharged material. The resulting stress-strain curve was plotted for the two strain measures (see Fig. 4.2).

Both strain measures are identical for the uncharged tissue while for the charged tissue some differences occur: The point of tension-compression nonlinearity (jump in slope) at zero reference strain corresponds to where the collagen fibres have their stress-free configuration (Fig. 4.2a). The global stress for the charged material is not zero at this point. If the stress-strain curves are plotted with respect to the apparent strain applied after free swelling both samples are globally stress-free at 0 strain (Fig. 4.2b). However, the collagen network is still in tension in the charged tissue at this stage. Therefore, the point of tension-compression nonlinearity is shifted to a negative strain value.
4. Mechanical Effects of the Collagen Architecture in Charged Media

Figure 4.2: (a) Axial Cauchy stress with respect to actual or reference strain. (b) Axial Cauchy stress with respect to applied or apparent strain (relative to FS configuration).

4.3.2. Geometry

As expected, without any fibre architecture to restrain swelling the largest sample volume was predicted in the isotropic sample (Fig. 4.3). The specimen volume successively decreased when swelling was inhibited by fibres in one \((\mathbf{e}_z)\), two \((\mathbf{e}_\varphi, \mathbf{e}_r)\) and all three directions (configuration “45°”), see Fig. 4.3. Swelling was inhibited primarily in the direction of fibres and samples swelled mostly in the directions without fibre reinforcement; e.g. horizontal fibre alignment produced the highest samples with the smallest diameters, whilst vertical fibre alignment produced the shortest samples with the largest cross section (Fig. 4.3). The Benninghoff architecture produces an average sample geometry in between that caused by the vertical and horizontal fibre arrangements. The geometry itself is predicted to be highly curved as a result of the heterogeneous fibre architecture compared to the ideal cylinders in the homogeneous cases. The deep zone exhibits significantly more lateral swelling than the superficial zone which concurs with experimental observations (Wong et al., 2000).

4.3.3. Stress-strain response

The “horizontal” fibre arrangement was predicted to produce the highest stresses in the uncharged material group (Fig. 4.4a). As expected, vertical fibres did not change the compressive material behaviour from the isotropic uncharged material response. At the strain levels investigated and for the material parameters used, the “45°” set of fibres also does not contribute to load bearing. Material line elements in the fibre directions are rotated but not stretched. The Benninghoff ar-
4. Mechanical Effects of the Collagen Architecture in Charged Media

Architecture produced only slightly higher average Cauchy stresses than the vertical arrangement due to a relatively thin superficial zone with horizontal fibres. For a charged tissue, the lowest stress values were predicted for the isotropic simulation (Fig. 4.4b). The “45°” simulation initially produced slightly higher Cauchy stress values than the “horizontal” one, however as the fibres stiffen this model predicts higher stresses than the “45°” sample at higher strain levels. A dramatic shift in the slope of the stress-strain curves is observed for the vertical fibre simulation. This occurs when the fibres reach their stress-free length. The second part of the curve has a slope that is almost parallel to the isotropic curve. Due to the heterogeneity of the sample with the Benninghoff architecture its top surface was not planar in the FS configuration. We plotted the average stress curves starting from the level of compression where full contact with the loading platen was established. Therefore, the stress-strain curve starts with an average initial Cauchy stress that does not equal zero. The tension-compression transition is smooth rather than sharp as seen in the vertical case. The stress magnitudes are comparable to those of the vertical architecture but stiffen at higher strains due to the horizontal fibres near the sample surface.

The total stress can be split up into the contributions by the osmotic pressure $\Delta \pi$ and the solid matrix extra stress (Fig. 4.5). The varying initial values of $\Delta \pi$ are predicted to depend on the fibre architecture. This in turn is directly related to the volume in the FS configuration (Fig. 4.3). During compression $\Delta \pi$ is predicted to increase due to a shrinking sample volume (Fig. 4.5a). The proportional increase in swelling pressure is predicted to be greatest for the horizontal simulations. At $\epsilon^{FS}_{zz} = 0$ the solid extra stress values (Fig. 4.5b are equal to $-\Delta \pi$ so that the total stress is zero (Fig. 4.4b). During the first part of the compression test the matrix
4. Mechanical Effects of the Collagen Architecture in Charged Media

4.3.4. Apparent Poisson's ratios

The predicted apparent Poisson's ratios are illustrated in Fig. 4.6. In the uncharged case (Fig. 4.6a) no homogeneous set of fibres but the “horizontal” case contributes to load bearing, hence these models exhibit the same Poisson's ratios. Only the horizontal fibres restrain lateral expansion and therefore produce much lower apparent Poisson effects (despite the same intrinsic value of $\nu$ for the

stresses remain positive as long as the osmotic pressure is greater than the applied load. When the compressive load reaches higher values, the matrix stresses are negative (compressive).
isotropic ground phase material). The thin superficial layer with horizontal fibres in the Benninghoff architecture therefore lowered slightly the average Poisson's ratio for this simulation compared to the vertical sample.

The results for the charged tissue are presented in Fig. 4.6b. The lowest Poisson's values are still predicted in the “horizontal” case. Isotropic and vertical fibres are predicted to produce the same curves, as in the vertical case all fibres are aligned in the loading direction (no rotation component and no restraint of lateral expansion). The highest Poisson's ratios are predicted in the “45°” case where the initial sample volume is lowest. For this fibre arrangement, the values progressively decrease as the already stressed fibres orient more horizontally and thus further restrain lateral expansion.

The Benninghoff architecture caused a nonlinear trend where the Poisson’s ratio is initially higher and decreases to a lower value. The magnitude is best comparable to the Poisson’s ratio of the vertical fibre alignment. A nonlinear behaviour of Poisson’s ratios in the transitional range between matrix tension and compression has been observed experimentally (Chahine et al., 2004).

![Figure 4.6: Apparent Poisson's ratio over axial strain \( \epsilon_{FS} \) for uncharged (4.6a) and charged (4.6b) materials. “Benn.” stands for Benninghoff architecture.](image)

### 4.3.5. Stiffness measures

The stiffness measures in eqs. 4.5 - 4.7 are all defined with respect to the nominal (technical) stress. The nominal Young’s modulus for the uncharged tissue (Fig. 4.7a) is highest for horizontal fibre alignment and increases progressively. The other homogeneous fibre arrangements produce lower and identical values. The
Benninghoff architecture is predicted to show only slightly higher values compared to these cases. The nominal Young’s modulus for the charged tissues is shown in Fig. 4.7b. It is lowest for the isotropic tissue, higher for the “horizontal” and “45°” samples and highest initially for the vertical tissue. As the applied strain increases, the predicted Young’s modulus for the vertical simulation reduces, tending towards the value predicted for the isotropic simulations. A similar trend is seen for the Benninghoff architecture. While the initial modulus values are lower than in the “vertical” case the two curves approach each other for higher strains.

The incremental modulus values for the charged materials (Fig. 4.7c) represent the slope of the technical-stress/applied-strain curve. The “vertical” material initially has the highest incremental modulus values. At the point of tension-compression nonlinearity the values suddenly drop to approximately the values of the isotropic tissue. While the “45°” simulation initially appears stiffer than the “horizontal” simulation, it is overtaken by the horizontal alignment at higher strain values. However, under the conditions simulated the effect is minor. In contrast to the sharp transition between compressive and tensile incremental moduli in the “vertical” simulations, the Benninghoff architecture causes a smooth transition. This is in good agreement with experimental observations (Chahine et al., 2004). The initial values are lower compared to the “vertical” case but approach it at higher strains.

Finally, including a tare strain into the evaluation can significantly alter the determined nominal Young’s modulus (Fig. 4.7d). Isotropic and vertical fibre alignment exhibit nearly identical values. The predicted Young’s modulus is greatest for the 45° simulation, followed by the constructs with horizontally aligned fibres – which exhibit the quickest stiffening. These differences compared to the model where no tare strain was included can be attributed to the unloading of the fibres from the FS state due to the offset displacement. At the beginning of the subsequent test they are not in a (tensile) loaded configuration anymore. The simulations with the Benninghoff architecture still exhibit stress softening although to a lesser degree than without a tare strain. Vertical fibres were not completely unloaded after 2% tare strain as can be observed from the incremental moduli in Fig. 4.7c so that tensile states still prevailed. This was due to a relative stiffening of the deep zone compared to the more superficial layers of the tissue driven by the fibre architecture.
Figure 4.7.: Nominal Young’s modulus $E_{nom}$ over axial strain $\varepsilon_{zz}^{FS}$ for uncharged (4.7a) and charged (4.7b) materials. Incremental Young’s modulus $E_{inc}$ over axial strain $\varepsilon_{zz}^{FS}$ for the charged tissue (4.7c). Nominal Young’s modulus after including an offset strain $E_{tare}$ over axial strain $\varepsilon_{zz}^{FS}$ for the charged material (4.7d). “Benn.” stands for Benninghoff architecture.

4.4. Materials & methods – ellipsoidal fibre model

The results will now be extended to continuous fibre distributions. Since material parameter estimation is difficult for this model class, an extensive parameter variation will be performed that allows their estimation as well as illustrates the relative influence of the various contributions. In this section the following definitions will be employed when referring to the “charged” or the “neutral” materials:

charged:  \[ T = T_{\text{fib}} - \Delta \pi J C^{-1} \]  
neutral:  \[ T = T_{\text{NH}} + T_{\text{fib}} \]  

The Neo-Hookean contribution (material parameters $C_1$ and $\nu$) followed from Eq. 3.67 while the fibre contribution was calculated using a continuous fibre distri-
bution (section 3.3.3) and a power law (Eq. 3.82) for the strain energy density functions (material parameters \( C_4, \beta \)). The Donnan osmotic pressure inside the tissue was given by (compare section 3.3.5)

\[
\Delta \pi = 2RT \left[ \sqrt{c_{ext}^2 + \frac{c_{F0}^2 c_{F0}^2}{4(J - \phi_{S0})^2}} - c_{ext} \right]
\] (4.11)

### 4.4.1. Isotropic behaviour

Biomaterials exhibit a range of properties: While arteries are usually assumed to be nearly incompressible \((\nu \rightarrow 0.5)\), agarose or porous scaffolds are very compressible \((\nu \approx 0.0)\). Furthermore, during transient loading of biphasic materials (e.g. agarose) the Poisson’s ratio can change from near incompressible values due to fluid pressurisation to the low values at equilibrium. Depending on the stiffness of the fibre network the material will display a versatile behaviour.

The principal model behaviour was illustrated for isotropic fibre architectures, i.e. \( \Xi = \text{diag}(1, 1, 1) = I \) and therefore \( C_4(a_0) = \bar{C}_4 \). Cubes with unit edge length were stretched and compressed to retrieve apparent material properties.

The following simulations were performed with illustrative parameter sets:

- neutral material: \( C_1 = 1.0 \text{MPa} \) (such that \( \bar{C}_4/C_1 = \bar{C}_4/\text{MPa} \) \( \beta = 2.5 \). fibre stiffness and intrinsic Poisson’s ratio were varied with the following parameter vectors:

\[
\begin{align*}
\bar{C}_4 &= [0.1, 0.5, 1.0, 1.5, 2.0, 3.0, 5.0, 10.0]^T \\
\nu_i &= [0.0, 0.1, 0.2, 0.3, 0.4, 0.49, 0.499, 0.4999]^T
\end{align*}
\]

Apparent Young’s moduli and Poisson’s ratios were evaluated at \( \pm 10\% \) strain for all 64 parameter combinations \( \bar{C}_4 \nu_i^T \).

- charged material: \( c_{F0} = 0.0002 \text{mEq mm}^{-3}, \ c_{ext} = 0.00015 \text{mmol mm}^{-3}, \ T = 298 K, \ \phi_{F0} = 0.8, \ \beta = 2.5 \) (parameter set applicable for articular cartilage modelling (Nagel and Kelly, 2010a; Ateshian et al., 2009)). The fibre stiffness \( \bar{C}_4 \) was varied using the same parameter vector as in the neutral material. Apparent Young’s moduli and Poisson’s ratios were determined at \( \pm 5\%, \pm 10\% \) and \( \pm 20\% \) strain with respect to the unloaded free swelling configuration.
4.4.2. Anisotropic behaviour

Fibre reinforced biological materials display varying anisotropic behaviour. Unit cubes aligned with the Cartesian coordinate system were stretched and compressed in all three directions $x$, $y$ and $z$ to retrieve the direction dependent apparent Young's moduli at ±10% strain. Three fibre architectures were evaluated with the objective of increasing the stiffness in the x-direction and decreasing it in the z-direction:

$$
\Xi = \text{diag}(\xi_k, 1.0, \xi_k^{-1}) \quad \text{with} \quad \xi_k = [1.0, 2.0, 4.0]^T
$$

(4.12)

The following simulations were performed with illustrative parameter sets:

- neutral material: $C_1 = 1.0 \text{MPa}$, $\beta = 2.5$, $C_4 = 2.0 \text{MPa}$. One compressible and one nearly incompressible material were simulated: $\nu_i = [0.1 \ 0.499]^T$.

- charged material: $\bar{C}_4 = 2.0$, other parameters as in the isotropic case (section 3.3.2). As will be seen in the results, the increasingly anisotropic definition of $\Xi$ is not adequately reflected in the results. Hence, this simulation set is further used to illustrate the effect of scaling the fibre stiffness distribution as described in the next section such that this can be overcome.

4.4.3. Scaling the ellipsoid

As stated earlier, one advantage of the definition proposed in Eq. 3.86 is that stiffness and anisotropy can be modulated separately using distinct parameters $\bar{C}_4$ and $\xi_i$. This can be advantageous in composition based modelling or remodelling simulations (Nagel and Kelly, 2012b). However, varying specifications of $\Xi$ do not only alter the architecture of the tissue but also overall stiffness of the fibrous material. In remodelling or composition based scenarios this can be undesired. In order to maintain the separation such that only $\bar{C}_4$ determines the average magnitude of the stiffness, it is possible to scale Eq. 3.86 with the inverse average value $\bar{\xi}$:

$$
C_4(a_0) = \frac{1}{\bar{\xi}} \left[ \frac{(\cos \Theta \sin \Phi)^2}{\xi_1^2} + \frac{(\sin \Theta \sin \Phi)^2}{\xi_2^2} + \frac{(\cos \Phi)^2}{\xi_3^2} \right]^{-\frac{1}{2}} \bar{C}_4
$$

(4.13)

where

$$
\bar{\xi} = \frac{1}{4\pi} \int_0^{2\pi} \int_0^{\pi} \left[ \frac{(\cos \theta \sin \phi)^2}{\xi_1^{2m}} + \frac{(\sin \theta \sin \phi)^2}{\xi_2^{2m}} + \frac{(\cos \phi)^2}{\xi_3^{2m}} \right]^{-\frac{1}{2}} \sin \phi \, d\phi \, d\theta
$$

(4.14)
The effect will be illustrated by comparing the results of the charged anisotropic material employing either Eq. 3.86 or 4.13. Additional details will be provided in section 5.3.3.

4.5. Results – ellipsoidal fibre model

4.5.1. Isotropic behaviour – neutral material

The predicted tensile Young’s moduli at 10% strain were primarily determined by the fibre stiffness. The influence of the intrinsic Poisson’s ratios only showed at low and high fibre stiffness values (Fig. 4.8a).

The apparent Poisson’s ratio in tension approached the intrinsic Poisson’s ratio for low fibre stiffness values (Fig. 4.8b). For compressible ground matrices (low $\nu_i$) the apparent Poisson’s ratios in tension increased quickly for higher fibre stiffness values due to fibres realigning into the direction of loading and pulling the ground substance along. For increasingly incompressible ground matrix materials the apparent Poisson’s ratio approached 0.5 for all fibre stiffness values (Fig. 4.8b).

Compressive Young’s moduli approached the intrinsic Young’s moduli (varying from 4.0 to $\approx 6.0$ MPa depending on $\nu_i$) for low fibre stiffness values and/or low intrinsic Poisson’s ratios (Fig. 4.8c). Apparent Young’s moduli only increased significantly in compression with high $\nu_i$ and high fibre stiffness, which led to the development of significant hydrostatic stresses in the material by fibres counteracting the lateral expansion (Fig. 4.8c). This is somewhat analogous to the situation when fibre reinforced soft tissues such as cartilage are initially loaded with sufficient strain rates. Here, the low permeability of the tissue significantly limits fluid exudation, and the material behaves as nearly incompressible.

In compression, low fibre stiffness values allowed the apparent Poisson’s ratio to approach the intrinsic Poisson’s ratio of the material (Fig. 4.8d). Increasing fibre contributions, however, increasingly inhibited lateral expansion leading to lower $\nu_a$. For increasingly incompressible ground matrices the apparent Poisson’s ratios also approached 0.5 in compression (Fig. 4.8d).

4.5.2. Isotropic behaviour – charged material

Increasing the fibre stiffness parameter led to increasing Young’s moduli in both tension and compression. A rapid stiffening of the material was predicted in tension
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![Graphs](image)

Figure 4.8.: Apparent Young's moduli $E_a$ and Poisson's ratios $\nu_a$ at $\pm$ 10% strain for various fibre stiffnesses and intrinsic Poisson's ratios of the isotropic ground substance material. Isotropic fibre architecture: $\mathbf{\Xi} = \mathbf{I}$. Note that the direction of axes is reversed in Fig. 4.8d.

with increasing strain due to the constitutive law ($\beta = 2.5$) and realignment of fibres into the loading direction (Fig. 4.9a). In compression, however, stress-softening was predicted with increasing strain due to the gradual unloading of fibres that were previously tensed by the swelling pressure (Fig. 4.9c).

In tension the Poisson's ratios increased with both increasing fibre stiffness and strain due to more fibres being rotated towards the direction of loading. Higher values than in the uncharged case were achieved (Fig. 4.9b). With increased compressive strain more fibres rotated to the horizontal direction and became tensed, thereby resisting lateral expansion and leading to a lower Poisson ratio (Fig. 4.9d). Higher fibre stiffness values further decreased the apparent Poisson's ratios.
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4.5.3. Anisotropic behaviour – neutral material

The intrinsic Poisson’s ratio had almost no effect on the tensile Young’s moduli in any direction (Fig. 4.10a). In compression however, a ground matrix with $\nu_t = 0.499$ caused lateral straining of the fibres and therefore anisotropic behaviour, while a ground matrix with $\nu_t = 0.1$ resulted in nearly identical compressive moduli in all three directions because lateral fibres were not sufficiently recruited (Fig. 4.10b). As a consequence, the nearly incompressible material appeared much stiffer in compression due to high hydrostatic stresses.

In tension, the x and z directions were the stiffest and softest, respectively. In compression, this trend was reversed for the nearly incompressible material such that the z direction became stiffest because the high tensile stiffness values in the x and y direction inhibited lateral expansion to a greater degree. Somewhat unexpectedly, increasing the degree of anisotropy to $\xi_k = 4$ resulted in lower moduli in all directions than observed with $\xi_k = 2$ (Fig. 4.10).
4. Mechanical Effects of the Collagen Architecture in Charged Media

4.5.4. Anisotropic behaviour – charged material & effect of scaling with $\tilde{\xi}^{-1}$

The charged material exhibited anisotropic behaviour in both tension and compression (Fig. 4.11). At 10% compressive strain, the material appears stiffest in the z-direction (Fig. 4.11b) as it does in the neutral material. At smaller strains however, the material is stiffest in the x-direction due to the high tensile modulus in that direction and the associated pre-stress (Nagel and Kelly, 2010a), see Fig. B.5d.

Again, increasing $\xi_k$ from 2 to 4 led to decreased tensile Young’s modulus predictions in all directions for the unscaled models. Maintaining the average radius of the ellipsoid, i.e. the amount of fibrous material, by employing the scaling procedure described above corrected the material behaviour in the desired way. Now when $\xi_1$ was increased from 2 to 4, the material became stiffer in tension in the x-direction while the z direction, where $\xi_3$ was decreased from 0.5 to 0.25 was still softer.

4.6. Discussion

The properties of complex tissues are often apparent in nature and parameter values obtained from experimental studies always depend on the material model used to interpret the test results. In this study we compared apparent properties of charged and uncharged tissues at varying fibre orientations obtained from simulations of unconfined compression tests. Besides four idealised homogeneous
4. Mechanical Effects of the Collagen Architecture in Charged Media

Figure 4.11.: Apparent Young's moduli $E_a$ at ±10% strain for various fibre stiffnesses and the charged ground substance material. Anisotropic fibre architecture with “aniso $\xi_k$” meaning $\Xi = \text{diag}(\xi_k, 1, \xi_k^{-1})$. Scaled simulations have $\xi^{-1}\Xi$.

cases of fibre directions a Benninghoff-type architecture was modelled creating a heterogeneous material to investigate a more physiologically relevant case. Three types of Young’s moduli were computed: Nominal with or without a tare strain and incremental. Furthermore, an apparent Poisson’s ratio was extracted.

The uncharged material behaved as expected with horizontal fibres producing the stiffest response in UC by inhibiting lateral expansion, an effect that was also reflected in the lower predicted Poisson’s ratio of this specimen compared with other fibre orientations. The Benninghoff architecture, characterised by vertical fibre alignment throughout most of its depth, generally exhibited a behaviour close to the sample with only vertical fibres. The charged tissues behaved quite differently. Due to tension-compression nonlinearity in conjunction with swelling the solid matrix is initially loaded in tension. This leads to tensile moduli being measured at initial small compressive strains (Ateshian et al., 2009; Chahine et al., 2004). These effects can cause a material with vertical fibres to appear stiffest initially. The stress-softening effect that follows may not be picked up if a sufficiently high tare strain is applied that unloads the fibres prior to actual testing. The results from the Benninghoff simulations were generally similar in magnitude to the results from the vertical fibre architecture. The reason again is that throughout most of the tissue vertical fibres dominate and the transition to a horizontal architecture only occurs in the upper part of the constructs. The Benninghoff architecture distinguished itself from the vertical case mainly by a smoothness during the transition from tensile to compressive states which can be attributed to the continuum of fibre angles present in the tissue. It is noteworthy that swelling is required for the Benninghoff structure to increase the equilibrium modulus of
the tissue, demonstrating the importance of the coupling between swelling and the collagen fibre architecture in determining the mechanics of articular cartilage.

Another aspect of fibre reinforcement that contributes to the different mechanical responses of the constructs with varying alignment is the amount of swelling in the uncompressed state. The more swelling is prevented, the greater the initial swelling pressures prior to loading will be.

In this study, for the purpose of comparison we made some simplifying assumptions. All the fibres in our model had their stress-free configuration exactly in the reference configuration (prior to swelling). In an actual tissue, the fibrils will have individual natural configurations (Humphrey and Rajagopal, 2002). The vertical fibre arrangement exhibited a sharp transition at the point of tension-compression nonlinearity, whereas experimentally a more smooth transition can be observed (Chahine et al., 2004) caused by heterogeneous tissue architecture and possibly different natural configurations of individual collagen fibrils. This transition will occur in models including a continuously changing fibre angle throughout the tissue domain (e.g. Benninghoff-type architecture) and / or fibre dispersion. These sophisticated models are also able to capture the transition from high to low Poisson’s ratios during the early stages of compression (Ateshian et al., 2009). Nevertheless, the presented modelling framework allows us to systematically investigate the influence of fibre architecture on the apparent mechanical properties of charged soft tissues.

Upon instantaneous loading biphasic media react quasi-incompressibly (Ateshian et al., 2007). In UC this would result in high lateral expansions. It is expected, therefore, that under dynamic loading conditions where fluid pressurisation plays a major role, the influence of horizontal and 45° fibres would be increased in both charged and uncharged constructs, leading to increased dynamic moduli.

While behaving similarly, continuous fibre distribution models lead to a more complex behaviour (Ateshian et al., 2009). In the neutral material the compressive properties are mainly determined by the ground matrix stiffness if the material is compressible (e.g. highly porous). For near incompressible ground matrices, fibre reinforcement plays a larger role in determining the compressive properties. The EFD model also led to a prediction of high tensile Poisson’s ratios due to realignment of fibres into the loading direction. An equally aligned standard orthotropic fibre model would not have the same effect (Ateshian et al., 2009). The charged materials exhibited the characteristic and complex interplay between stiffening effects in tension and stress softening in compression. The fibre stiffness had a
4. Mechanical Effects of the Collagen Architecture in Charged Media

Significant effect in both tension and compression for this material class.

A modification of the anisotropy tensors can change the overall stiffness of the fibrous material in relation to the ground matrix. This can either be desired or undesired. A decoupling of the specification of the fibre architecture and the fibre properties can be preferable in some cases, e.g. remodelling simulations or composition based constitutive modelling. A scaling factor was introduced for this purpose that prevented the overall stiffness decline when transitioning, in the presented case, from lower ($\Xi = \text{diag}(2,1,0.5)$) to higher anisotropy values ($\Xi = \text{diag}(4,1,0.25)$). This resulted in a more anisotropic tissue that was noticeably stiffer in the x-direction and softer in the z-direction. Depending on the purpose of the study this scaling methodology can be a useful tool. In some applications this distinction will not of special interest, e.g. when simply fitting a constitutive model to experimental data in order to simulate its apparent behaviour.

In summary, when interpreting experimental results the swelling nature of a tissue should be considered even if it will not be included explicitly in a model of the experiment. The results underline the importance of the collagen network in restraining swelling pressures. Through this mechanism collagen fibres increase the equilibrium stiffness of cartilage. Thus, they indirectly contribute to load bearing even if they are not actively stretched under specific loading conditions. Furthermore, when aligned parallel to the direction of load, collagen fibres can dramatically increase the equilibrium modulus of a charged soft tissue at small strains while they are still in a state of tension. Understanding structure-function relationships of constituents will be important for understanding the behaviour of native soft tissues and tissue engineered constructs.

The collagen architecture was static in the above simulations. A fibre remodelling methodology will be introduced in the next chapter that will close the loop between fibres influencing the mechanical environment in the tissue and this environment in turn influencing tissue architecture.
5 Collagen Remodelling

5.1. Introduction

The term “collagen remodelling” usually refers to a change in the collagen fibre orientation. Cells and collagen fibres have been shown to align with respect to the principal strain in collagen lattices (Eastwood et al., 1998). Cell activity based ECM remodelling includes the realignment of existing fibres, the secretion of matrix enhancing or degrading products and their inhibitors as well as the production of new (aligned) matrix components (Baaijens et al., 2010). In addition to cell mediated mechanisms, collagenase degradation was found to be strain dependent with a local minimum at 4% strain (Huang and Yannas, 1977). This can lead to alignment when fibres perpendicular to the direction of loading become resorbed via altered kinetics of collagenase-collagen interactions (Nabeshima et al., 1996; Ruberti and Hallab, 2005). ECM alignment with respect to principal directions of mechanical stimuli has been shown in a variety of tissues. Bone adaptation to mechanical forces has long been known (Roux, 1885) and the trabecular architecture is aligned in correspondence with the experienced mechanical forces (Carter et al., 1989; Huiskes et al., 2000). The periosteal collagen architecture has been shown to align with characteristic directions of growth during development (Foolen et al., 2008) and collagen alignment with respect to loading has been shown in heart valves (Sacks et al., 1998), blood vessels (Hariton et al., 2007a), cartilage (Benninghoff, 1925b; Wilson et al., 2006a) and tendons (Calve et al., 2004).

As collagen fibres can only bear tensile loads (Chandran and Barocas, 2006; Canal Guterl et al., 2010), the degree of fibre crimp determines the deformation at which it becomes stressed. This deformation, for which various terms exist in the literature, e.g. “transition stretch” (Foolen et al., 2010), “recruitment stretch” (Watton et al., 2004), “engagement strain” (Zulliger et al., 2004), “zero force stretchability” (Cacho et al., 2007), therefore determines the natural,
5. Collagen Remodelling

i.e. stress-free, configuration of the collagen network and can be altered when the collagen network is remodelled. There are numerous examples in the literature where changes in the configuration of the collagen network led to alterations in the geometry and mechanical properties of the tissue. For example, a change in tendon stiffness following exercise has been partially attributed to altered collagen molecule crimp angles (Wood et al., 1988; Magnusson et al., 2008). Pretension in normal skin has been attributed to cell-ECM interactions as well as collagen-network tension established during development (Silver et al., 2003; Farahani and Kloth, 2008). For dermal wound contraction several mechanisms have been proposed, e.g. cellular contraction (fibroblasts and myofibroblasts) in the granulation tissue as well as a structural collagen reorganisation (see Farahani and Kloth (2008) and references therein). Thomopoulos et al. (2005) seeded uniaxially and biaxially constrained collagen I gels with tendon and cardiac fibroblasts or left them acellular. The study showed that the gels contracted in the unconstrained directions and that uniaxially constrained gels developed a high degree of structural and mechanical anisotropy while biaxial and unconstrained gels remained isotropic. The authors found that for the observed changes boundary conditions were relevant but cell presence was not required. When collagen lattices were attached at opposing ends contraction caused the development of an hourglass shape (Tomasek et al., 2002).

Investigating how periosteum is involved in long bone growth, Foolen et al. (2010) demonstrated transient collagen remodelling in periosteum held at fixed lengths such that the transition stretch approximated the applied stretch after 3 to 4 days. The mechanism was cell mediated as cytochalasin D application inhibited the observed changes. Tomasek et al. (2002) proposed a “slip and ratched” mechanism whereby connective tissue contracture involves an incremental shortening of the ECM material that is initially cell mediated and is then imprinted onto the ECM as evidenced by a total loss of force in contracting collagen lattices when cytochalasin D is applied early, and a residual tension when it is applied much later (Tomasek et al. (2002) and related references). Soft tissue contracture also plays an important role in burn-scar pathologies, Dupuytren’s disease and others (Tomasek et al., 2002).

The effect of collagen crimp has been considered in constitutive models of soft tissues where at a given level of material deformation individual fibres are stretched differently (Lanir, 1979, 1983). Later models have expanded on the idea using various statistical fibre and recruitment stretch distributions, e.g. Cacho et al. (2007);
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Zulliger et al. (2004). Recently, collagen fibre recruitment was directly measured in arterial tissue and found to occur statistically distributed around finite stretch values (Hill et al., 2012). Theoretical studies have also used remodelling of collagen natural configuration to investigate various phenomena. Watton et al. (2004) used remodelling of the recruitment stretch to simulate the evolution of abdominal aneurysms. It has been hypothesised (van Donkelaar and Wilson, 2012) that matrix turnover is required for chondrocyte hypertrophy and involves degradation of collagen and unstretched re-synthesis in a new configuration. Humphrey (1999) presented a model whereby the kinetics of collagen deposition is assumed to be similar regardless of the tissue deformation at which it is secreted and used it to study collagenous tissue remodelling at fixed lengths. Based on the idea developed by Rodriguez et al. (1994) in the context of growth, some authors have used multiplicative decompositions of the deformation gradient to capture evolving natural configurations of ECM constituents to model growth or remodelling towards various homeostatic states (Garikipati et al., 2006; Thomas et al., 2009; Davol et al., 2008; Ramasubramanian and Taber, 2008; Raykin et al., 2009). For a recent review on computational models of collagen remodelling see Baaijens et al. (2010).

In the sequel the approach to describing collagen remodelling in terms of the orientation and recruitment stretch developed in this work will be introduced.

5.2. Entropy considerations

Remodelling can lead to a stiffening of the material. For illustration, consider the following strain energy density function composed of a Neo-Hookean part and two families of fibres

$$\psi = C_1(I_1 - \ln I_3 - 3) + D_2(\ln I_3)^2 + C_4(I_4 - 1)^\beta + C_5(I_6 - 1)^\beta$$  \hspace{1cm} (5.1)

The deformation gradient and right Cauchy-Green tensors for simple shear read

$$F_{i,j} = \begin{pmatrix} 1 & \gamma & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \end{pmatrix} \quad \text{and} \quad C_{ij} = \begin{pmatrix} 1 & \gamma & 0 \\ \gamma & 1 + \gamma^2 & 0 \\ 0 & 0 & 1 \end{pmatrix}$$  \hspace{1cm} (5.2)

where the shear strain is given as $\gamma = \tan \alpha$, where $\alpha$ is the shear angle. Therefore, the invariants become $I_1 = 3 + \gamma^2$ and $I_3 = 1$, i.e. the deformation is isochoric. The material is defined as orthotropic such that the two fibre directions obey $a_0 \cdot g_0 = 0$. 

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5. Collagen Remodelling

They will be defined as \( a_0 = \cos \phi e_1 + \sin \phi e_2 \) and \( g_0 = -\sin \phi e_1 + \cos \phi e_2 \). Furthermore,

\[
I_4 = M_a : C \quad I_6 = M_g : C \quad \text{with} \quad M_a = a_0 \otimes a_0, \quad M_g = g_0 \otimes g_0
\] (5.3)

The invariants for simple shear and an arbitrary in-plane fibre orientation follow as

\[
I_4 = 2\gamma \cos \phi \sin \phi + \gamma^2 \sin^2 \phi + 1
\] (5.4)
\[
I_6 = -2\gamma \cos \phi \sin \phi + \gamma^2 \cos^2 \phi + 1
\] (5.5)

The strain energy density can now be expressed as

\[
\psi = C_1 \gamma^2 + C_4(2\gamma \cos \phi \sin \phi + \gamma^2 \sin^2 \phi)^\beta + C_5(-2\gamma \cos \phi \sin \phi + \gamma^2 \cos^2 \phi)^\beta
\] (5.6)

The analysis of \( C \) for simple shear yields the eigenvalues (the squares of the principal stretches) in the 1-2-plane

\[
\lambda_{1,2}^2 = \frac{2 + \gamma^2 \pm \gamma \sqrt{4 + \gamma^2}}{2}
\] (5.7)

and for the corresponding in-plane eigenvectors

\[
n_1 = \left(\frac{8 + 2\gamma^2 - 2\gamma \sqrt{4 + \gamma^2}}{4}\right)^{-\frac{1}{2}} \begin{pmatrix} \sqrt{4 + \gamma^2 - \gamma} \\ \frac{\gamma}{2} \end{pmatrix}
\]
(5.8)

Eqs. 5.7 and 5.8 can be used to verify the numerical implementation of fibre remodelling. Together with Eq. 5.6 they illustrate Vianello's coaxiality theorems (Vianello, 1996a,b) which will have implications for the dissipation inequality in remodelling tissues.

The function in Eq. 5.6 was evaluated for \( C_1 = 1 \) MPa, \( C_4 = 5 \) MPa, \( C_5 = 2 \) MPa and \( \beta = 2 \) for \( \alpha \in [-30^\circ; 30^\circ] \) and \( \phi \in [0; 180^\circ] \) (Fig. 5.1a). It can be seen that \( \psi \) increases with shear in both directions and that several extrema occur with respect to the fibre angle. In Fig. 5.1b \( \psi \) is plotted for three different shear angles. Additionally, the principal stretch direction predicted from Eq. 5.8 is graphed (vertical lines in Fig. 5.1b). It becomes evident that the free Helmholtz
energy attains a maximum when the structural and deformation tensors commute such that the stiffest fibre direction is aligned with the maximum principal strain direction (Menzel, 2007). This is a consequence of Vianello’s coaxiality theorems (Vianello, 1996a,b).

For anisotropic materials the strain energy depends on the structural tensors $M$ as internal variables in addition to the usual deformation measure. Hence, Eq. 3.60 can be expanded to

$$-\dot{\psi}(C, M) + \frac{1}{2} T : \dot{C} \geq 0$$

(5.9)

When fibre remodelling is considered and hyperelasticity assumed the equation reduces to

$$-\frac{\partial \psi}{\partial M} : \dot{M} \geq 0$$

(5.10)

As illustrated in Fig. 5.1a, fibre remodelling can lead to a stiffening of the material, which violates the entropy inequality in Eq. 5.10. In other words, from a thermodynamical point of view a purely mechanical theory is inadmissible in the presence of remodelling processes that might stiffen the material (Garikipati et al., 2006). Considering heat conduction (compare Eq. 3.20) is a possibility to satisfy the entropy inequality. Under the limitation of isothermal processes, Eq. 5.10 can either be extended by an entropy production density term $\dot{s}$ or the energy term is
5. Collagen Remodelling

split into mechanical ($\psi_m$) and chemical ($\psi_c$) parts (Garikipati et al., 2006):

$$-\frac{\partial \psi_m}{\partial \hat{M}} : \dot{\hat{M}} - \dot{\psi}_c \geq 0$$  \hspace{1cm} (5.11)

$$-\frac{\partial \psi}{\partial \hat{M}} : \dot{\hat{M}} - \frac{\theta \dot{\hat{M}}}{\theta} \geq 0$$  \hspace{1cm} (5.12)

The first variant implies that in order to reorient fibres and thus stiffen the material the cell has to consume its chemical energy. If a constitutive model for $\psi_c$ is not available or as long as the interplay between thermal and chemical contributions remains unclear, the second equation is suitable to ensure thermodynamical compatibility. An alternative to open system thermodynamics (Kuhl and Steinmann, 2003) are mixture models in which interactions between the phases can lead to more explicit relationships in the context of multiphasic materials (e.g. capturing the removal and addition of certain constituents at the expense of others) (Humphrey and Rajagopal, 2002). Until these complex interactions are further characterised and quantified, however, purely mechanical theories remain a valuable phenomenological tool to study tissue remodelling (Himpel et al., 2008; Garikipati et al., 2006). This approach will be adopted here.

5.3. Angular fibre remodelling

5.3.1. The Euler-Rodrigues formula and the exponential map

![Geometrical relationships for the rotation of a vector $\alpha$ about an axis $e$ by an angle $\theta$, adapted from Itskov (2009).](image)

Before the remodelling scheme is introduced, some geometrical considerations useful for the description of finite rotations will be presented. Consider a vector
a that is rotated around an axis designated by the unit vector e by an angle \( \theta \) to yield the vector \( \hat{a} \) (Fig. 5.2). A rotation tensor \( \mathbf{R} \) can be derived that causes the equivalent rotation via the linear mapping \( \hat{a} = \mathbf{R}a \) as follows. Using the decomposition of \( a \) into components parallel and perpendicular to \( e \) (i.e. \( a = a' + x \)), the vector \( \hat{a} \) can be written as

\[
\hat{a} = a' + \cos \theta x + \sin \theta y 
= a + (\cos \theta - 1)x + \sin \theta y
\]  

Using

\[
y = e \times x = e \times (a - a') = e \times a = \hat{e}a
\]

where \( \hat{e} \) is the second order skew symmetric tensor defined by the last identity in Eq. 5.15 (see also Eq. A.64), as well as \( x = y \times e = -e \times y \), the following can be formulated:

\[
\hat{a} = a + \sin \theta \hat{e}a + (1 - \cos \theta) (\hat{e})^2 a
\]

This yields the Euler-Rodrigues formula

\[
\mathbf{R} = \mathbf{I} + \sin \theta \hat{e} + (1 - \cos \theta)(\hat{e})^2
\]

The tensors \( \mathbf{R} \) and \( \hat{e} \) belong to the special orthogonal group \( \text{SO}(3) \) and the set of skew-symmetric matrices \( \text{so}(3) \), respectively (Simo and Hughes, 1998):

\[
\mathbf{R} \in \text{SO}(3) = \{ \mathbf{A} : \mathbb{R}^3 \to \mathbb{R}^3 | \mathbf{A}^T \mathbf{A} = \mathbf{I} \text{ det} \mathbf{A} = 1 \} \\
\hat{e} \in \text{so}(3) = \{ \hat{\mathbf{A}} : \mathbb{R}^3 \to \mathbb{R}^3 | \hat{\mathbf{A}} + (\hat{\mathbf{A}})^T = 0 \}
\]

The exponential map can be used for the transformation of skew-symmetric matrices to orthogonal matrices:

\[
\exp[\hat{\mathbf{A}}] = \sum_{n=0}^{\infty} \frac{1}{n!}[\hat{\mathbf{A}}]^n
\]

By applying the exponential map to the product \( \theta \hat{\omega} \) where \( \mathbf{\omega} \cdot \mathbf{\omega} = 1 \) and considering Eq. A.65, one finds

\[
\exp[\theta \hat{\omega}] = \mathbf{I} + \hat{\omega} \left( \theta - \frac{\theta^3}{3!} + \frac{\theta^5}{5!} - \ldots \right) + \hat{\omega}^2 \left( \frac{\theta^2}{2!} - \frac{\theta^4}{4!} + \frac{\theta^6}{6!} - \ldots \right) \\
= \mathbf{I} + \sin \theta \hat{\omega} + (1 - \cos \theta)\hat{\omega}^2
\]
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Thus, by comparison with Eq. 5.17 one finds that the exponential map yields the rotation tensor corresponding to the rotation around \( \omega \) by an angle \( \theta \):

\[
R = \exp[\theta \hat{\omega}] \iff (\theta \omega) \times a = \theta \omega a \quad \text{and} \quad ||\omega|| = 1
\]

For an arbitrary skew symmetric tensor, where \( ||\omega|| \neq 1 \), the normalised version of the Rodrigues formula reads

\[
\exp \hat{\omega} = I + \sin ||\omega|| \frac{\hat{\omega}}{||\omega||} + (1 - \cos ||\omega||) \frac{\hat{\omega}^2}{||\omega||^2}
\]

Using standard trigonometric relationships\(^1\) the final version of the Rodrigues equation reads (Simo and Hughes, 1998)

\[
\exp \hat{\omega} = I + \frac{\sin ||\omega||}{||\omega||} \hat{\omega} + \frac{1}{2} \left( \frac{\sin(||\omega||/2)}{||\omega||/2} \right)^2 \hat{\omega}^2
\]

While the exponential map provides the transformation \( \text{so}(3) \rightarrow \text{SO}(3) \), the inverse transformation is needed as well. In this work, the extraction of the rotation vector was based on a singularity-free algorithm to compute the quaternion \( \hat{q} = [q_0, \hat{q}] = [\cos(\theta/2), \sin(\theta/2)e] \), for details see Spurrier (1978); Menzel (2007) and algorithm 1 in appendix A.8

5.3.2. Evolution of \( \Xi \)

In this section, a fibre remodelling methodology for 1, 2 and 4 families of fibres as well as a continuous fibre distribution will be developed. The common basis for all said variants of the algorithm is the definition of the anisotropy tensor \( \Xi \) in Eq. 3.85. A deformation driven remodelling approach is chosen. Consider the spectral decompositions

\[
C = \sum_{i=1}^{3} \lambda_i^2 n_i \otimes n_i \quad \text{and} \quad \Xi = \sum_{i=1}^{3} \xi_i w_i \otimes w_i
\]

where the \( n_i \) represent the principal stretch directions (and will be called stimulus directions) and \( w_i \) the eigenvectors of the anisotropy tensor. The fundamental assumption of the remodelling algorithm is that over time the anisotropy tensor approaches the right Cauchy-Green tensor: \( \Xi \rightarrow C \) (Fig. 5.3). As \( \{w_i\} \) represents an orthonormal basis, the reorientation model for orthotropic continua

\(^1\) \( 1 - \cos^2 x = \sin^2 x \) as well as \( \cos^2 x = \cos 2x + \sin^2 x \).
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Figure 5.3.: The fundamental assumption of the remodelling algorithm is that over time the anisotropy tensor approaches the right Cauchy-Green tensor: $\Xi \rightarrow C$.

proposed in Menzel (2007) can be adopted. The eigenvalues of $\Xi$ are sorted so that $\xi_1 \geq \xi_2 \geq \xi_3$. The ordering of the eigenvalues of $C$ is performed such that $w_1$ reorients to the closest stimulus direction, $w_2$ to the closest of the remaining two and $w_3$ to the last one. The implications of this choice are illustrated in Fig. 5.4. Maintenance of right handed coordinate systems is assured. A rotation tensor is then established

$$R = n_i \otimes w_i$$

(5.27)

where the summation over $i$ is implicit. Corresponding to $R$ is a rotation vector $\theta = \theta e$ where $e$ is the axis of rotation and $\theta$ the angle. As outlined in the previous section, both were extracted using algorithm 1 in appendix A.8. Now a time constant $\tau_f$ can be introduced to define the angular velocity vector:

$$\omega = \frac{\theta}{\pi \tau_f} e$$

(5.28)

Using the equivalence $\omega \times a = \dot{\omega} a$ the skew-symmetric matrix corresponding to $\omega$ is $\dot{\omega}$. The Euler theorem states that the exponential map $\exp(\omega \Delta t) \in SO(3)$ performs a rotation about the vector $\omega$ by the angle $||\omega|| \Delta t$, so that

$$w_{i}^{n+1} = \exp(\omega \Delta t)w_i^n$$

(5.29)

The exponential map is calculated from the Rodrigues formula

$$\exp(\omega \Delta t) = I + \frac{\sin(||\omega\Delta t||)}{||\omega\Delta t||} \omega \Delta t + \frac{1}{2} \left[ \frac{\sin(||\omega\Delta t||/2)}{||\omega\Delta t||/2} \right]^2 (\omega \Delta t)^2$$

(5.30)

Now the basis $\{w_i\}$ can be reoriented towards the basis $\{n_i\}$. The eigenvalues are subsequently updated based on the linear rate equation

$$\dot{\xi}_i = \frac{1}{\tau_f}(\lambda_i^2 - \xi_i)$$

(5.31)
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Figure 5.4.: Possible rotation schemes illustrated for the following special case: The current anisotropy (state A) and the target state (state B) are characterised by identical eigenvalues (i.e. half axes) but are rotated 90° to each other. There are different possibilities for the transition from state A to state B. Possibility 1 maintains the eigenvalues (i.e. the ellipticity) and rotates the ellipsoid via a modification of its eigenvectors (top). Possibility 2 maintains the eigenvectors (half axis directions) but modifies the eigenvalues, thus transitioning through a temporary state of isotropy. The reorientation scheme presented here prefers the second route. In the general case of arbitrary differences between states A and B a combination of eigenvalue and eigenvector modification occurs.

that can be solved explicitly via integration (here written for the increment \([n; n + 1]\)):

\[
\xi_i^{n+1} = \lambda_i^2 + (\xi_i^n - \lambda_i^2)e^{-\frac{\Delta t}{\tau_f}}
\]  

(5.32)

where the same rate constant \(\tau_f\) has been used for both the reorientation of the eigenvectors and the modification of the eigenvalues (ellipticity) of \(\Xi\). Eq. 5.32 means that the material gets reinforced by fibres in directions of higher stretch as the eigenvalues of \(\Xi\) approach those of \(C\). Principal verification using a simple shear test is found in appendix B.4.
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5.3.3. Deriving the fibre architecture from $\Xi$

Continuous fibre distribution

Since $\Xi$ can be represented as an ellipsoid, the ellipsoidal fibre stiffness distribution can be directly derived using Eq. 3.86. A scaling of the ellipsoid can be introduced such that the anisotropy is derived from $\Xi^m = \sum_{i=1}^{3} \xi^m_i w_i \otimes w_i$:

$$C_4(a_0) = \left[ \frac{(\cos \Theta \sin \Phi)^2}{\xi_1^{2m}} + \frac{(\sin \Theta \sin \Phi)^2}{\xi_2^{2m}} + \frac{(\cos \Phi)^2}{\xi_3^{2m}} \right]^{-\frac{1}{2}} \tilde{C}_4$$  \hspace{1cm} (5.33)

An overall increase in stiffness (i.e. fibrous material) can be included into evolution equations for $\tilde{C}_4$. However, the scaling with the exponent $m$ additionally alters the overall stiffness. In order to maintain the split between $\tilde{C}_4$ designating the amount of fibrous material and $\Xi^m$ designating its orientational distribution, the following normalisation is performed (compare also appendix B.3.3):

$$C_4(a_0) = \frac{1}{\tilde{\xi}} \left[ \frac{(\cos \Theta \sin \Phi)^2}{\xi_1^{2m}} + \frac{(\sin \Theta \sin \Phi)^2}{\xi_2^{2m}} + \frac{(\cos \Phi)^2}{\xi_3^{2m}} \right]^{-\frac{1}{2}} \tilde{C}_4$$  \hspace{1cm} (5.34)

where

$$\tilde{\xi} = \frac{1}{4\pi} \int_{0}^{2\pi} \int_{0}^{\pi} \left[ \frac{(\cos \theta \sin \phi)^2}{\xi_1^{2m}} + \frac{(\sin \theta \sin \phi)^2}{\xi_2^{2m}} + \frac{(\cos \phi)^2}{\xi_3^{2m}} \right]^{-\frac{1}{2}} \sin \phi \, d\phi \, d\theta$$  \hspace{1cm} (5.35)

An alternative derivation of the fibre architecture follows from the constitutive assumption that $C_4(a_0) \propto \lambda^2(a_0)$. Since an individual fibre is both rotated and stretched ($a = F a_0$) during deformation but the stiffness distribution is attributed to the original spherical vector cloud in the undeformed configuration, this ansatz leads to a dumbbell shaped stiffness distribution. The equation for the direction dependent fibre stiffness simply reads:

$$C_4(a_0) = \frac{\Xi^m : (a_0 \otimes a_0)}{r_m} \tilde{C}_4$$  \hspace{1cm} (5.36)

where $r_m$ is used for scaling similar to $\tilde{\xi}$. Both distributions coincide at the peaks and are compared in Fig. 5.5.

As can be seen from Fig. 5.5, the dumbbell shape in itself would lead to a higher overall fibre stiffness. But via the scaling performed in Eqs. 5.34 and 5.36 both distributions will amount to the same average stiffness values\(^2\) but differ in the distribution of the fibrous material (Fig. 5.6).

\(^2\tilde{\xi} \approx 0.8848\) for this case and $r_m = 7/6$.  

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(a) $\Xi = \text{diag}(1.5, 0.8)$  

(b) $\Xi = \text{diag}(2.0, 0.6)$

Figure 5.5.: Comparison of the ellipsoidal and the dumbbell shaped stiffness distribution for two in-plane examples. Distributions derived from Eqs. 5.33 (ellipsoid, $m=1$) and 5.36 (dumbbell). The green vectors illustrate the initial vector cloud. Each vector’s stiffness is represented by the distance the corresponding marker (red cross - ellipsoid; blue x - dumbbell) has to the origin.

(a) unscaled  

(b) scaled

Figure 5.6.: Unscaled (a) and scaled (b) representations of the fibre distribution in the 1-3-plane for $\Xi = \text{diag}(2.0, 1.0, 0.5)$. Scaling equalises the average fibre stiffness but maintains the distribution characteristics.

**Discrete families of fibres**

The directions of discrete families of fibres can be derived from $\Xi$ as well by aligning them between its principal axes in a manner dependent on the eigenvalue magnitudes:

\[
a_0^1 = \frac{\xi_1 w_1 + \xi_2 w_2 + \xi_3 w_3}{\sqrt{\xi_1^2 + \xi_2^2 + \xi_3^2}} \\
a_0^2 = \frac{\xi_1 w_1 + \xi_2 w_2 - \xi_3 w_3}{\sqrt{\xi_1^2 + \xi_2^2 + \xi_3^2}} \\
a_0^3 = \frac{\xi_1 w_1 - \xi_2 w_2 + \xi_3 w_3}{\sqrt{\xi_1^2 + \xi_2^2 + \xi_3^2}} \\
a_0^4 = \frac{\xi_1 w_1 - \xi_2 w_2 - \xi_3 w_3}{\sqrt{\xi_1^2 + \xi_2^2 + \xi_3^2}}
\]

(5.37)  

(5.38)  

(5.39)  

(5.40)
Collagen Remodelling

Four families of fibres therefore appear in this 3D setting (Fig. 5.7). Most discrete fibre remodelling algorithms assume that fibre reorientation is only driven by tensile stresses or strains. These formulations can be recovered by using only tensile eigenvalues in Eqs. 5.37 - 5.40. In the case of $\mathbf{E} \rightarrow \mathbf{C}$ this implies $\xi_i \geq 1$. In other words, for three tensile principal strains the model will predict four families of fibres, for $\xi_3 < 1$ it will reduce to two fibre families, for $\xi_3, \xi_2 < 1$ to transverse isotropy and for $\xi_3, \xi_2, \xi_1 < 1$ no fibre directions will be predicted at all. Many remodelling algorithms have been developed for cardiovascular tissues which are usually assumed to be incompressible. In that case, the options $\xi_3, \xi_2, \xi_1 < 1$ and $\xi_3, \xi_2, \xi_1 > 1$ will not occur and the only relevant outcomes are those with one and two families of fibres. All directions are assigned identical properties. An alternative, though mechanically not equivalent way of defining an orthotropic material, would be to align three families of fibres directly with the eigenvectors of $\mathbf{E}$ and assigning each fibre direction individual mechanical properties. However, the assumptions presented in Eqs. 5.37 - 5.40 are more common and have compared more favourably to histological observations in heart valves, arteries and articular cartilage, as reviewed in Baaijens et al. (2010). Regardless, in cartilage mechanics continuous fibre distribution models have been shown to capture aspects of the (strain-dependent) mechanical behaviour that cannot be captured with discrete-fibre orthotropic models of either kind (Ateshian et al., 2009).
5.4. Remodelling the collagen network’s recruitment configuration

5.4.1. The recruitment configuration

![Image of recruitment configuration](image.png)

Figure 5.8.: As long as only the (blue) ground substance is stretched, the force stretch curve exhibits a comparatively low slope. Once collagen fibres are stretched beyond their recruitment stretch (are “uncrimped”) they contribute to load bearing and the slope of the curve increases. This can be illustrated using a one dimensional rheological model (top row of images) in which the spring representing the collagen only stores energy once the angular joint is straightened out.

A major part of the model presented below is a change in the natural configuration of the collagen molecules via a modification of their transition stretch, i.e. the minimum stretch at which the fibre begins to bear load. Since collagen fibres can be thought of as long, slender, cable-like structures, they experience buckling at a certain point of axial compression. Due to the comparatively low flexural rigidity this is considered to occur without significant stress contribution. Conversely, when stretched from a crimped state they start bearing tension only once the applied stretch exceeds a certain value (Fig. 5.8). While this can be described by a transition stretch for an individual fibre, a single stretch value is insufficient to convey this information on a tissue level with many multidirectional fibres in a 3D setting. Therefore, an evolving recruitment configuration is introduced by a multiplicative decomposition of the deformation gradient to calculate the energy.
5. Collagen Remodelling

potentials for the stresses in the collagen network (Fig. 5.9):

\[ F = F_e F_r \]  \hspace{1cm} (5.41)

While this and related definitions are local quantities, the explicit dependence on the particles \( X \) and time \( t \) will be omitted in the notation. The recruitment tensor \( F_r \) maps a material line element from the static reference configuration \( Q_0 \) into the recruitment configuration \( Q_r \), which defines the deformation state (stretch) at which collagen fibres begin to contribute to the stress response. The elastic deformation gradient \( F_e \) then maps this line element into the current configuration \( Q \) (Fig. 5.9). Note that the same intermediate recruitment configuration is used here for all families of fibres. If more complexity is desired, analogous decompositions could be conceived for each fibre family. The elastic and recruitment right Cauchy-Green tensors follow as

\[ \tilde{C}_e = F_e^T F_e \hspace{1cm} \text{and} \hspace{1cm} C_r = F_r^T F_r \]  \hspace{1cm} (5.42)

Assuming that the reference configuration of the isotropic matrix material does not change its deformation will be described with respect to \( Q_0 \). The anisotropic energy potential will be formulated with respect to \( Q_r \). Therefore, the following
split of the free Helmholtz energy density is used

\[ \psi = \psi_{\text{iso}}(C) + \sum_{i=1}^{N} \psi_{\text{aniso}}(\tilde{C}_e, \tilde{M}^i) \]

\[ = \psi_{\text{iso}}(I_1, I_2, I_3) + \sum_{i=1}^{N} \psi_{\text{aniso}}(I_{4e}^i) \]  

(5.43)

Here, \( \tilde{M}^i \) is the unit structure tensor of the \( i \)th family of fibres in \( \Omega_r \) (for a similar definition of structural tensors in the context of viscoelastic and elastoplastic theories see e.g. Reese (2003); Nguyen et al. (2007)):

\[ \tilde{M}^i = \hat{a}_0^i \otimes \hat{a}_0^i = \frac{F_r \cdot M^i \cdot F^T_r}{\text{tr}(M^i \cdot C_r)} \]

with \( \hat{a}_0^i = \frac{F_r \cdot a_0^i}{||F_r \cdot a_0^i||} \)  

(5.44)

A material line element in the \( i \)th fibre direction experiences a stretch \( \lambda_r^i = \sqrt{\text{tr}(M^i \cdot C_r)} \) in the recruitment configuration. According to the potentials defined in Eq. 5.43 this stretch can be interpreted as the transition stretch. Thus, only fibres stretched by \( \lambda^i > \lambda_r^i \) will contribute to the stress response. The fibre stretch \( \lambda_f^i \) is the square root of the elastic invariant \( I_{4e}^i \) that is defined in the intermediate recruitment configuration:

\[ I_{4e}^i = \text{tr}(\tilde{M}^i \cdot \tilde{C}_e) \]  

(5.45)

The individual stress contributions were added as second Piola-Kirchhoff stresses in the reference configuration:

\[ T = T_{\text{iso}} + T_{\text{aniso}} \]  

(5.46)

and hence \( I_{4e}^i \) was expressed in terms of quantities defined in the reference configuration for numerical treatment:

\[ I_{4e}^i = \frac{\text{tr}(M^i \cdot C)}{\text{tr}(M^i \cdot C_r)} = (\lambda_{f}^i)^2 = \left( \frac{\lambda^i}{\lambda_f^i} \right)^2 \]  

(5.47)

\( F_r \) was defined similar to a relation used in orthotropic growth (Menzel, 2007) as

\[ F_r = I + \sum_{i=1}^{3} [\lambda_r(w_i) - 1] w_i \otimes w_i \]

\[ = \sum_{i=1}^{3} \lambda_r(w_i) w_i \otimes w_i \]  

(5.48)

with \( \lambda_r(w_i) \) being the transition stretch in one of the mutually orthogonal directions \( w_i \).
5.4.2. Evolution of $F_r$

As outlined in Humphrey (1999), many aspects of growth and remodelling can be captured when “the kinetics of collagen deposition and degradation is similar regardless of the configuration of the body at which it occurs”. This implies that the collagen network will tend to a homeostatic stress or strain state if turnover is ongoing. Further evidence for this idea stems from transition stretch adaptation to applied deformation (Foolen et al., 2010), aneurism growth (Watton et al., 2004) and others (Tomasek et al., 2002).

For each characteristic material direction remodelling of the current transition stretch $\lambda_r$ towards a desired transition stretch $\lambda_0$ was modelled with the linear rate equation:

$$\dot{\lambda}_r = \frac{1}{\tau}(\lambda_0 - \lambda_r)$$  \hspace{1cm} (5.49)

so that the transition stretch in a certain fibre direction in the next iteration (n+1) was determined via

$$\lambda_{r}^{n+1} = \lambda_0 + (\lambda_r^n - \lambda_0)e^{-\frac{\Delta t}{\tau}}$$  \hspace{1cm} (5.50)

where $\Delta t$ is the time step between iterations n and n+1 and $\tau$ a time constant determining the rate at which collagen gets turned over. The desired transition stretch was determined based on either of the two assumptions:

1. Fibres are incorporated into the matrix at a homeostatic stretch $\lambda_h$, such that over time the fibre network remodels to that homeostatic stretch value. Hence the transition stretch is determined based on the multiplicative decomposition

$$\lambda_0 = \frac{\sqrt{\text{tr}(MC)}}{\lambda_h}$$  \hspace{1cm} (5.51)

Here, $\lambda_h > 1$ would mean that fibres are incorporated into the matrix in a stretched configuration while $\lambda_h = 1$ corresponds to an unstretched and $\lambda_h < 1$ to a crimped incorporation.

2. Fibres are incorporated into the matrix at a homeostatic stress $\sigma_h$, such that over time the fibre network remodels to that homeostatic stress value. Hence the transition stretch is determined iteratively via a local Newton iteration such that the fibre stretch in the current configuration causes the fibres to attain that homeostatic stress value

$$\lambda_0 \leftarrow \sigma_h = \sigma_f \left( \lambda_f = \frac{\sqrt{\text{tr}(MC)}}{\lambda_0} \right)$$  \hspace{1cm} (5.52)
If fibres are put down stress free then $\sigma_h = 0$ will be used in the above relation, whereas for fibres with pre-stress a $\sigma_h > 0$ has to be specified.

Further illustrative tests of the algorithm performance can be found in appendix B.5.

5.5. Framework validation

5.5.1. Uniaxial example problems

The mode of loading, the driving stimulus for remodelling, the time constants and the material parameters can potentially interact and influence the apparent behaviour. To understand this basic model behaviour, some uniaxial example problems were investigated using an exemplary non-physiological set of material parameters for illustration purposes.

A quadratic bar was modelled using the transversely isotropic material with fibres running parallel to its long axis. Along this axis loading was applied either strain controlled (constant stretch $\lambda = 1.2$) or stress controlled (constant Cauchy stress $\sigma = 1$ MPa). Loading was applied within 0.01 s, held for 1000 s, reversed ($\lambda = -1.2$ or $\sigma = -1$ MPa) within 0.02 s and held for another 1000 s. Fibres were modelled with an initial transition stretch of $\lambda_r(t = 0) = 1$ i.e. $\Omega_r$ and $\Omega_0$ were coinciding. Simulations were performed with the following parameter set$^3$: $\lambda_h = 1.05$, $\sigma_h = 0.2$ MPa, $C_1 = 1$ MPa, $\nu = 0.3$, $\beta = 10$. Since tissues with more or less significant fibre reinforcement are expected to react differently to changes in that network, the fibre-to-matrix stiffness ratio was varied. The fibre stiffness $C_4$ was expressed with respect to the substrate stiffness $C_1$ such that a “stiff” ($C_4 = 10$ MPa), a “normal” ($C_4 = 1$ MPa) and a “soft” ($C_4 = 0.1$ MPa) reinforcement were created. The time constant was identical for all simulations and chosen such that sufficient remodelling could take place in the simulation time ($\tau = 50$ s).

Under displacement control, stretch driven remodelling exhibited identical transient behaviour for all materials. Remodelling occurred with the same apparent time constant and the fibre stretch reached its homeostatic value for all cases (Fig. 5.10b). This corresponded to identical transition stretch values for all degrees of fibre reinforcement (Fig. 5.10a). Due to the different fibre stiffness values stresses

$^3$All simulations in this section were modelled with a Neo-Hookean ground matrix and the exponential constitutive model for the collagen fibres (Eq. 3.80) unless otherwise stated.
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![Diagram](image1)

Figure 5.10.: Uniaxial remodelling; displacement controlled loading and stretch driven remodelling.

![Diagram](image2)

Figure 5.11.: Uniaxial remodelling; force controlled loading and stretch driven remodelling.

in the fibres were different. Load reversal completely unloaded all fibres until remodelling restored the state of tension (Fig. 5.10c).

Under load control the remodelling response differed for the various stiffness ratios. Remodelling was slower the stiffer the fibre material. While the homeostatic stretch approached by all materials was identical (Fig. 5.11b), the corresponding transition stretch values differed strongly (Fig. 5.11a). In case of the stiff material the transition stretch took on values of less than 1 even for an applied tensile stress load in order to create enough stress in the fibre material (via the opposing

![Diagram](image3)

Figure 5.12.: Uniaxial remodelling; displacement controlled loading and stress driven remodelling.
5. Collagen Remodelling

Figure 5.13.: Uniaxial remodelling; force controlled loading and stress driven remodelling.

compressive stress of the base matrix) to reach the homeostatic fibre stretch. A load reversal did not unload the stiff fibres completely anymore due to the low transition stretch (Fig. 5.11c). Under displacement control, the apparent time constants were identical also for stress driven remodelling. The stiffer the fibre the more stress had to be relieved to achieve the same homeostatic stress state for each of the fibre materials (Fig. 5.12c). Due to the varying stiffnesses this corresponded to different fibre stretch values (Fig. 5.12b) and to different transition stretch values (Fig. 5.12a) under displacement control.

Stress controlled remodelling under load control showed varying apparent transient behaviour. The stiffer the fibre material the slower the approach to homeostatic stress values (Fig. 5.13c). Transition stretch (Fig. 5.13a) and fibre stretch values (Fig. 5.13b) developed towards stiffness specific final values over time.

5.5.2. Remodelling of periosteum held at fixed lengths

The model described above was applied to a recent experimental study in which chick periosteum was held at fixed stretches with respect to in vivo length (Foolen et al., 2010). The periosteal geometry was approximated as a tube-like structure with a diameter of 4 mm and a length of 12 mm (Foolen et al., 2008) and a wall thickness of 50 μm (Bertram et al., 1998). The initial transition stretch was set at the in vivo length: \( \lambda_r(t = 0) = 1 \) (Foolen et al., 2010). The periosteum was then shortened to 75% and stretched to 120% to perform a tensile test. Subsequently, two cases from Foolen et al. (2010) were modelled: The periosteum was either stretched by 5% of its in vivo length (extension at a fixed stretch of \( \lambda = 1.05 \)) or compressed by 10% of the in vivo length (shortening at a fixed stretch of \( \lambda = 0.9 \)). The applied stretch was held for 4 days and a tensile test as
5. Collagen Remodelling

(a) Force-stretch curves (left) and transition stretch (right) for periosteum compressed by 5% as they change over time.

(b) Two-Photon laser scanning microscopy images of collagen in chick periosteum (tibiotarsi). Collagen morphology was imaged before, immediately after and 72h after the application of 10% compression. Scale bars represent 25 μm.

Figure 5.14.: Experimental measurements of a Remodelling transition stretch in periosteum due to sustained shortening or lengthening, adapted from Foolen et al. (2010).

described above simulated after each day. It was observed experimentally (Foolen et al., 2010) that the transition stretch approached the applied stretch after 3 to 4 days (Fig. 5.14a and Fig. 4b in Foolen et al. (2010)). This was accompanied by a change in collagen network morphology: Collagen fibres were straight in the initial state, crimped after the application of shortening and straight again after 72 hours of sustained shortening (Fig. 5.14b). Therefore, stretch driven remodelling was applied towards an unstretched configuration: \( \lambda_h = 1.0 \). The isotropic base
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matrix was modelled very weak in comparison to the collagen fibres and nearly incompressible. The material parameters were fit to roughly approximate the force-stretch curves seen in Foolen et al. (2010) such that $C_1 = 0.001$ MPa, $C_4 = 1.2$ MPa and $\beta = 0.0$. A remodelling constant of $\tau = 1$ d was chosen.

![Graphs showing force against stretch and development transition stretch](image)

Figure 5.15.: Remodelling of the transition stretch in periosteum due to sustained shortening or lengthening - Simulation results.

Remodelling of the transition stretch occurred towards the applied stretch as expected with the strongest changes at early time points and levelled off towards day 4 (Figs. 5.15b and 5.15d). The shifting point of the tension-compression transition can be seen in the predicted force-stretch curves of the materials tested after each day in culture. No heel region that was evident in the experiment (Fig. 5.14a) was observed in the simulations (Figs. 5.15a and 5.15c). This is due to a statistical distribution of the transition stretch in biological tissues and can be incorporated into models either phenomenologically as progressively stiffening stress-strain formulations or mechanistically via modelling a statistical distribution of the transition stretch (Hill et al., 2012; Cacho et al., 2007; Zulliger et al., 2004; Lanir, 1979). For day 0 periosteum ($\lambda_r = 1.0$) the following model variations were tested conceptually:
• **Standard:** As described above. Anisotropic strain energy density as in Eq. 3.80 (exponential law), 1 unidirectional family of fibres, $C_4 = 1.2 \text{ MPa}$ and $\beta = 0.0$.

• **Exponential:** Anisotropic strain energy density as in Eq. 3.80 (exponential law), 1 unidirectional family of fibres, $C_4 = 0.8 \text{ MPa}$ and $\beta = 10.0$.

• **Power law:** Anisotropic strain energy density as in Eq. 3.82 (power law), 1 unidirectional family of fibres, $C_4 = 1.7 \text{ MPa}$ and $\beta = 2.9$.

• **Ellipsoid:** Ellipsoidal fibre distribution ($\Xi = \text{diag}(10,1,1)$) as described above. Anisotropic strain energy density as in Eq. 3.80 (exponential law), $\bar{C}_4 = 1.2 \text{ MPa}$ and $\beta = 0.0$.

• **Statistical:** As "standard" model. However, instead of 1 unidirectional family of fibres, a set of 100 coaligned fibres was modelled with one hundredth of the total stiffness $C_4$ each. The transition stretch for each fibre was randomly varied in the interval $[0.95\lambda_r; 1.05\lambda_r)$ so that each fibre had a different transition stretch.

The comparison of the force-stretch behaviour is shown in Fig. 5.16. The standard model shows the sharp transition described above. Equally, the ellipsoidal model does not produce a smooth transition since a large portion of the fibre distribution starts bearing load at the same time and the constitutive behaviour of the individual fibres is identical to the standard model. A slight stiffening at higher...
strains was observed due to subsequent recruitment of more fibres. The exponential model exhibits a heel region but the transition is not smooth. The power law model however produced a smooth transition between tension and compression as well as a pronounced heel region. Both the exponential and the power law model however show the progressively stiffening behaviour over a wide range of strains while the experimental curves (Foolen et al., 2010) consist of a narrow heel region followed by quasi-linear behaviour. When the transition stretch is modelled as statistically distributed, this behaviour can be captured. Although each fibre individually undergoes a sharp tension-compression transition the combination of the 100 fibres causes a smooth transition on the tissue level. This transition is confined to a narrow strain region around the mean recruitment stretch $\lambda_r$ and the curve subsequently approaches the linear behaviour of the standard model.

![Diagram](image)

Figure 5.17.: Boundary conditions for the unconstrained, uniaxially and biaxially constrained collagen gels and the clamped gel. While in the uniaxially (b) and biaxially (c) constrained gels only displacements normal to the respective edges are constrained, both normal and tangential displacements are constrained in the clamped gel (d).

### 5.5.3. Remodelling in collagen gels

Collagen gels were modelled as thin quadratic sheets. Since the interplay between the material parameters, remodelling constants and boundary conditions strongly determines the rate and extent of remodelling, values were estimated for the following simulations to demonstrate the desired effects. The material was modelled as a soft isotropic ground substance ($C_1 = 0.03 \text{kPa}$, $\nu = 0.4$) with a continuous fibre distribution with $\beta = 0$ and $C_4 = 1 \text{kPa}$. This produced gels with (strain dependent) tensile moduli on the order of 10's of kPa (which is in the range of experimentally reported values for 3mg/ml collagen gels (Roeder et al., 2002), the same concentration used in (Thomopoulos et al., 2005)) and compressive moduli in the range of $\approx 0.3 \text{kPa}$ creating a material much softer in compression than in tension. Gels where either left unconstrained, constrained uniaxially or constrained biaxi-
ally (Figs. 5.17a to 5.17c). Constraints were imposed by fixing displacements in the reference configuration in either the x (uniaxial) or both the x and y (biaxial) direction. Fibres where remodelled to achieve a homeostatic stretch $\lambda_h = 1.01$ leading to a tendency to contract the collagen gels. The developing deformation was driving the fibre angular remodelling. A culture time of 72 h was modelled with a remodelling time constant of $\tau = 0.5$ h. Anisotropy was quantified in terms of the ratio $\xi_1/\xi_2$ (i.e. the ratio of the major and minor in-plane half-axes of the anisotropy ellipsoid described by $\Xi$), while gel contraction was quantified in terms of the apparent stretch. The “clamped” case was modelled by constraining two opposing edges in the x and y direction (Fig. 5.17d). The evolving shape and principal fibre direction were evaluated for this set of boundary conditions.

![Graph](image1)

Figure 5.18.: Anisotropy ratio $\xi_1/\xi_2$ in the plane of the gel due to deformation driven remodelling of the fibre angular distribution.

![Graph](image2)

Figure 5.19.: Apparent stretch occurring in the gels due to contraction in the unconstrained direction by remodelling collagen fibres.

Unconstrained and biaxial gels maintained structural isotropy as shown by an anisotropy ratio of $\xi_1/\xi_2 = 1$ (Figs. 5.18a and 5.18c). This was a direct consequence of the identical gel deformation in both directions observed in these gels – the unconstrained gel contracted equally in both directions by just under 20% over time (Fig. 5.19a) while the biaxially constrained gel could not contract at all since the constraints counteracted the increasing fibre stress (Fig. 5.19c). The uniaxially constrained gel however showed a strong contraction in the unconstrained
direction by more than 20% while contraction was inhibited in the constrained direction (Fig. 5.19b). This deformation led to a structural anisotropy where the ratio of the anisotropy ellipsoid’s half axes $\xi_1/\xi_2$ in the plane of the gel increased to $\approx 1.6$ over time (Fig. 5.18b). This was due to fibres weakening over time in the unconstrained direction.

The clamped gel developed the typical hourglass shape (Tomasek et al., 2002) and the long axes of the ellipsoids followed the principal strain trajectories (see Figs. 5.20a and 5.20b).

Figure 5.20.: (a) The clamped gel developed the typical hourglass shape. Contours show the vertical component of $F_r$. (b) The long half-axis of the anisotropy ellipsoid as a representation for the fibre angular remodelling of the principal fibre direction plotted over the undeformed geometry of the clamped gel.

Figure 5.21.: (a) Fibrin gels used in experimental studies (adapted from Sander et al. (2010)). Boundary conditions and schematic specimen shape for the 1:1 (b) and 1:0.5 (c) fibrin gels. Displacements constrained normal and tangential at the edges.
5. Collagen Remodelling

5.5.4. Remodelling of fibrin gels

Cruciform fibrin gels as used in Sander et al. (2010) were modelled with the parameter set from the collagen gel simulations for simplicity. One of the fibrin cruciforms was symmetric in that its arms were of equal width (Fig. 5.21b). It will be referred to as the 1:1 gel. The second gel was modified to have vertical arms with half the thickness of the horizontal arms and will hence be called the 1:0.5 gel (Fig. 5.21c). The ends of the arms were constrained not to displace. The anisotropy ratio and principal fibre direction were evaluated at the end of the simulations.

The remodelling transition stretch caused a contraction of the cruciforms both in plane and out of plane due to the continuous fibre distribution leading to volumetric compaction of the gels. In the 1:1 gels a region of isotropy was correctly predicted in the centre of the construct. In the 1:0.5 gels two isotropic regions developed which were located away from the centre of the construct towards the narrower arms with a more aligned fibre architecture developing in between these regions (Figs. 5.22a and 5.22b), which is consistent with the experimental findings (Sander et al., 2010). In the arms of the crosses fibre alignment was predicted along the arms' axes (Figs. 5.23a and 5.23b) as determined experimentally by polarimetric fiber alignment imaging (Sander et al., 2010), Fig. 5.23c.

Figure 5.22.: Anisotropy ratio in the 1:1 (a) and 1:0.5 (b) gels. The white asterix marks the centre of the isotropic regions.
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Figure 5.23.: Principal fibre direction in the 1:1 (a) and 1:0.5 (b) gels (a,b) and anisotropy as determined from polarimetric fibre alignment imaging (adapted from Sander et al. (2010)). The regions of isotropy coincide with those predicted by the model (Figs. 5.22a, 5.22b).

5.6. Discussion

In this chapter a phenomenological model was established that combines an evolving natural configuration of the collagen network with its angular reorientation. The natural configuration is modelled as an intermediate configuration and locally defines the onset of the anisotropic stress contribution. First, the predictions of the model were illustrated with regard to uniaxial stress and strain based remod-
5. Collagen Remodelling

elling under load and displacement control to facilitate a basic understanding of the modelled mechanisms. The model was then applied to the transition stretch adaptation of periosteum (Foolen et al., 2010) using a transversely isotropic material law. Finally, cell seeded hydrogel compaction was modelled combining the evolving natural configuration with a fibre reorientation method for a continuous angular fibre distribution. The model is phenomenological and as such does not mechanistically make a distinction between cell-mediated and non cell-mediated remodelling, which, depending on the purpose of the study, can be both an advantage and a disadvantage.

In both stretch and stress controlled remodelling displacement controlled loading was predicted to create a very stable environment and hence stimulus. In contrast, during force controlled loading a more pronounced feedback loop exists between the deforming material and fibre remodelling. Stiffer fibres stress shield the base matrix and exacerbate this feedback loop which leads to extended time scales of remodelling. This has implications when choosing a remodelling algorithm for a specific material and/or experimental scenario as well as for fitting the time constants for remodelling.

The simulations of the periosteum held at fixed lengths captured the experimentally observed transient adaptation of the transition stretch over a few days (Foolen et al., 2010). Statistical distributions of fibre quantities can be incorporated into the constitutive model and were shown to capture such effects as the experimentally observed toe region in the force-stretch curves. However, no dependence of the amount of ECM on the mechanical environment was modelled but the changing orientation and natural configuration of the collagen network. More comprehensive evolution equations incorporating the dependence of absolute matrix synthesis are of course conceivable (Taber, 1995; Menzel, 2007).

For the collagen gel simulations, the collagen network was assumed to remodel to reach a homeostatic tensile state of 1% strain. In order to generate this level of tensile strain, the collagen network contracts, which can be either cell mediated or not. If the boundary conditions acting on the gel (e.g. supports) prevent contraction, a state of tension quickly develops in the collagen network. If the gel is unconstrained, the gel contracts until the internal compressive stresses within the compacted base matrix balance the tensile stresses in the collagen network. Due to the soft isotropic ground substance compared to the fibres the small value for the homeostatic stretch lead to quite large compaction strains on the order of 20%. Interpreted in the context of a cell-mediated mechanism this means that
cells contract the scaffold by 20% before they have established the desired state of tension, which is here designated in terms of the homeostatic stretch. At 20% compaction the scaffold provides enough resistance to further compaction in order to maintain this level of stretch. When choosing stretch driven remodelling over the stress driven approach for a phenomenological model this effect has to be considered for materials that are highly tension-compression nonlinear. Thomopoulos et al. (2007) used affine and nonaffine kinematics as well as continuum and network modelling approaches to show that fibre alignment alone is not sufficient to explain the mechanical anisotropy in their collagen gels. With the current model it was therefore not attempted to quantify the mechanical anisotropy but a set of material parameters roughly in the range of collagen gel properties was used to capture some structural aspects of the experimental observations. The model predicted the contraction in the unconstrained directions qualitatively and the resulting structural anisotropy of the gels. It further was able to capture the developing hourglass shape in the clamped gel and the resulting heterogeneous fibre architecture. With regard to the fibrin gel study (Sander et al., 2010), the characteristic regions of isotropy observed in the cruciforms were predicted consistently with the experimental findings and a previous small strain modelling study (Ohsumi et al., 2008). Our goal was to show the model behaviour and the possible influences of transition stretch remodelling. For a more quantitatively accurate fit the appropriate constitutive relations and parameter values need to be determined by suitable experiments and inverse modelling. There is data available on the amount of pre-stress in various tissues, e.g. skin (Silver et al., 2003) or arteries (Vaishnav and Vossoughi, 1987), and on a cellular level, cf. Farahani and Kloth (2008); Wrobel et al. (2002). Furthermore, compaction of tissue engineered constructs provides an indirect quantitative measure to extract knowledge on cell contractility if a suitable model is used (Ohsumi et al., 2008). Combining experimental data on different scales with iterative and inverse modelling will be useful for parameter identification, model refinement and experimental design.

For this presentation of the modelling framework we used experimental studies from different sources to provide indirect validation of the modelling assumptions and performance. When focussing on one specific application the model needs refinement. Other phenomena will have to be captured depending on the subject of the study. The free Helmholtz energy potentials can be exchanged against other formulations and the model can be extended to a biphasic framework in a straight forward way (Görke et al., 2010). Besides adapting the constitutive equa-
5. Collagen Remodelling

tions, other biological and mechanical aspects are likely to be necessary. Load
dependent ECM synthesis rates, both of collagenous and non-collagenous proteins,
are one example, especially when long term cell and tissue culture is considered
(Sander et al., 2010; Grassl et al., 2003). The main purpose of the presented
model is capturing structural aspects associated with changes in tissue architec­
ture, i.e. collagen fibre alignment, anisotropy and stress-free configuration. We
did not account for changing amounts of collagen. Previous models have included
deformation dependent changes in collagen content (e.g. Driessen et al. (2003b))
or fibre strength (Menzel, 2007). If necessary in future applications, this can be
added via appropriate evolution equations for the material parameters associated
with fibre stiffness (e.g. $C_4$, $\beta$) such that stiffness increases represent more or
thicker fibres in a macroscopic point.

While growth and remodelling theories can – as has been done here – be based
on local evolving intermediate configurations, cf. Taber (1995); Garikipati et al.
(2006) and others, a recent and conceptually different approach has been intro­
duced in Ateshian and Ricken (2010) where material is deposited in multiple gen­
erations each having their own invariant reference configuration. This is a very
appealing ansatz with regard to the growth of biological tissues. Future studies
should explore its relation to theories based on intermediate configurations as used
in the context of various engineering disciplines (Lubarda, 2004) and the differ­
ences in model predictions and / or parameter identification.

In summary, we have investigated differences between stress and strain based re­
modelling of the transition stretch of collagen fibres in uniaxial examples of force
and displacement based loading scenarios. We further applied the theory of an
evolving natural configuration based on a multiplicative decomposition of the de­
formation gradient to different experimental examples to conceptually show the
implications and coupled it with a fibre reorientation methodology. In the follow­
ing chapters, this framework will be used to study observations from in vitro and
in vivo experiments and test mechanoregulation hypotheses related to an evolving
collagen network.
6 Evolution of Structure-Function Relationships During Bioreactor Culture of Tissue Engineered Cartilage

6.1. Introduction

The aim of functional tissue engineering is to create viable substitutes to repair damaged tissues. Many tissue engineering strategies rely on some form of scaffold or hydrogel that is seeded with and infiltrated by cells. The cells then synthesise phenotype-specific extracellular matrix (ECM), ideally generating a mechanically functional tissue. The specific biomolecules synthesised by the cells and incorporated into the ECM make up the tissue composition which in turn dictates the basic biomechanical properties of the tissue. The relationships between tissue composition and mechanical function have been the subject of many studies on articular cartilage (e.g. Williamson et al. (2001); Wilson et al. (2007); Julkunen et al. (2010b)).

Besides the biochemical composition another key determinant of biomechanical performance is the structural arrangement of the various constituents and their interactions. Connective tissues usually have a very distinct collagen architecture: Articular cartilage exhibits a typical zonal variation in its collagen network ranging from parallel to the articular surface in the superficial zone to perpendicular in the deep zone; collagen fibres in the menisci are predominantly oriented circumferentially leading to a transversely isotropic material with a very high circumferential stiffness; other examples of soft tissues with a highly organised collagen structure include tendons, ligaments, periosteum and arteries.
Biological tissues adapt their structure to their mechanical environment (Taber, 1995). A collagen architecture responsive to the mechanical environment has been observed and computational models have been used to study this phenomenon in many tissues, including cardiovascular (Gleason and Humphrey, 2004; Driessen et al., 2005; Kuhl and Holzapfel, 2007), articular cartilage (Grodzinsky et al., 2000; Wilson et al., 2006a; Klisch et al., 2008; van Turnhout et al., 2011), tendon (Giori et al., 1993) and during skeletal tissue regeneration (Cullinane et al., 2002; Nagel and Kelly, 2010b), see also chapter 8. In many of these studies the collagen network has been hypothesised to align with respect to the principal directions of local mechanical regulators. In addition to orientational remodelling the notion of natural configurations has been documented. Since proteins are incorporated into the extracellular matrix at different time points and different deformation states, they have individual stress-free configurations (Humphrey, 1999). Due to ongoing synthesis, degradation and remodelling the tissue’s stress-free configurations evolve. In the context of a collagen fibre, an implication of this is that the recruitment stretch, i.e. the stretch at which the fibre becomes uncrimped and begins to bear load, can evolve. Remodelling of this stress-free state plays a role in scaffold contraction (Thomopoulos et al., 2005) and tissue growth and remodelling (Tomasek et al., 2002; Foolen et al., 2010; van Donkelaar and Wilson, 2012).

Articular cartilage has been in the focus of numerous tissue engineering studies (Temenoff and Mikos, 2000; Hunziker, 2002; Koga et al., 2009). Many of these have shown increases in metabolic or synthetic cell activities due to dynamic loading in addition to enhanced mechanical properties (Mauck et al., 2000; Buschmann et al., 1995; Davisson et al., 2002; Tsuang et al., 2008; Nicodemus and Bryant, 2010). Other studies on chondrocyte seeded hydrogels in bioreactor culture have found increased mechanical properties of mechanically loaded constructs compared to unloaded free swelling controls despite no significant differences in biochemical composition (Mauck et al., 2003a; Lima et al., 2007; Bian et al., 2010; Hoenig et al., 2011). Similar results have been observed for mesenchymal stem cells undergoing chondrogenesis while subjected to dynamic compression (Huang et al., 2010). Enhanced structural organisation has been suggested as one possible explanation for these latter results in the experimental literature (Mauck et al., 2003b; Kelly et al., 2006; Lima et al., 2007; Hoenig et al., 2011). However, this has not been directly tested experimentally and its influence – both in nature and in magnitude – on the mechanical properties remains largely unknown. Dynamic loading causes biochemical, biomechanical, compositional and nutritional alterations that are all

Potential contributors to the observed changes. Unravelling the relative contribution of the involved mechanisms is complex and challenging. Computational models offer the advantage of allowing for systematic investigation of individual mechanisms without altering other aspects of the system, which is often not feasible experimentally. The hypothesis under investigation in this study is that changes in the local collagen orientation and/or stress-free configuration in response to loading can lead to enhanced bulk mechanical properties of tissue engineered cartilaginous constructs due to mechanical loading in the absence of alterations to the biochemical composition, i.e. material parameters. To test this hypothesis, model predictions of changing construct geometry and mechanical properties due to structural changes in the collagen network in response to dynamic compression will be compared to the results of bioreactor studies where chondrocyte seeded agarose constructs are subjected to dynamic compression (Lima et al., 2007; Bian et al., 2010). Structural changes to the engineered tissues were studied using a previously developed remodelling framework (chapter 5 in this thesis and Nagel and Kelly (2012c)). This and similar frameworks have been successful in predicting changes in collagen fibre orientation and stress-free configuration in a large number of biological tissues and cell seeded hydrogel systems which suggests that the underlying principles are of general significance in load-bearing tissues.

6.2. Materials & methods

Bioreactor culture typically lasts several weeks for cartilage tissue engineering. During this time mechanical loading may be applied for several hours a day. A common loading frequency for dynamic compression is 1 Hz. Assuming 3 hours of loading per day this accumulates to more than 60,000 load cycles. Simulating one loading cycle alone requires many increments and iterations due to the nonlinear nature of the equations that govern material behaviour. Finally, adding the free-swelling time to the simulation process during which modelling and remodelling processes continue would lead to a size of the simulation that exceeds practicality. Therefore, in order to simulate several weeks of bioreactor culture (Bian et al., 2010; Lima et al., 2007) a set of approximations will be developed in the following sections based on the equilibration of cyclically loaded biphasic materials as well as the distinct time scales of loading and biological remodelling activities.

6.2.1. Equilibration of cyclically loaded biphasic tissues

The "gel diffusion time", the approximate time it takes for a cylindrical biphasic material in unconfined compression to reach equilibrium, can be calculated from the aggregate modulus $H_A$, the sample radius $r_0$ and its hydraulic permeability $k$ as (Armstrong et al., 1984):

$$\tau_g = \frac{r_0^2}{H_A k}$$  \hspace{1cm} (6.1)

For articular cartilage with the approximate material parameters $H_A = 1 \text{ MPa}$, $k = 1.0 \cdot 10^{-15} \text{ m}^4 \text{N}^{-1} \text{s}^{-1}$ and the sample radius relevant for current tissue engineering studies $r_0 = 2 \text{ mm}$ we find a characteristic time constant of $\tau_g \approx 67 \text{ min}$. For agarose with the same geometrical dimensions and the material parameters $H_A = 1.5 \text{ kPa}$, $k = 6.61 \cdot 10^{-14} \text{ m}^4 \text{N}^{-1} \text{s}^{-1}$ (Gu et al., 2003) we find $\tau_g \approx 67 \text{ min}$ as well. These rough estimates show a comparable order of magnitude for $\tau_g$ of the two materials.

![Ramp-and-hold behaviour compared to cyclic loading](image)

Figure 6.1.: Ramp-and-hold behaviour compared to cyclic loading. (a) Applied axial displacement relative to construct height over time normalised by the gel diffusion time; (b) resulting lateral displacement relative to sample radius over time normalised by the gel diffusion time.

During cyclic (harmonic) loading an initial transient phase in the dynamic response of a biphasic tissue is followed by a steady-state (Suh et al., 1995). Consider a displacement controlled harmonic load $u_z(t) = u_m + u_a \sin \omega t$ applied to a biphasic tissue where $u_m$ is the offset compression (mean axial deformation) and $u_a$ the amplitude. The resulting lateral displacement $u_r$ and reaction force $F$ will be cyclical curves themselves with a mean corresponding to the value of $u_r$ or $F$ that would follow from step and hold test with $u_z(t) = u_m$. This has been confirmed by simulations with an exemplary parameter set, see Fig. 6.1.
6.2.2. Incompressible approximation during instantaneous loading of biphasic tissues

At equilibrium all hydraulic fluid pressurisation has decayed in a free draining porous medium ($p = 0$) so that the stress response equals that of a single phasic material with the constitutive relation of the solid matrix extended by the contribution of the osmotic pressure $\Delta \pi$. On the other hand, upon sudden loading the pore liquid is unable to escape the compressed matrix except for a thin boundary layer at free draining surfaces. The bulk material therefore behaves like a single phasic incompressible material (Ateshian et al., 2007).

![Figure 6.2: (a) Volume ratio $J$ over applied compressive strain. (b) Reaction force over applied compressive strain. Lines represent results of biphasic models, the markers designate the single phasic approximation. Note, that the apparent strains for agarose and cartilage are different, as cartilage exhibits swelling and the compression in the bioreactor is applied with respect to day 0 (agarose) dimensions.](image)

In this study, the biphasic material was approximated by its two limiting single phasic solutions. The free swelling and the offset steps were modelled as equilibrium loading. For the final dynamic load step, the parameter $D_2$ had to be modified to yield a nearly incompressible material (using $\nu = 0.495$). To maintain the current sample volume ratio $\tilde{J}$ at the point of transition $\tilde{t}$ an additional isotropic pressure term $-\tilde{p}I$ was added to the Cauchy stress tensor during the dynamic load step. With the stress relation

$$\sigma = \frac{2}{J} \frac{\partial \psi}{\partial I_1} b + 2J \frac{\partial \psi}{\partial I_3} I \quad (6.2)$$

the condition

$$\left( \frac{\partial \psi}{\partial I_3} \right)_{I_3 = \tilde{J}^2} + k = \left( \frac{\partial \psi}{\partial I_3} \right)_{I_3 = \tilde{J}^2} \quad (6.3)$$
can be derived, where (compare appendix A.4)

\[
\left( \frac{\partial \psi}{\partial I_3} \right)^{c,i} = 2D_2^{c,i} \ln I_3 - C_1 \quad \text{and} \quad D_2^{c,i} = \frac{C_1 \nu^{c,i}}{2(1 - 2\nu^{c,i})} \tag{6.4}
\]

Thus, the pressure term \( \tilde{p} \) can be found as

\[
\tilde{p} = 2 \left[ \left( \frac{\partial \psi}{\partial I_3} \right)^c - \left( \frac{\partial \psi}{\partial I_3} \right)^i \right] \frac{J}{J} = \frac{4(D_2^{SL} - D_2^{DL}) \ln \tilde{J}^2}{J} \tag{6.5}
\]

where \( D_2^{SL} \) is the parameter value during the quasistatic load steps \((D_2^c)\) and \( D_2^{DL} \) the value during dynamic loading \((D_2^i)\). All other material parameters were kept constant.

To test the accuracy of this approach we compared the volume ratio \( J \) and the contact reaction force on the loading platen in a full biphasic model of unconfined compression to the results of the single phasic approach. To cover the range of material properties of interest in this study we modelled an agarose sample and an isotropic cartilage sample \((C_1 = 0.11 \text{ MPa}, \text{ other material properties as in table 6.2, no fibres})\). The loading platen displacement was defined with respect to the agarose construct height (i.e. day 0) to yield 10\% mean compression (equilibrium properties) followed by another 5\% compression at a rate corresponding to cyclic loading at 1 Hz (transient properties).

Due to swelling of the cartilaginous specimen the loading protocol induced over 20\% strain compared to 10\% in the agarose (Fig. 6.2) paralleling experimental observations (Lima et al., 2007). The boundary layer where the material does not behave quasi-incompressibly was < 100 \( \mu \text{m} \) of the sample radius for agarose and < 60 \( \mu \text{m} \) of the sample radius for cartilage. Good quantitative agreement was found between both approaches. The volume ratio decreases during the equilibrium load step and stays nearly constant during the dynamic load step (Fig. 6.2a). The reaction force in the biphasic models increases more steeply with applied strain in the dynamic loading regimen as compared to the equilibrium load step due to fluid pressurisation (Fig. 6.2b). We conclude that under the present loading conditions the approximation of the biphasic medium as a compressible single phasic medium during the tare strain load step and an incompressible single phasic medium during the dynamic load step is sufficiently accurate.

6.2.3. The stimulus configuration concept – biological inertia

Consider uniaxial remodelling of the recruitment stretch $\lambda_r$ towards a desired recruitment stretch $\lambda_0$ with the linear rate equation

$$\dot{\lambda}_r = \frac{1}{\tau_r} (\lambda_0 - \lambda_r)$$

and $\lambda_r(t = 0) = 1$. The specimen is loaded with a stretch $\lambda_a$ in form of a square wave alternating every second between $\lambda_l$ and $\lambda_c$, so that the mean deformation over time is equal to $\lambda_m = 0.5(\lambda_l + \lambda_c)$ (Fig. 6.3). With the characteristic time scale of loading $\tau_l$ we compare the transient evolution of the recruitment stretch for $\tau_r = 0.1\tau_l$, $\tau_r = \tau_l$, $\tau_r = 10\tau_l$ and $\tau_r = 100\tau_l$ for exemplary values of $\lambda_l = 1.3$, $\lambda_c = 0.4$ and hence $\lambda_m = 0.85$. Remodelling is driven either by the applied stretch ($\lambda_0 = \lambda_a$) or by the mean stretch ($\lambda_0 = \lambda_m$).

Calculations show that for $\tau_r \gg \tau_l$ remodelling driven by the current configuration can be approximated very well by remodelling with respect to the mean deformation rather than the actual current configuration (Figs. 6.4a to 6.4d). This approach allows for easier modelling and coarser time steps.

6.2.4. Material model & remodelling

A large strain biphasic material model with osmotic swelling effects was used (chapter 3). The total stress $\sigma$ in the biphasic medium is given as:

$$\sigma = -(p + \Delta \pi)I + \sigma_E$$

Here, $p$ is the hydraulic pore pressure, $\sigma_E$ the solid extra Cauchy stress and $\Delta \pi$ the Donnan osmotic pressure inside the tissue. The solid extra stresses were derived

Figure 6.4.: Remodelling recruitment stretch for increasing remodelling time constants. Red curve with crosses: Remodelling with respect to current stretch \( \lambda_0 = \lambda_r \); blue curve with crosses: Remodelling with respect to mean stretch \( \lambda_0 = \lambda_m \); green curve: mean stretch \( \lambda_m \).

from free Helmholtz energy density functions that were split into isotropic and anisotropic parts. For the isotropic part we used a Neo-Hookean formulation.

To describe structural remodelling of the collagen network an evolving recruitment configuration was introduced via a multiplicative decomposition of the deformation gradient

\[
F = F_e F_r
\]  

(6.8)

The part of the deformation denoted by \( F_r \) occurred stress-free in the collagen network while the elastic deformation \( F_e \) contributed to the stress response. With the definition of elastic right Cauchy-Green tensor \( \tilde{C}_e = F_e^T F_e \) the energy potential for the anisotropic tissue response was modelled using a continuous angular
fibre distribution following a formulation used in Ateshian et al. (2009)

\[ \psi_{\text{aniso}} = C_4(a_0) \left[ I_{4e} - 1 \right]^{\beta(a_0)} \]  

(6.9)

with \( I_{4e} \geq 1 \) and \( \beta(a_0) \geq 2 \)

with the anisotropic material parameters \( C_4(a_0) \) and \( \beta(a_0) \) defining the (strain dependent) stiffness in a fibre direction \( a_0 \). The modified invariant \( I_{4e} = \text{tr} (\tilde{M} \tilde{C}_e) = \lambda_j^2 \) ensured that collagen fibres only contributed stresses once the fibre stretch reached a certain transition value. Here, \( \tilde{M} \) is the unit structure tensor of a family of fibres in the recruitment configuration. More details can be found in chapter 5.

Collagen network anisotropy was described using the anisotropy tensor \( \Xi \) (Eq. 3.85). The material parameter \( C_4 \) in a fibre direction was derived via an ellipsoid representation that could be scaled with a parameter \( m \) to allow for adjustment of the degree of anisotropy (increasing \( m \) leads to a higher degree of ellipticity in the material parameter distribution than given by \( \Xi \)), see Eq. 5.34. The scaling defined in Eq. 5.34 was performed to ensure a “constant” amount of collagen irrespective of the degree of anisotropy.

The collagen network was assumed to remodel its orientation with respect to the local deformation such that the fibre network is reinforced in stretched directions and weakened in compressed directions. The formulation developed in section 5.3.2 was used. The stress-free configuration of the collagen network was modelled to evolve based on the assumption that collagen fibres remodel towards a homeostatic stretch value \( \lambda_h \) at which they reside in the matrix. Once loading disturbs the homeostatic state of the network and fibres are no longer stretched at their homeostatic value \( \lambda_h \) the recruitment stretch has to change in order to restore tensional network homeostasis. The implementation of this remodelling scheme described in section 5.4 was used.

### 6.2.5. ECM synthesis

The accumulation of extracellular matrix (ECM) components such as proteoglycans (PG) and collagen (COL) in cell seeded hydrogels leads to increases in their mechanical properties which changes the stimuli imposed by the bioreactor. Since only the basic phenomenological aspects of biomolecule deposition were of interest, a bilinear model of constituent synthesis was assumed: Initially, a constituent is secreted at a constant rate. Once it reaches a designated final concentration \( \tilde{m}_\alpha \)
that level is maintained constant:

\[
\dot{m}_\alpha = \text{const.}, \quad 0 \leq m_\alpha < \bar{m}_\alpha
\]  \hspace{1cm} (6.10)

\[
\dot{m}_e = 0, \quad m = \bar{m}_\alpha
\]  \hspace{1cm} (6.11)

where \( m_\alpha \) is the current mass of constituent \( \alpha \), i.e. PG or COL. Final amounts of PG and COL were chosen to be 8\% w/w and 16\% w/w, respectively. We assumed that PG production levels off after 42 days and that collagen production is 6 times slower. This is in line with experimental observations showing that after 42 days in culture COL content reaches only a fraction of native values whereas PG builds up more quickly (Lima et al., 2007; Bian et al., 2010). In accordance with the study objective and experimental observations (Mauck et al., 2003a; Lima et al., 2007; Bian et al., 2010), synthesis rates were modelled independent of mechanical stimuli. Therefore, differences in the predicted mechanical properties of dynamically compressed and free swelling constructs in these simulations are purely due to changes in collagen orientation and configuration due to loading.

It was assumed that the material properties of the isotropic ground phase \( C_1, D_2 \) remained at agarose values. The fixed charge density \( c_{f0} \) was directly related to the current proteoglycan content. The anisotropic material parameter \( C_4 \) was related to collagen content. This is in general agreement with studies that relate tissue composition to mechanical properties (Williamson et al., 2001; Korhonen et al., 2003; Ficklin et al., 2007; Wilson et al., 2007; Julkunen et al., 2010b). For simplicity, we assumed a simple linear connection between the material parameters and the constituent levels:

\[
c_{f0} = c_{f0}(\text{Cart}) \frac{m_{PG}}{\bar{m}_{PG}}
\]  \hspace{1cm} (6.12)

\[
\bar{C}_4 = \bar{C}_4(\text{Cart}) \frac{m_{COL}}{\bar{m}_{COL}}
\]  \hspace{1cm} (6.13)

6.2.6. Boundary conditions

In this study boundary conditions were modelled according to the bioreactor culture protocols used in Lima et al. (2007) and Bian et al. (2010). Cylindrical cell seeded constructs were either left free swelling for the entire culture period (FS group) or loaded for 3 hours a day in cyclic unconfined compression and left to swell freely during the remaining 21 hours (DL group).

For remodelling studies the current tissue deformation is of interest. Free swelling

could be modelled as an equilibrium load step \((p = 0)\). However, it is impractical due to computational limitations to model 3 hours of cyclic loading at 1 Hz (10800 cycles) of a highly nonlinear material. Therefore two simplifying model assumptions were compared:

1. Based on the behaviour of cyclically loaded biphasic materials relaxing to a mean deformation state (see section 6.2.1) and the assumption that characteristic time scales at which remodelling occurs are long compared to 1 s ("biological inertia", see section 6.2.3) cyclic loading was modelled as an equilibrium load step to the mean level of compression (10%).

2. Since remodelling could alternatively be directed towards the maximum deformation during a cycle the equilibrium step was followed by a quasi-incompressible compression by the dynamic amplitude (additional 5%). Incompressibility could be assumed due to the high loading rate (see section 6.2.2).

6.2.7. Evaluated quantities

Construct properties were evaluated in terms of the nominal equilibrium modulus \(E_{\text{nom}}\) and the apparent equilibrium Poisson's ratio \(\nu_{\text{app}}\). Additionally, the FS geometry was evaluated in terms of volume and aspect ratio (defined as the ratio of lateral to axial strain in the FS state with respect to the reference geometry prior to swelling). The direction dependent recruitment stretch \(\lambda_r\) and collagen fibre reinforcement \(\xi\) were plotted as well.

6.2.8. Performed simulations

The performed simulations are listed in table 6.1. Both free swelling (FS) and dynamically loaded (DL) experiments were simulated. Unless otherwise stated in table 6.1, loading was applied for 3 hours per day. Remodelling either took place with respect to the mean deformation (DL) or the maximum deformation (DL max) during compression.

In "configuration only" simulations, only the recruitment stretch was remodelled and an isotropic fibre stiffness assumed \((\Xi = I)\). The value of the homeostatic strain \(\epsilon_h\) was subjected to a parameter variation.

In "orientation only" simulations, the recruitment stretch was kept unchanged \((F_r = I)\) while the fibre stiffness representing orientational effects was allowed to

115
remodel. Since varying \( m \) does not produce additional qualitative insight, only one representative simulation was performed.

The "combination" simulations finally combined both effects. This group was used to study the effect of extending the duration of dynamic compression to 6 and 9 hours per day with the \( m = 50 \) & \( \epsilon_h = 2\% \) parameter set. To study the relative effects of \( m \) and \( \epsilon_h \), \( m \) was decreased to 10 in a second set of simulations. The parameter values used for all simulations are listed in table 6.2.

Unless otherwise stated, predicted values at day 56 are provided in the results section. Since both loading and geometry were axisymmetric, the material homogeneous and the model deterministic, constructs remained homogeneous throughout culture. Hence, presented results are representative of any point in the construct geometry.

### 6.3. Results

#### 6.3.1. Fibre distribution

Remodelling of the fibre orientation was predicted to lead to anisotropy in loaded samples whereas free swelling samples were predicted to remain isotropic (Fig. 6.5). Simulations where only fibre reorientation was considered (Fig. 6.5a) showed...

Table 6.2.: Material parameters.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>agarose</th>
<th>cartilage</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_1$ [MPa]</td>
<td>$4.34 \cdot 10^{-3}$</td>
<td>$4.34 \cdot 10^{-3}$</td>
</tr>
<tr>
<td>$\nu$ [-]</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>$\phi_{F_0}$ [-]</td>
<td>0.98</td>
<td>0.8</td>
</tr>
<tr>
<td>$\tilde{C}_4$ [MPa]</td>
<td>0.0</td>
<td>2.0</td>
</tr>
<tr>
<td>$\beta$ [-]</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>$c_{F_0}$ [mEq mm$^{-3}$]</td>
<td>0.0</td>
<td>0.0002</td>
</tr>
<tr>
<td>$k$ [m$^4$ (N s)$^{-1}$]</td>
<td>$6.61 \cdot 10^{-13}$</td>
<td>$7.5 \cdot 10^{-15}$</td>
</tr>
<tr>
<td>$c_{ext}$ [mmol mm$^{-3}$]</td>
<td>0.00015</td>
<td>0.00015</td>
</tr>
<tr>
<td>$T$ [K]</td>
<td>298</td>
<td>298</td>
</tr>
<tr>
<td>$\gamma$ [d]</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

a slightly more anisotropic tissue when remodelling towards the maximum deformation during dynamic loading (DL max) than when remodelling to the mean configuration (DL). Maximum stiffness values were observed in the radial (and circumferential) direction (0° to horizontal) due to fibre reorientation in this direction, whereas the lowest fibre stiffness was predicted in the axial direction (90° to horizontal).

This trend was maintained in the simulation where realignment was combined with reconfiguration (Figs. 6.5b and 6.5c). Increasing the time of dynamic loading from 3 to 6 and 9 hours further increased the anisotropy of the tissue. When the scaling parameter $m$ was decreased from 50 to 10, the collagen architecture itself was predicted to be more anisotropic (Fig. 6.5c).

6.3.2. Recruitment stretch distribution

The recruitment stretch distribution remained isotropic in the free swelling samples with anisotropy developing in the loaded samples (Figs. 6.6a to 6.6e). The transition stretch with respect to the reference configuration was predicted to increase in the horizontal (radial and circumferential) direction in loaded samples, while it was predicted to decrease in the axial direction. The anisotropy was slightly more pronounced when remodelling was driven by the maximum compressed deformation. The parameter variation of $\epsilon_h$ yielded lower recruitment stretch values for higher values of $\epsilon_h$ as well as a slightly decreasing degree of anisotropy (Figs. 6.6a to 6.6e).

Figure 6.5.: Direction dependent relative structural anisotropy $\xi(\alpha)/\xi_m$ with $\xi_m = (\xi_1 + \xi_2 + \xi_3)/3$ at day 56. $\alpha$ is the angle to the horizontal (radial) direction. (a) reorientation only simulation; (b,c) simulation with combined effects. A value greater than one indicates higher than average fibre reinforcement while a value smaller than one indicates a lower than average fibre reinforcement.

6.6a to 6.6c). In the combined simulations (where both reorientation and reconfiguration occurred), the recruitment stretch distribution was less anisotropic with generally lower values for longer compression times (Fig. 6.6d).

6.3.3. Sample Geometry

A higher recruitment stretch in the horizontal direction will lead to preferred swelling into that direction, since fibres start to inhibit deformation at a later stage. A higher fibre stiffness in the horizontal direction however will lead to more swelling into the vertical direction. Hence, in the reconfiguration only simulations, samples with an aspect ratio of $\epsilon_r^0/\epsilon_z^0 \geq 1$, i.e. lower and wider samples, were predicted (Figs. 6.7a to 6.7c). In the reorientation only simulations however, the opposite trend, i.e. a higher and more slender sample was predicted (Fig. 4d).

![Graphs showing direction dependent recruitment stretch distribution](image)

(a) $\epsilon_h = 1.5\%$

(b) $\epsilon_h = 2.0\%$

(c) $\epsilon_h = 3.0\%$

(d) $m = 50.0 \& \epsilon_h = 2.0\%$

(e) $m = 10.0 \& \epsilon_h = 2.0\%$

Figure 6.6.: Direction dependent recruitment stretch distribution $\lambda_r(\alpha)$ at day 56. $\alpha$ is the angle to the horizontal (radial) direction.

For all simulation cases, the free swelling samples remained isotropic and hence maintained an aspect ratio of 1 (Figs. 6.7a to 6.7d).

With increasing homeostatic strain $\epsilon_h$ the FS volume of the samples decreased significantly from $\approx 66\, \text{mm}^3$ for $\epsilon_h = 1.5\%$ to $\approx 39\, \text{mm}^3$ for $\epsilon_h = 3.0\%$.

In the combined simulations, fibre reorientation led to a decreasing aspect ratio when the scaling parameter $m$ was high (Fig. 6.7e) but increasing aspect ratios
Figure 6.7.: Sample volumes and aspect ratios in the free swelling state at day 56. The effect of extending the duration of dynamic compression per day has been studied in the $m = 50, \epsilon_h = 2\%$ group (e).

Strain dependent Young's modulus predictions exhibited stress softening effects (Figs. 6.8a to 6.8d). Reconfiguration lead to a stiffness increase in the loaded samples with little difference between remodelling to the maximum or mean de-
formation (Figs. 6.8a to 6.8c). The magnitude of homeostatic network strain $\epsilon_h$ furthermore has a pronounced effect on overall sample stiffness, covering a stiffness range at 10% strain from $\approx 0.4$ to $\approx 1.4$ MPa when increasing $\epsilon_h$ from 1.5 to 3%. This was due to the increased amount of pre-strain in the fibre network as well as the associated sample compaction (compare sample volumes in Figs. 6.8a to 6.8c). Reorientation on the other hand led to less fibres pre-strained in the loading direction and hence initially softer samples when the constructs were loaded (Fig. 6.8d). Combining realignment and reconfiguration caused combined effects. Loaded samples appeared softer than free swelling samples initially but were predicted to have higher stiffnesses with increasing deformation (Figs. 6.8e and 6.8f). This effect was more pronounced when loading for 6 h instead of 3 h. However, increasing the time of loading even further did not translate into increased mechanical properties but decreased construct stiffness again (Fig. 6.8e).

For the combined simulation with $m = 50$ and $\epsilon_h = 2\%$ the transient construct development was evaluated at days 0, 12, 28 and 56 in terms of Young’s moduli of the FS and DL3 samples (Fig. 6.10a). Loaded samples were predicted to be stiffer at all time points. The moduli increased monotonically until day 42. Despite an increasing collagen content after day 42, construct properties at day 56 were lower due to the ongoing remodelling of the collagen network than at day 42 (Fig. 6.10a).

### 6.3.5. Poisson’s ratios

Poisson’s ratios were only marginally influenced by dynamic loading in the reconfiguration only simulations (Figs. 6.9a to 6.9c). Fibre realignment, however, led to significant decreases in the Poisson’s ratios due to dynamic loading (Fig. 6.9d). The orientational effect was even more pronounced in the combined simulations with an increasing time of loading leading to decreasing Poisson’s ratios and less pronounced nonlinear behaviour (Fig. 6.9e). For a lower value of $m$ the decrease in Poisson’s ratio was less pronounced but generally similar to the $m = 50$ simulation (Fig. 6.9f).

Figure 6.9.: Poisson's ratios at day 56. The effect of extending the duration of dynamic compression per day has been studied in the $m = 50$, $\epsilon_h = 2\%$ group (c).

6.4. Discussion

In this study we used a computational model based on previous work (Nagel and Kelly, 2012c) to investigate changes in the mechanical properties of cell seeded agarose constructs in bioreactor culture from a structural perspective. To the

Figure 6.10.: Transient construct development for 0 (FS) and 3 hours (DL) of loading at various time points in culture for the $m = 50$, $e_h = 2\%$ simulations. Young’s moduli at 10% strain (bars), collagen and GAG content (lines) (a); free swelling volumes (bars) and sample thicknesses (lines) (b).

best of our knowledge, this study represents the first theoretical investigation of mechanically induced changes in the orientation and stress-free configuration of the collagen network in a swelling hydrogel during bioreactor culture, and furthermore demonstrates for the first time how alterations to this network can lead to improvements in the mechanical functionality of engineered cartilage tissue. We hypothesised that remodelling of the collagen architecture of tissue engineered constructs in response to dynamic compression can lead to enhanced bulk mechanical properties in the absence of alterations to the biochemical composition, as reported experimentally (Mauck et al., 2003a; Lima et al., 2007; Bian et al., 2010; Huang et al., 2010; Hoenig et al., 2011). Traditionally, collagen fibre orientation is the main architectural feature considered. Fibre reorientation in response to loading, however, caused decreases in bulk construct compressive stiffness due to the charged nature of the material (Nagel and Kelly (2010a), chapter 4). The present model additionally considered the local natural configuration of the collagen network. The model predicted that collagen network reconfiguration leads to increased equilibrium moduli while reorientation leads to lowered Poisson’s ratios. The model further predicted that the FS geometry of loaded and unloaded samples differs depending on the dominant remodelling mechanism involved. Only when both conformational and orientational changes were considered, could the trends in Young’s modulus, Poisson’s ratio and sample geometry be predicted simultaneously.

Changes to the collagen’s natural configuration were predicted to impact tissue properties by altering the volumetric compaction of the developing tissue and the

state of pre-strain in the collagen network. During loading the constructs occupy less volume than in their FS state due to the exudation of fluid. Collagen fibres are partially laid down and/or remodelled in this configuration. Therefore, for a given homeostatic strain value, the DL samples are compacted to a smaller volume in their unloaded states and hence appeared stiffer due to higher swelling pressures (Nagel and Kelly, 2010a). A lower volume in DL constructs (52.9 mm$^3$) compared to FS samples (53.1 mm$^3$) at day 42 has been observed experimentally (Kelly et al., 2006). In addition to altering the volumetric compaction, remodelling the natural configuration also caused changes in the stress softening phenomena associated with the tension-compression nonlinearity (Chahine et al., 2004; Ateshian et al., 2009; Nagel and Kelly, 2010a), with higher collagen network pre-strains predicted to increase the apparent mechanical properties of the construct at equilibrium. Additionally, altering the stress-free state of the collagen network due to loading was predicted to lead to increased aspect ratios, i.e. increased sample radii and decreased heights. Corresponding results have also been reported experimentally (Kelly et al., 2006) where flattening and widening of the samples were observed with a height to radius ratio of 1.02 and 0.91 for FS and DL samples, respectively. Dynamically loaded samples that were up to 20% thinner in the loading direction than free swelling controls has also been reported (Mauck et al., 2003a).

That collagen in the cartilage ECM is under pre-stretch with respect to the free swelling configuration can be demonstrated by digesting the collagen in a cartilage plug and observing its subsequent re-swelling (e.g. Maroudas (1976); Bank et al. (2000)). A pilot study investigating the influence a modulation of the swelling properties during culture has on the apparent properties of tissue engineered cartilage can be found in appendix B.7. The actual value of $\epsilon_h$ is speculative and the natural configurations can only be approximated using inverse simulations. The parameter variation of $\epsilon_h$ showed that increasing its value will have a significant effect on construct stiffness.

Experimental evidence is also available for reorientation of the collagen network within engineered cartilaginous constructs in response to extrinsic mechanical signals. For example, no preferred collagen angle has been found using polarised light microscopy in free swelling tissue engineered cartilage while a maximum intensity perpendicular to the loading axis indicates some horizontal fibre alignment in dynamically compressed samples (Kelly et al., 2006). The model also predicted an isotropic fibre architecture in free swelling samples while the maximum value of the anisotropy tensor $\Xi$ was predicted in the horizontal direction within the loaded
samples. The apparent Poisson’s ratio of engineered cartilaginous constructs has been shown to be lower for loaded (≈ 0.17) than for free swelling (≈ 0.23) samples (Kelly et al., 2006). Reorientation of the collagen network was predicted to result in similar changes to the Poisson’s ratio of dynamically compressed constructs. Horizontal fibres are more efficient at resisting lateral expansion, which explains the decreases in the Poisson’s ratio of the loaded samples. In contrast, the experimentally observed increases in the equilibrium moduli could not be explained by fibre reorientation. Fibre reorientation alone was predicted to lead to higher constructs with a smaller diameter, not consistent with experimental observations (Mauck et al., 2003a; Kelly et al., 2006). Therefore while fibre reorientation can potentially explain certain experimentally observed phenomena during bioreactor culture such as decreasing Poisson’s ratio, it alone cannot explain the effect of dynamic compression on the structural development of engineered cartilaginous constructs.

When combining collagen network realignment and recruitment stretch reconfiguration, a combination of the individual results was predicted. While lower construct properties in the loaded samples were predicted in the initial small strain range, loading led to increased equilibrium properties at higher strains (≈ 5% strain and higher). A lower Poisson’s ratio was also predicted. Thus, combining reorientation and reconfiguration allowed the simultaneous prediction of increased Young’s moduli and decreased Poisson’s ratios as well as geometrical changes. The interaction of the various constituents was predicted to lead to initial decreases in construct thickness and volume during the initial 14 days of culture followed by an increase thereafter. Initial decreases in sample thickness with subsequent thickening have also been reported experimentally (Mauck et al., 2003a). Our model also predicted a slight increase in equilibrium modulus for 6 h of dynamic compression compared to 3 h (at 10% applied strain). However, for 9 h of loading the modulus was predicted to decrease again to the level reached after 3 h of loading. This should be seen as a qualitative result. The exact duration of dynamic compression that will produce the stiffest constructs will depend on the parameter values chosen, which are yet to be identified, and other biological effects. Experimentally, no increase in the Young’s modulus of engineered constructs has been observed for increasing the daily duration of dynamic compression from 3 to 6 hours (Ng et al., 2009).

In addition to the daily duration of applied dynamic compression, the total duration of culture will also determine construct mechanical properties. For example, a

levelling off of GAG accumulation has been reported for the last two weeks of an 8 week study, while the collagen content continued to increase (Mauck et al., 2003a). Despite that, stiffness values plateaued or even decreased (Mauck et al., 2003a). Our model similarly predicted that after day 42 construct mechanical properties decreased despite increasing collagen content. This was due to ongoing remodelling, namely reconfiguration of the collagen fibres slowly releasing any excess tension in the collagen network previously built up due to increasing swelling pressures. This result emphasises the importance of tissue structure and can partially explain the difficulties in obtaining composition-function relationships. The latter are usually obtained relating bulk biochemical content to biomechanical properties and, as the simulations show, can only be an estimate if structural aspects are neglected.

A number of assumptions had to be made in developing this model. To capture the evolution of the material parameters used in the constitutive model they were related to the main ECM constituents: PG and COL. This evolution was described by a simple bilinear relationship capturing the basic trends observed in bioreactor culture: Faster PG and slower COL production as well as the resulting increase of construct properties over time. If the synthesis (and degradation) of the constituents itself becomes the focus of study, more sophisticated models will be required. The increases in PGs led to increasing construct volumes due to swelling. Because the offset strain associated with the dynamic compression regime is applied with respect to day 0 geometry, this caused the applied tare strain in the model to change over time in culture. In the isotropic case an increase from 10% to over 20% (see Fig. 6.2), paralleling experimental observations (Lima et al., 2007), was predicted.

Both agarose and cartilage are porous media in which the pore liquid contributes significantly to the material behaviour. Flow-dependent viscoelastic behaviours can be captured with biphasic models (Mow et al., 1980). The bioreactor loading regimen used in this study could be conveniently split up into an equilibrium part, where hydraulic fluid pressurisation was negligible, and a short term quasi-instantaneous part, where fluid flow is negligible. This enabled us to use single phasic constitutive models and compare remodelling towards the mean and the maximum deformation. Based on the equilibration behaviour of cyclically loaded biphasic tissues and the assumption that biological remodelling takes place on a time scale significantly larger than 1 s we were able to simulate a complete day of loading rather than merely one representative loading cycle. Due to the small time
step size it would be computationally infeasible to simulate the complete loading protocol of a day in detail for 56 days with a full biphasic model.

Our phenomenological model makes no mechanistic distinction between cell-mediated and non cell-mediated remodelling. Collagen network remodelling has been observed both in the presence and absence of cells (Thomopoulos et al., 2005). In tissues with a lowcellularity, such as articular cartilage, non cell-mediated mechanisms might play an important role. Strain dependent collagen-collagenase interactions have been reported (Huang and Yannas, 1977) such that fibres perpendicular to the direction of tensile loading become resorbed which ultimately causes alignment. As long as the mechanism of remodelling in cartilaginous constructs has not been resolved experimentally, phenomenological simulations can potentially provide insight into the consequences of remodelling.

Using a computational approach, we have been able to provide support for the hypothesis that a mechanoregulated collagen architecture can lead to enhanced bulk mechanical properties of tissue engineered constructs due to mechanical loading with the same biochemical composition as free swelling controls. We further showed that reorientation alone, the traditionally considered architectural feature, is insufficient to capture the experimental observations. This does not invalidate or exclude other hypotheses related to the collagen network that could explain the observed phenomena. Alternative mechanisms likely to be involved include collagen cross-linking and the synthesis of other ECM proteins, such as other collagen types (Kelly et al., 2004, 2006; Yan et al., 2009). Studies on heart valve

tissue engineering Balguid et al. (2007) have observed that dynamic loading did not enhance or even decreased bulk collagen content but did lead to increased cross-linking and mechanical properties of the tissue engineered constructs. Yan et al. (2009) found low levels of collagen IX and mature collagen cross-linking to be a major contributing factor to poor mechanical properties of in vitro engineered cartilage. This study also demonstrated that physical stimulation, via centrifugal forces, enhances the mechanical properties of tissue engineered cartilage, implicating enhanced levels of collagen IX and collagen cross-linking as contributors to improvements in construct functionality. These studies suggest an important role for cross-linking and its promotion via dynamic loading in engineering living tissue substitutes. In phenomenological constitutive models both increases in cross-linking and bulk collagen content could be captured via elevated material parameters. This will clearly lead to increases in the apparent mechanical properties in the simulations but does not elucidate whether such phenomena are responsible for the increased mechanical properties reported. While increased properties due to increases in material parameters is an intuitively obvious result, changes in configurational parameters such as fibre orientation and stress-free configuration might be less transparent especially in charged swelling materials and require appropriate models to investigate their possible contribution to the observed changes. The altered stress-free configuration predicted by our model and parameterised in terms of the macroscopic recruitment stretch for the collagen network can be interpreted in the context of enhanced cross-linking. Forming a cross-link between two lax collagen fibrils will lead to earlier recruitment when the network is stretched (Fig. 6.11). Intramolecular cross-linking might be observable on the macroscopic level as earlier recruitment as well. Thus, rather than being a separate explanation, an increased cross-link density can form one mechanistic explanation of the altered recruitment configuration in these simulations.

Similarly, a number of experimental studies have also shown that dynamic loading can lead to both changes in biochemical composition and mechanical properties (Mauck et al., 2000; Buschmann et al., 1995; Davisson et al., 2002; Tsuang et al., 2008; Nicodemus and Bryant, 2010). Models such as that presented here might in the future be able to help decouple the relative roles played by compositional and structural changes in determining the mechanical properties of engineered tissues. Another possible mechanism is the altered diffusion of ECM proteins within the samples due to DL. Despite equivalence of the bulk biochemical content DL could lead to different distributions of the ECM proteins and hence affect mechanical
properties (Kelly et al., 2004). However, finite element studies (Sengers et al., 2004) on the local distribution of ECM in tissue engineered cartilage concluded that the global aggregate modulus and permeability were largely insensitive to the microscopic matrix distribution.

While biochemical assays can help determine composition-function relationships, tissue organisation is a determinant of biomechanical functionality in its own right. The effect of organisational alterations is difficult to investigate experimentally, since tissue structure is not easily altered and certain structural features aside from orientation such as natural configurations are difficult to quantify. Uncoupling the relative roles of tissue composition, distribution and organisation at various hierarchical levels is a task amenable to simulation methods. In the future this modelling framework will be extended to other cell types, particularly MSCs, combined with tissue differentiation algorithms and applied to the study of in vivo healing scenarios (Nagel and Kelly, 2010b), see chapter 8. For example, a native-like zonal architecture is crucial for successful chondral and osteochondral defect repair (chapter 7). It is for this reason that mechanoregulation algorithms need to include both tissue differentiation and architecture simultaneously in order to understand how environmental factors regulate tissue form and function during skeletal regeneration.
7 The Composition of Engineered Cartilage at the Time of Implantation Determines the Likelihood of Recapitulating a Benninghoff Architecture

7.1. Introduction

The articular cartilage of synovial joints is subjected to a complex and challenging mechanical environment. Both the intricate architecture and biochemical composition of the tissue contribute to it achieving its normal biomechanical function (Mow et al., 1992; Julkunen et al., 2010b; van Turnhout et al., 2011; Nagel and Kelly, 2010a, 2012b). The heterogeneous composition and structure of this avascular tissue are maintained by a sparse population of cells which survives under significant nutrient constraints. Within limits these chondrocytes are capable of adjusting their metabolic and catabolic activities in response to external factors such as the mechanical environment. This allows for structural and compositional alterations and hence an adaptation of the tissue to physiological loads (Mow et al., 1992; Grodzinsky et al., 2000).

The limits within which adaptation is possible are, however, exceeded when the cartilage is injured. The lack of spontaneous repair of chondral defects has been attributed to the lack of proliferating cells and a scaffold into which these cells can migrate (Nehrer et al., 1998). Numerous clinical treatments for chondral defects exist, none of which can be regarded as satisfactory or consistently successful (for a review see Hunziker (2002)). Untreated defects, even focal defects of limited
7. The Composition of Engineered Cartilage at the Time of Implantation Determines the Likelihood of Recapitulating a Benninghoff Architecture

size, often extend to full thickness defects over time and can eventually lead to the degeneration of the surrounding healthy cartilage tissue (Hunziker, 2002; Schinhan et al., 2012).

Numerous tissue engineering strategies, including autologous chondrocyte implantation (Brittberg et al., 1994), chondrocyte transplantation in scaffolds (e.g. MACI, matrix associated autologous chondrocyte implantation) (Nehrer et al., 1998), growth factor guided cell therapies (Hunziker and Rosenberg, 1996; Hunziker, 2001) and the delivery of progenitor cells (Im et al., 2001; Uematsu et al., 2005) are either in clinical practise or under current investigation. Bioreactor culture is often used with the intention of engineering a more functional tissue in vitro (Thorpe et al., 2010; Meyer et al., 2010; Martin et al., 2004). In vitro pre-culture of engineered implants or pre-differentiation of MSCs have generally resulted in promising in vivo outcomes (Steck et al., 2009; Marquass et al., 2011), although recapitulating the spatial organisation of the native tissue has proven difficult and the overall repair tissue quality is inferior to normal articular cartilage (Steck et al., 2009; Gotterbarm et al., 2008; Hunziker, 2001). The debate regarding the optimal mechanical properties of the construct at the time of implantation into a (chondral or osteochondral) defect is therefore ongoing (Miot et al., 2012; Khoshgoftar et al., 2012).

Given that a failure to recapitulate the normal architecture of articular cartilage most likely will lead to the failure of the repair tissue, this study sought to develop a theoretical framework within which the role of the joint mechanical environment on the organisation of repair tissue in cartilage defects could be better understood. It has been shown that composition based constitutive modelling in combination with collagen remodelling algorithms is capable of predicting the Benninghoff architecture in tibial plateau cartilage based on the interplay between swelling pressures and external loading (Wilson et al., 2006a). We further showed that a collagen remodelling algorithm (Nagel and Kelly, 2012c) can explain aspects of altered structure-function relationships in tissue engineered cartilage due to mechanical loading during bioreactor culture (Nagel and Kelly, 2012b). In this chapter, this modelling framework is first employed to investigate the effect of chondral defect size and depth on remodelling of the collagen architecture in the surrounding undamaged cartilage. This model is then used to predict how the repair and undamaged surrounding tissue architecture would adapt over time following the implantation of tissue engineered cartilage of varying biochemical composition. It was hypothesised that a) in untreated defects, the degree of remodelling away
The Composition of Engineered Cartilage at the Time of Implantation Determines the Likelihood of Recapitulating a Benninghoff Architecture

from a normal Benninghoff architecture in the surrounding undamaged cartilage is a function of the defect size and b) successful recapitulation of a Benninghoff architecture in tissue engineered cartilage depends on the construct composition at the time of implantation. In the final part of the study it was speculated that the repair tissue’s ability to adapt to the mechanical environment might decrease with increasing extracellular matrix content and then asked, in such a scenario, whether it is more beneficial to implant an immature tissue that can adapt rapidly to its mechanical environment, or a more functional graft that provides greater initial stability but may remodel at a slower rate.

7.2. Materials & methods

Computational models of collagen remodelling in response to mechanical cues have been used to demonstrate that a Benninghoff architecture develops in intact tibial plateau cartilage in response to external loading and internal swelling pressures (Wilson et al., 2006a). The geometry and the mathematical relationships regarding the depth dependent composition used in that study were adopted here and combined with a model of collagen remodelling in cartilaginous tissues outlined in the previous chapters (Nagel and Kelly, 2012b,c) to establish a numerical ("undamaged" or "intact" tissue) control against which the predictions of the defect simulations were compared (see section 7.2.5).

7.2.1. Depth-dependent composition of articular cartilage

With the introduction of a dimensionless coordinate $\tilde{z} \in [0; 1]$ ($\tilde{z} = 0$ corresponds to the articular surface, $\tilde{z} = 1$ to the cartilage-bone interface), the initial fixed charge density $c_{F0}$, initial porosity / fluid volume fraction $\phi_{F0}$ and the collagen solid mass fraction (collagen per dry weight) $\mu^S_{COL}$ served as compositional input (Wilson et al., 2007) from which the material behaviour was derived (see Fig. 7.1):

$$c_{F0} = -0.1 \tilde{z}^2 + 0.24 \tilde{z} + 0.035 \frac{\text{mEq}}{\text{ml}}$$  \hspace{1cm} (7.1)

$$\phi_{F0} = 0.9 - 0.2 \tilde{z}$$  \hspace{1cm} (7.2)

$$\mu^S_{COL} = \frac{dmC}{dmS} = 1.4 \tilde{z}^2 - 1.1 \tilde{z} + 0.59$$  \hspace{1cm} (7.3)

Under the assumption of equal true mass densities for all solid constituents the
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Figure 7.1.: Depth dependent input for cartilage composition defined in Eqs. 7.1 to 7.3.

The total collagen mass fraction (collagen per wet weight) could be calculated using

$$\mu_{\text{COL}} = (1 - \mu_F)\phi_C^S = \left(1 - \frac{\phi_F}{\phi_F + (1 - \phi_F)\tilde{\rho}}\right)\mu_{\text{COL}}^S \quad (7.4)$$

where $\tilde{\rho} = 1.43$ is the ratio of the true solid (proteoglycan, collagen) to fluid densities. The quantity $\mu_F$ represents the fluid mass fraction and $\phi_C^S$ the collagen solid volume fraction.

### 7.2.2. Material model

Chondrocyte implantation assisted by a scaffold (hydrogel) was assumed. The scaffold was modelled as a Neo-Hookean contribution $T_{\text{NH}}$ to the solid matrix stress and remained unchanged throughout the simulation with parameters derived for agarose as in our previous study (Nagel and Kelly, 2012b). Cartilaginous tissues were modelled as charged biphasic materials such that the total second Piola-Kirchhoff stress $T$ was given as

$$T = -(p + \Delta\pi)JC^{-1} + \kappa T_f + T_{\text{NH}} \quad \text{with} \quad \kappa = \frac{\phi_{C0}(\tilde{z})}{\phi_{C0}(\text{DZ})} \quad (7.5)$$

where $C$ is the right Cauchy-Green tensor and $J = \sqrt{\det C}$. The stress in the collagen network $T_f$ has been scaled using the ratio of the collagen volume fraction $\phi_{C0}(\tilde{z})$ to its deep zone value $\phi_{C0}(\text{DZ})$ for which we supply the material parameters. In other words, the stress in the fibrous part of the mixture has been weighted with the amount of collagen in a particular region in relation to the deep zone. The hydraulic pore pressure $p$ is determined via Darcy’s law and the swelling pressure $\Delta\pi$

$$\Delta\pi = 2RT \left[\sqrt{c_{\text{ext}}^2 + \frac{c_{F,e}^2}{4} - c_{\text{ext}}} \right] \quad (7.6)$$

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is calculated based on the medium salt concentration \( c_{\text{ext}} \), the absolute temperature \( T \), the universal gas constant \( R \) and the fixed charge density \( c_{F,e} \) which relates the number of charges to the extracellular fluid volume and can be derived\(^1\) from the standard fixed charge density (calculated with respect to total fluid content) \( c_F \) as

\[
c_{F,e} = \frac{\phi_F}{\phi_{F,e}} c_F
\]

(7.7)

where the fluid volume fraction \( \phi_F \) and the extracellular fluid volume fraction

\[
\phi_{F,e} = \frac{\tilde{\rho} \mu_{F,e}}{1 - \mu_F + \tilde{\rho} \mu_F}
\]

(7.8)

have been used. The extracellular fluid mass fraction \( \mu_{F,e} \) increases with increasing swelling pressure and decreasing collagen mass fraction (Han et al., 2011). The nonlinear coupled equations are solved numerically in a local iteration loop. For further details on said relationships, see e.g. (Wilson et al., 2007; Han et al., 2011). The stress in the collagen network balancing the swelling pressures was calculated as a tension-only contribution according to

\[
T_f = 2 \int_0^{2\pi} \int_0^\pi H(I_4 - 1) \frac{\partial \tilde{\nu}_{\text{aniso}}}{\partial \vec{C}} \sin \phi \, d\phi \, d\theta
\]

(7.9)

using an ellipsoid fibre distribution model. The strain energy density function was chosen following Ateshian et al. (2009) (Eq. 3.82) with parameters for articular cartilage taken from our previous study (Nagel and Kelly (2012b); chapter 6, table 6.2). The fibre stiffness in a point was direction dependent based on the fibre architecture (orientation and alignment) in that point (Nagel and Kelly (2012c); chapters 3, 5, 6).

The permeability was scaled using

\[
k = k_0 (1 - \phi_{F_0})^M
\]

(7.10)

with \( M \) chosen such that a permeability of \( k = 6.61 \cdot 10^{-13} \text{m}^4\text{N}^{-1}\text{s}^{-1} \) was attained for 2% agarose and \( k = 7.5 \cdot 10^{-15} \text{m}^4\text{N}^{-1}\text{s}^{-1} \) for cartilage with \( \phi_{F_0} = 0.8 \). These constraints were fulfilled for \( k_0 = 3.28 \cdot 10^{-16} \text{m}^4\text{N}^{-1}\text{s}^{-1} \) and \( M = -1.945 \).

\(^1\)The approach to calculating the extracellular water volume fraction adopted here differs from that performed in Wilson et al. (2007), see appendix A.9.
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7.2.3. Fibre remodelling

Angular fibre remodelling was implemented as described in chapter 5. Stress-free configurations of fibres were not remodelled but fixed in the reference configuration. Briefly, the collagen fibre distributions in a point were allowed to gradually change their orientation and degree of alignment depending on the deformation at that location. Generally speaking, fibres were modelled to preferentially align with respect to directions of tensile strain, governed by a time constant $\tau$.

![Figure 7.2. Axisymmetric model of tibial plateau cartilage. The mesh was refined in regions of increased interest (centre of the tibial plateau, where defects will be created).](image)

7.2.4. Geometry and boundary conditions

The axisymmetric geometry of the tibial plateau used in Wilson et al. (2006a) was adopted here, see Fig. 7.2. A compressive load of 800 N was applied to the tibial plateau within 0.5 s (Kelly and Prendergast, 2005; Donahue et al., 2002) via a rigid impermeable platen after the initial swelling deformations had converged and established the pre-stress in the collagen network. Free surfaces were modelled as free draining, others as sealed. All displacements were suppressed at the sub-chondral bone junction. Mixed formulation elements were used with biquadratic interpolation for the displacements and bilinear interpolation for the pore pressure.

At maximum load the deformations were analysed and used to drive remodelling as described in the previous section. The iterational loop was then repeated starting with the free swelling step and based on the new material properties and structure to derive the deformations in the next iteration of the simulation.

7.2.5. Study design

Several simulations were performed to establish the influence of defect size and implanted engineered tissue composition on tissue architecture which was parameterised in terms of the principal fibre direction. Near isotropic regions, i.e. spherical
distributions, were excluded from the evaluation due to ill-defined angles in this case.

1. **Control group:** Defect free simulations were run until no change in fibre architecture was observed. The resulting Benninghoff collagen architecture that is predicted to form in the tissue in response to proteoglycan induced swelling and external joint loading serves as a numerical control and defines the healthy architecture against which all other predictions will be compared. Establishment of a numerical control is necessary as the details of the predicted fibre architecture will depend on the exact loading protocol, geometry and material properties inherent in the model. These assumptions are common to all subsequent models that constitute perturbed versions of the "healthy" numerical control.

2. **Empty defect group:** 3 full thickness and 3 partial thickness (extending 1.2 mm into the tissue) defects were created with diameters of 2, 4 and 6 mm. Defect surfaces were modelled as free draining. Simulations were again run until steady state and the resulting collagen architecture compared to the control group.

3. **Implants with constant biochemical composition:** The 3 full thickness defects were filled with cartilage-like engineered tissue that exhibited no depth dependency in its composition. Depending on extracellular matrix content these constructs were classified as either
   - **immature:** porosity $\phi_{F_0} = 0.98$, FCD $c_{F_0} = 1.4 \cdot 10^{-5} \text{mEq mm}^{-3}$, 1.6\%w/ww collagen content,
   - **medium:** porosity $\phi_{F_0} = 0.85$, FCD $c_{F_0} = 7.1 \cdot 10^{-5} \text{mEq mm}^{-3}$, 7.9\%w/ww collagen content or
   - **mature:** porosity $\phi_{F_0} = 0.77$, FCD $c_{F_0} = 1.41 \cdot 10^{-4} \text{mEq mm}^{-3}$, 15.8\%w/ww collagen content.

   These values for the fixed charge density and collagen content correspond to 100\% (mature), 50\% (medium) and 10\% (immature) of the average deep zone values (bottom 75\% of the tissue thickness) in native articular cartilage. Simulations were run until steady state and the resulting collagen architecture compared to the control group. No further ECM synthesis was assumed to occur.

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2Simply speaking, a sphere does not have a preferred direction. A meaningful angular measure of alignment can therefore not be assigned.
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4. Implants with ongoing matrix synthesis: There is very little data on collagen remodelling in vivo. While collagen in adult articular cartilage is considered quite stable (Mankin and Lippiello, 1969), it has also been suggested that remodelling might be more significant than often assumed and upregulated after injury (Eyre, 2002; Eyre et al., 2006). Hence, a final simulation was considered where the ability of the tissue to remodel was assumed to decrease with increasing matrix accumulation. Constructs specified as mature, medium and immature in the previous section were implanted into the 4 mm full thickness defect. In this simulation it was assumed that cells continued to secrete ECM constituents until the native depth dependent composition was achieved. The rate of collagen remodelling was assumed to decrease with increasing ECM accumulation. The predicted repair tissue architecture was evaluated at 6 months, 1 year, 4 and 10 years post implantation. Synthesis and its effect on remodelling are outlined in the next section. The remodelling time constant was scaled with the solid matrix mass fraction as follows

\[ \tau = \tau_\infty \frac{\mu_s}{0.22} \]  

(7.11)

where an average composition of 6%w/ww proteoglycans and 16%w/ww collagen was assumed for mature cartilage. The parameter \( \tau_\infty \) (the remodelling time when levels of matrix accumulation have reached that of mature tissue) was systematically varied to be 100 days, 1 year, 10 years and 100 years to model increasingly limiting constraints on remodelling.

7.2.6. ECM synthesis and ECM dependent remodelling

Neglecting the influence of cells and minor ECM constituents the fluid (\( \mu_F \)) and solid constituent (proteoglycan (PG): \( \mu_{PG} \), collagen (COL): \( \mu_{COL} \)) mass fractions obey the constraint

\[ \mu_F = 1 - \mu_S = 1 - \mu_{PG} - \mu_{COL} \]  

(7.12)

or in terms of their rates of change

\[ \dot{\mu}_F + \dot{\mu}_{PG} + \dot{\mu}_{COL} = 0 \]  

(7.13)

It is assumed that the solid matrix constituents \( \alpha = \{\text{PG, COL}\} \) are synthesised at constant rates (collagen is assumed to be synthesised six times slower than proteoglycans, see Nagel and Kelly (2012b) or chapter 6) until the depth dependent target concentration \( \tilde{\mu}_\alpha(z) \) of each constituent is achieved. The following iterative
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Updates are then performed to obtain the location specific composition triplet \( \{ \mu_{\text{COL}}^S, c_{F_0}, \phi_{F_0} \} \) for the next iteration \( n + 1 \):

1. Increment solid constituent mass fractions: \( \mu_{\alpha}^{n+1} = \mu_{\alpha}^n + \mu_{\alpha}\Delta t \). If a target value \( \tilde{\mu}_{\alpha}( \tilde{x} ) \) is reached, set \( \mu_{\alpha} = 0 \).

2. Calculate water fraction as \( \mu_F = 1 - \mu_{\text{PG}} - \mu_{\text{COL}} \).

3. Determine solid fraction of collagen \( \mu_{\text{COL}}^S \) from \( \mu_{\text{COL}} \) and \( \mu_F \).

4. Determine porosity \( \phi_{F_0} \) from \( \mu_F \).

5. Determine FCD via linear scaling: \( c_{F_0}^{n+1} = \frac{c_{F_0}( \tilde{x} )}{\mu_{\text{PG}}( \tilde{x} )} \mu_{\text{PG}}^{n+1} \).

---

Figure 7.3.: Fibre architecture in intact tibial plateau. Fibres are assumed to align with principal strain directions in response to swelling and external loading. Directions plotted designate the long axis of the ellipsoid anisotropy representation.
Figure 7.4: Fibre architecture in tibial plateau with defects. Blue corresponds to less than 5° difference compared to intact Benninghoff architecture, green to less than 25°, orange to less than 50° and red to more than 50°. Dashed boxes represent defect areas.
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7.3. Results

7.3.1. Numerical control

The defect free model, or numerical control, predicted a Benninghoff-type collagen architecture (Fig. 7.3). The principal fibre direction, designated by the long axis of the ellipsoidal fibre distribution, was predicted to be aligned vertically in the deep zone and went through a symmetric transition via an isotropic (i.e. more random) state towards a horizontal alignment in the superficial zone. With increasing distance from the center of the tibial plateau, the transition was increasingly asymmetric, i.e. fibres preferentially bend over towards the periphery and did so earlier than in the central regions.

![Figure 7.5: Average angular deviation of predicted steady state architecture from the normal Benninghoff architecture in the tibial plateau with empty defects.](image)

7.3.2. Empty defects

Creating a defect within the articular surface is predicted to alter the mechanical environment within the surrounding tissue, leading to remodelling of this initially undamaged tissue away from a normal Benninghoff-type collagen architecture. Significant deviations between the fibre angle in the normal tissue and the damaged tissue ($\geq 50^\circ$) were predicted far into the intact tibial plateau (Fig. 7.4). The asymmetric fibre transition reversed from a peripheral (outward) to a central (inward) direction in the regions adjacent to the defect. The affected regions within the tissue increased with defect size (Fig. 7.5). For a 6mm defect, only a small peripheral region of the articular surface had angular deviations of less than $5^\circ$. 

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from the Benninghoff architecture. Collagen alignment was predicted to deviate more than 50° from the Benninghoff architecture in a region extending to 4 times the defect radius. Similar trends were predicted in the partial thickness simulations where the surrounding cartilage was only slightly less affected than in the full thickness defect. Remodelling was also observed in the cartilage underlying the partial thickness defect. When averaged over the entire tibial plateau deviations were between 15 and 25° (Fig. 7.5).

**Figure 7.6.:** Average angular deviation of predicted steady state architecture in the SZ, MZ and DZ of defects treated with tissue engineered constructs (a - c) as well as the surrounding native tissue (d). No synthetic activity is assumed. SZ: superficial zone (top 10% of the defect tissue), MZ: middle zone (next 15% of the defect tissue), DZ: deep zone (remaining 75% of the tissue), AC: articular cartilage outside of defect.

### 7.3.3. Influence of construct maturity

Independent of defect size, implanting a more mature engineered tissue was predicted to result in remodelling towards a more native-like architecture (Figs. 7.6a – 7.6c). For engineered tissues with low or intermediate ECM content, the repair tissue failed to recapitulate a native-like collagen architecture, particularly in
the superficial and middle zone. A more Benninghoff-like architecture was predicted following implantation of a mature construct (Fig. 7.7). The predicted fibre architecture can be explained by the composition-function relationships of the regenerating tissue. The immature implants had a low compressive stiffness and intrinsic Poisson's ratio due to the small concentration of ECM constituents and thus did not experience normal levels of deformations in the superficial and middle zones, leading to remodelling away from a normal Benninghoff architecture in these regions of the repair tissue. The mature constructs had a composition akin to deep zone cartilage, however their superficial and middle regions were stiffer than the equivalent zones of the native cartilage. This explains the prediction of remodelling away from a normal Benninghoff architecture in the deeper regions of the repair tissue, as the applied load gets transferred to the deep zones causing increased compressive strains. However, in contrast to the immature constructs, the mature engineered cartilage was predicted to successfully recapitulate the collagen architecture in the superficial zone. All implants stabilised the surrounding tissue significantly during dynamic loading with a tendency of better protection due to a higher implant stiffness (Figs. 7.6d and 7.7).

7.3.4. Influence of the rate of remodelling

Metabolic and catabolic cell activities depend on the cell's biophysical environment in complex ways which was not explicitly accounted for in this study. If one assumes the successful establishment of a native compositional gradient in the repair tissue it is intuitively obvious that the repair architecture will remodel towards the native Benninghoff architecture (Sec. 7.3.2). Based on the slow collagen turnover observed in adult articular cartilage, the question arises, though, how the prospects of recapitulating the native architecture are affected if the tissue gradually loses its ability to remodel as it matures and whether under these conditions it is advantageous to implant a mature, as suggested by the previous results, or an immature tissue with a theoretically greater ability to remodel.

If synthetic cell activities continue until a native depth dependent compositional gradient is restored, the repair tissue architecture can remodel towards the original architecture. In this model, the synthesis of native collagen levels took 8 to 9 months. If the tissue is able to quickly remodel itself ($\tau_\infty = 100$ days for the fully mature tissue), the Benninghoff architecture was predicted to be restored by the 4 year time point and deviated on average less than $5^\circ$ from the Benninghoff
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Figure 7.7.: Fibre architecture in and around defects when a constant biochemical composition is maintained both spatially and temporally. Composition at implantation classified as mature, medium or immature. Blue corresponds to less than 5° difference to intact Benninghoff architecture, green to less than 25°, orange to less than 50° and red to more than 50°. Empty areas correspond to circumferential alignment of the preferential fibre direction. SZ: superficial zone (top 10% of the defect tissue), MZ: middle zone (next 15% of the defect tissue), DZ: deep zone (remaining 75% of the tissue), AC: articular cartilage outside of defect.

architecture after 1 year (Fig. 7.8a). For a remodelling time constant $\tau_\infty = 1$ year, re-establishment of the architecture was not completed until the 10 year timepoint (Fig. 7.8b). For even longer remodelling times, the improvement with time 10 years post implantation was marginal (Figs. 7.8c, 7.8d). In general and contrary to our expectations, no large differences were predicted between mature (improved remodelling stimulus but slower remodelling) and immature (faster remodelling but initially worse stimuli) constructs. Of course, whether immature engineered implants can continue to synthesise ECM and reach normal levels of proteoglycans and collagen is open to debate, particularly in the earlier stages of repair where adverse mechanical conditions within the regenerating tissue may not be conducive to the maintenance of a chondrocyte phenotype.
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7.4. Discussion

In this study the effects of defect size and the composition of tissue engineered cartilage implanted to treat such defects on the resulting tissue architecture were evaluated using a computational model of collagen remodelling. The model predicts that a) even small defects (≤2 mm) have adverse effects on the mechanical environment in a significant portion of the surrounding healthy tissue volume; b) these effects increased with defect size; c) mature tissue engineered cartilage is more successful at stabilising the surrounding cartilage than immature implants; d) mature tissue engineered constructs create a mechanical environment in the regenerating tissue that better facilitates the re-establishment of a Benninghoff-type architecture; e) remodelling towards a Benninghoff architecture is achieved within engineered tissues assuming native compositional gradients are eventually
established post-implantation but the time course for such remodelling depends on the tissue's level of adaptivity.

The prediction of the Benninghoff architecture in intact cartilage with native-like gradients in its biochemical composition was in agreement with previous models (Wilson et al., 2006a) and experimental observations (Benninghoff, 1925b; Clark, 1991; Kääb et al., 1998). The earlier transition towards horizontal alignment closer to peripheral regions has been documented in SEM studies (Clark, 1991; Kääb et al., 1998). The symmetric transition of ellipsoidal fibre representations from vertical to horizontal alignment has been observed in central high load bearing regions and the asymmetric transition with “turning” ellipsoids from vertical to horizontal alignment has been associated with peripheral joint regions (Xia, 2008) and was predicted by the model. These correlations between model predictions and experimental observations provide strong support for the hypothesis that collagen fibre alignment in articular cartilage is driven by mechanical cues.

Untreated chondral defects have been shown to increase in size and frequency (Wang et al., 2006) and generally lead to degeneration and osteoarthritic changes in the tissue (Schinhan et al., 2012). Mechanical loading influences the synthesis of MMPs, TIMPs and aggrecanases that are relevant for tissue maintenance, degradation and remodelling (Torzilli et al., 2011). The altered mechanical environment in the presence of defects most likely disturbs the mechanobiological homeostasis of the resident cell population. Under such conditions the model predicts that, aggravated by increasing defect size, the collagen architecture deviates from the native architecture present in healthy tissue. This indicates that the native architecture in these regions is no longer ideally aligned to support the applied loads. The altered strain states in such a collagen network that does not remodel successfully can make it more susceptible to biochemical degradation (Huang and Yannas, 1977) and lead to further deterioration of the structural integrity of the extracellular matrix.

In order to prevent or hinder the occurrence of these adverse effects, the tissue needs to be stabilised. The unsatisfactory results of current clinical treatments for chondral defects present a strong motivation for tissue engineering strategies (Hunziker, 2002). There is debate as to the required functionality of an engineered tissue at the time of implantation and it has been argued that due to the adaptive nature of the joint as a whole the ideal properties of a tissue replacement vary over time and can be anywhere from below native properties to higher than native depending on the state of the surrounding tissues (Frank et al., 2004).
The primary goal of tissue engineering in this context is to restore joint function (Butler et al., 2000). The engineered graft should also serve to prevent further degeneration in the surrounding tissue by stabilising the adjacent cartilage and provide a continuous smooth surface to avoid stress concentrations and excessive shear. In the simulations presented in this paper the mature implant was most successful in stabilising the native cartilage and creating an internal mechanical environment within the regenerating tissue that provides appropriate stimuli to form (or support) a native-like Benninghoff architecture. The immature implants are very soft compared to articular cartilage and thus carry a low proportion of the load which limits their effectiveness for stabilising the defect walls against bulging into the defect. This effect will become more pronounced during transient loading with loss of fluid load support. It has been recognised that the depth dependent organisation and composition of articular cartilage are prerequisites for its function and that tissue engineers should strive to recapitulate these gradients via different cell sources, biomaterials, gradients in scaffold mechanical properties and signalling molecules (Klein et al., 2009). The gradients that developed in the mature implants \textit{in vivo} and resulted in a depth dependent architecture will not develop \textit{in vitro} in implants engineered under homogeneous environmental culture conditions.

In the final part of the study the existence of a target state ECM concentration that depends on the biophysical and chemical environment of the chondrocyte (Wilson et al., 2002) was explored. We did not make any assumption as to the nature of the environmental cues regulating synthesis. Instead, a simple phenomenological approach was used where the native depth dependent composition was assumed to be restored over time. This created the necessary stimuli for the development of a native tissue architecture. The effect of a varying ability of the tissue to remodel under these conditions was then investigated. The current model predicted little differences with regard to mature vs. immature implantation conditions with only a slight benefit predicted for implanting the mature implant. When fast remodelling of the tissue was assumed ($t_{\infty} = 100$ days), recapitulation of a native architecture was predicted to follow the establishment of the native depth dependent composition. However, if the ability of the mature tissue to remodel was assumed to be low ($t_{\infty} > 10$ years) an optimal architecture was not achieved within the time frame modelled. Failure to recapitulate a Benninghoff architecture leads to higher peak strains in the tissue that might have mechanobiological implications and are potentially damaging. In that case it has been suggested that
implantation of immature or isotropic implants will unlikely be successful (Hunziker et al., 2007) and it appears advisable to implant a “tissue that manifested a high degree of structural anisotropy from the very onset of the healing process, in order to ensure its longevity and mechanical competence” (Hunziker et al., 2007). One way to achieve the desired architecture might be using scaffold architectures as guiding structures (Moutos et al., 2007; Ateshian, 2007), or to modulate the environment through the depth of the developing tissue (Thorpe et al., 2011).

Basic parts of the presented model were based on previously published work (Wilson et al., 2006a, 2007), namely the depth dependent composition and the geometry, with some associated limitations. The employed material model, however, was different and the loading protocol was simplified due to high computational demands associated with the material model and the time scales considered. Despite these model differences, the physiological Benninghoff architecture was recovered in accordance with the previous models (Wilson et al., 2006a). This confirms that the relevant model aspects for this prediction are the depth dependent mechanical properties and composition, specifically the depth dependent swelling pressures, as well as joint loading. External loading compresses top layers more than the deep zone which is in line with the established cartilage biomechanics literature (Chen et al., 2001a; Han et al., 2011). While the actual joint geometry, kinematics and contact conditions are significantly more complex than our model representation, this interplay between depth-dependent swelling and external loading is expected to be a common characteristic in the knee joint and could be a major driving factor in the establishment of articular cartilage architecture. Thus, the assumption that the common contact area covered by the femoral condyle and the meniscus can be lumped into one representation is not expected to influence the results qualitatively. Meniscus and tibia have been combined into one representation in other knee models (Manda and Eriksson, 2012) and loading via rigid rather than deformable contact models has been shown to lead to a more challenging mechanical environment being predicted in the tissue (Owen and Wayne, 2011). The meniscus has further been shown to have a sealing function that maintains fluid load support in the tissue (Haemer et al., 2012), justifying the fluid boundary condition used in the present model. However, the interaction of deformable contact bodies becomes important for large defects where rim stresses will be elevated significantly (Peña et al., 2007) which is why the current study was restricted to comparatively small defects. Larger defects and the investigation of the influence of defect location would also necessitate a three dimensional knee model with a more accurate ge-
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ometry (Peña et al., 2007; Shirazi and Shirazi-Adl, 2009). Model improvements in contact mechanics and simulations of larger defects will likely predominantly affect the predicted architecture around the defect rim. It is further expected that the importance of construct maturity and strength increases in larger defects where the joint load is distributed onto a smaller undamaged rest area. Ideal integration between the repair tissue and the native cartilage was further assumed. Promising results towards the integration of cartilage replacements have been achieved (Hunziker, 2001; Theodoropoulos et al., 2011) and can be aided by, for example, removing proteoglycans from the defect surface (Hunziker and Rosenberg, 1996). Failure to achieve integration potentially disturbs the continuity of the displacement fields and negatively influences tissue mechanics and remodelling.

Recommendations for future work therefore include a) the use of three dimensional models to investigate the effect of defect size and therefore load bearing magnitude and joint curvature on the presented results; b) the use of deformable-deformable contact models to extend this work to larger defect sizes and c) more extensive experimental evaluations of the transient development of cartilage architecture in and around defects.

In conclusion, implantation of a mature engineered tissue with near native properties was found to induce the most favourable stimuli for remodelling towards a Benninghoff-like architecture. In the presence of active remodelling, the establishment of native compositional gradients is predicted to result in the development of a native architecture. If active remodelling cannot occur in vivo, the results of this study suggest that the architecture should be formed prior to implantation. Independent of the extent to which internal remodelling occurs in native cartilage, remodelling simulations can provide an illustrative tool to evaluate varying implant conditions in terms of the mechanical environment in the regenerate itself as well as the extent to which an adverse mechanical environment extends into the tissue.
8 Mechanoregulation of Collagen Organisation during Skeletal Tissue Repair

8.1. Introduction

Biological tissues, especially those whose primary function is mechanical in nature, such as orthopaedic or cardiovascular tissues, have been shown to be mechanosensitive, i.e. they adapt to changes in their mechanical environment. These adaptations occur during all stages of development – from the embryo to the adult organism. Healing of skeletal injuries has been intensively used for mechanobiological studies. During the healing process the granulation tissue filling the site of injury is infiltrated by mesenchymal stem cells that eventually differentiate into osteoblasts, fibroblasts or chondrocytes etc. and secrete matrix of differing biochemical compositions. Many authors have studied the relationship between this differentiation process and the mechanical environment and proposed mechanoregulation theories. Pauwels (1941, 1960) related the mechanical environment, as described by shear stress and hydrostatic stress, to the tissue phenotype. Carter et al. (1988, 1998) expanded on this idea and used hydrostatic stress and maximum principal strain as regulators, with quantitative boundaries of the mechanoregulation scheme proposed later (Isaksson et al., 2006). It was used among others in a study where pseudoarthrosis formation was studied in oblique fractures (Loboa et al., 2001). Specifically, this model has been applied frequently in tendon mechanobiology, such as fibrocartilaginous metaplasia emergence in tendons wrapping around bony formations (Giori et al., 1993; Wren et al., 2000). Claes et al. (1998) and Claes and Heigele (1999) also used principal strain and hydrostatic stress to predict tissue phenotypes during fracture healing. Other mechanical stimuli proposed as
mechanoregulators during skeletal regeneration include certain strain invariants (García-Aznar et al., 2007; Reina-Romo et al., 2009) and strain energy density (Ament and Hofer, 2000). Recognising the biphasic nature of most skeletal tissues, Prendergast et al. (1997); Huiskes et al. (1997) based their mechanoregulation algorithm on octahedral shear strain and fluid velocity. It has been successfully used to predict key events during fracture healing (Lacroix and Prendergast, 2002a; Lacroix et al., 2002; Isaksson et al., 2006a), distraction osteogenesis (Isaksson et al., 2007; Boccaccio et al., 2007, 2008) osteochondral defect healing (Kelly and Prendergast, 2005, 2006), in vivo bone chamber experiments (Khayyeri et al., 2009, 2010) and implant integration (Huiskes et al., 1997).

Cullinane et al. (2002, 2003) have demonstrated that the mechanical environment during bone defect healing can influence both tissue differentiation and the organisation (collagen fibre architecture) of the repair tissue. They showed that cyclic bending applied daily to an experimental mid-femoral defect (Fig. 8.1) results in the formation of cartilage as opposed to bone tissue. These neoarthroses exhibited preferred fibre angles consistent with those seen in articular cartilage. Hayward and Morgan (2009) further demonstrated that the patterns of tissue differentiation observed experimentally could be predicted using the mechanoregulation theory of Prendergast et al. (1997), providing further evidence to suggest that both strain and fluid flow are key regulators of tissue differentiation. What remains to be elucidated is the exact relationship between the mechanical environment and the structural organisation of extracellular matrix at the repair site during skeletal tissue differentiation.

A number of theories have been proposed for the remodelling and/or growth of biological tissues (Humphrey and Rajagopal, 2003; Garikipati et al., 2006, 2004). Collagen remodelling has been extensively studied in the context of cardiovascular tissues. In these studies collagen fibres have been assumed to align with respect to a local mechanical regulator such as stress (Taber and Humphrey, 2001; Gleason and Humphrey, 2004; Hariton et al., 2007b,a) or strain (Driessen et al., 2003b,a; Kuhl and Holzapfel, 2007; Driessen et al., 2008). Strain driven remodelling algorithms have also been successfully used to predict the collagen architecture in articular cartilage (Wilson et al. (2006a) and chapter 7). In studies on fibrocartilage formation in tendons, fibres were similarly hypothesised to align with respect to the local maximum tensile strain direction (Giori et al., 1993; Wren et al., 2000). The hypothesis under investigation in this study is that collagen fibres synthesised during tissue differentiation align between the positive principal directions of ei-
8. Mechanoregulation of Collagen Organisation during Skeletal Tissue Repair

Figure 8.1.: Experimental setup to induce cyclic bending in a rat femoral fracture. Adapted from Cullinane et al. (2003).

ther local stress or strain tensors. In this chapter the mechanoregulation model of Prendergast et al. (1997), as implemented by Lacroix et al. (2002), is extended to include a fibre reinforced constitutive model for soft tissues (chapter 3), where the organisation of the fibre network is regulated by the mechanical environment (chapter 5). To test the hypothesis, the model will be used to simulate the effect of bending on bone defect repair, and the predicted patterns of differentiation and collagen fibre orientations in the repair tissue will be compared to those observed experimentally by Cullinane et al. (2002).

8.2. Materials & methods

Adaptation of biological tissues is complex and generally includes volumetric growth, synthesis and resorption of extracellular matrix constituents and their reorientation. Here, the terms remodelling and synthesis will be used loosely to refer to a variety of processes: The reorientation of collagen fibres, the synthesis of new fibrils, their crosslinking etc. The intention is not to make a clean distinction between the involved mechanisms but to macroscopically and phenomenologically capture the resulting structurally relevant anisotropic effects. Therefore, the term remodelling will be used to represent both modelling and remodelling of the repair tissue architecture in response to the mechanical environment.
8.2.1. Constitutive model

All soft tissues were modelled as fluid saturated porous media. The total mixture stress of a biphasic medium with solid and fluid volume fractions $\phi_S$ and $\phi_F$ is given as

$$\sigma = \frac{-p\phi_F I}{\sigma_F} + \frac{(-p\phi_S I + \sigma_F^E)}{\sigma_S}$$

(8.1)

where $p$ is the pore pressure and $\sigma_F^E$ the extra stress in the solid skeleton for which the large strain hyperelastic material model developed in chapter 3 has been used. The solid matrix of all tissues was modelled using a compressible Neo-Hookean material law with the material parameters $C_1$ and $D_2$. At small strains they are related to the linear elastic Young’s modulus and Poisson’s ratio via relation 3.68. While granulation tissue, bone marrow and bone were modelled by this strain energy function alone, fibrous tissue and cartilage were further characterised as anisotropic materials by two fibre directions. These can be identified in the undeformed configuration by the two unit vector fields $a_0(X)$ and $g_0(X)$. Subsequently, the dependence on $X$ will be omitted in the notation. During deformation the fibres are stretched and rotated. Their mapping into the current configuration follows from

$$a = Fa_0 \quad \text{and} \quad g = Fg_0$$

(8.2)

The fibre stretch $\lambda$ is independent of a rigid body motion and motivates the introduction of the two invariants $I_4$ and $I_6$ as

$$I_4 = I : M C = \lambda_a^2 \quad \text{and} \quad I_6 = I : M' C = \lambda_g^2$$

(8.3)

where the structural tensors $M = a_0 \otimes a_0$ and $M' = g_0 \otimes g_0$ have been used. For the strain energy density functions characterising the constitutive behaviour of the collagen fibres an exponential formulation (compare Eq. 3.80) was chosen:

$$\psi_{\text{aniso}} = \frac{C_4}{2\beta_a} \left[ e^{\beta_a(I_4-1)^2} - 1 \right] + \frac{C_5}{2\beta_g} \left[ e^{\beta_g(I_6-1)^2} - 1 \right]$$

(8.4)

The material parameters $C_4$ and $C_5$ give the fibres a baseline stiffness while $\beta_a$ and $\beta_g$ describe the stiffening with increasing fibre stretch. If the $\beta$-values are set to zero, a linear fibre model is recovered. Fibres were only allowed to bear tensile loads and were assumed to buckle without stress contributions under compressive loads thus introducing tension-compression nonlinearity into the model.
8.2.2. Material parameters

The isotropic material parameters $C_1$ and $D_2$ were determined via relation (3.68) from the material parameters used in many previous studies (Isaksson et al., 2006a,b; Byrne et al., 2007; Lacroix and Prendergast, 2002b,a) for bone, bone marrow and granulation tissue. However, due to the switch from isotropic to anisotropic models the material parameters for the remaining soft tissues were reconsidered. Early studies on tissue differentiation during fracture healing often modelled the tissues as single phasic elastic materials neglecting fluid flow (Claes et al., 1998; Claes and Heigele, 1999). The high stiffness values chosen for cartilage in these studies might have been intended to represent a dynamic stiffness. For biphasic models, however, the equilibrium stiffness has to be supplied as a consistent input parameter. Also, these former studies did not take into account tension-compression nonlinearities but employed isotropic material laws. For example, a Young's modulus of 10 MPa was commonly chosen for cartilage. Cartilage properties are species-, location- and type dependent. Compressive aggregate moduli of articular cartilage vary between 0.1 and 2 MPa and tensile moduli between 5 and 40 MPa have been reported (Mow and Guo, 2002; Akizuki et al., 1986; Roth and Mow, 1980).

An intermediate value of 1 MPa was chosen for the Young's modulus of the isotropic ground phase. As many researchers report tensile moduli in the split line direction that are roughly 10 fold higher than compressive properties (Roth and Mow, 1980; Akizuki et al., 1987; Huang et al., 2005) we chose a tensile modulus of 10 MPa. To transform these values into the parameters for the material model described above a Poisson’s ratio for articular cartilage of $\nu = 0.167$ was assumed – a value well within the spectrum of reported values (e.g. Jurvelin et al. (1997)) and typically used in mechanoregulation studies. Using relation (3.68) the values for $C_1$ and $D_2$ can be determined from a compressive Young's modulus of $E_\perp = 1$ MPa. To determine tensile material parameters consider an articular cartilage specimen harvested from the superficial zone parallel to the split-line direction (Roth and Mow, 1980; Akizuki et al., 1987). Both families of fibres were assumed to coincide with the direction of loading and given identical properties. To get a ten times stiffer reaction in tension than in compression up to $\approx 20\%$ strain, the values for the fibre stiffnesses were set to $C_4 = C_5 = 0.964$ MPa. The corresponding values for fibrous tissue were approximated to be one fifth of the cartilage values in accordance with previous mechanoregulation studies (Isaksson et al., 2006a,b; Byrne
et al., 2007; Lacroix and Prendergast, 2002b). At physiological loading rates most soft tissues exhibit significant fluid flow independent viscoelasticity. One way to account for the immediate viscoelastic effects without modelling relaxation phenomena is to use a momentary stiffness value instead of an equilibrium modulus. Note however that these values can be specific to certain loading regimes and strain rates. To partially address this uncertainty in soft tissue mechanical properties, we undertook a parameter variation study with 3 sets termed “low” (derived from equilibrium properties), “mid” (intermediate values) and “high” (values previously used in isotropic models were taken as compressive properties for the current fibre reinforced model). The predicted phenotypes and differentiation patterns were compared to those predicted by the traditional approach (i.e. assuming all tissues to be isotropic). Material properties are summarised in table 8.1. Linear fibre behaviour was assumed, so that $\beta_a = \beta_q = 0$. The fluid bulk modulus of water was used for the pore fluid in all tissues: $K = 2300$ MPa.

8.2.3. Mechanoregulation model

Mechanoregulation simulations usually follow a common loop: A typical loading cycle is simulated to compute the regulatory stimuli under present conditions. The implemented regulation algorithm then predicts the tissue’s response. Specifically, the mechanical properties at a specific region of the tissue are updated based on the predicted change in tissue phenotype and organisation, as described below. The simulation of the representative loading cycle is then repeated with the updated material properties. Subsequently, these loops will be referred to as iterations $i$. The following mechanoregulation algorithm was implemented in each integration point of the finite element mesh.

At the beginning of the simulation the entire fracture callus was modelled as granulation tissue. Stem cell infiltration from the periosteum, the outer cortical
Table 8.1.: Material parameter sets “low” / “mid” / “high” / “iso”. $E_-$ and $E_+$ are compressive and tensile Young’s moduli, respectively, while for the isotropic parameter set the same modulus $E_\pm$ was used in tension and compression. Other variables: $\nu$ - Poisson’s ratio, $k$ - permeability, $\phi_{F0}$ - initial porosity.

<table>
<thead>
<tr>
<th>property</th>
<th>granulation tissue</th>
<th>fibrous tissue</th>
<th>cartilage</th>
<th>bone</th>
<th>immature bone</th>
<th>mature bone</th>
<th>cortical bone</th>
</tr>
</thead>
<tbody>
<tr>
<td>$E_-$ (low)</td>
<td>[MPa]</td>
<td>0.2</td>
<td>0.2</td>
<td>1.0</td>
<td>2.0</td>
<td>1000</td>
<td>6000</td>
</tr>
<tr>
<td>$E_+$ (low)</td>
<td>[MPa]</td>
<td>0.2</td>
<td>2.0</td>
<td>10.0</td>
<td>2.0</td>
<td>1000</td>
<td>6000</td>
</tr>
<tr>
<td>$E_-$ (mid)</td>
<td>[MPa]</td>
<td>0.2</td>
<td>1.0</td>
<td>5.0</td>
<td>2.0</td>
<td>1000</td>
<td>6000</td>
</tr>
<tr>
<td>$E_+$ (mid)</td>
<td>[MPa]</td>
<td>0.2</td>
<td>10.0</td>
<td>50.0</td>
<td>2.0</td>
<td>1000</td>
<td>6000</td>
</tr>
<tr>
<td>$E_-$ (high)</td>
<td>[MPa]</td>
<td>0.2</td>
<td>2.0</td>
<td>10.0</td>
<td>2.0</td>
<td>1000</td>
<td>6000</td>
</tr>
<tr>
<td>$E_+$ (high)</td>
<td>[MPa]</td>
<td>0.2</td>
<td>20.0</td>
<td>100.0</td>
<td>2.0</td>
<td>1000</td>
<td>6000</td>
</tr>
<tr>
<td>$E_\pm$ (iso)</td>
<td>[MPa]</td>
<td>0.2</td>
<td>2.0</td>
<td>10.0</td>
<td>2.0</td>
<td>1000</td>
<td>6000</td>
</tr>
<tr>
<td>$\nu$</td>
<td>[-]</td>
<td>0.167</td>
<td>0.167</td>
<td>0.167</td>
<td>0.167</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>$k$</td>
<td>$[\frac{m^3}{Ns}]$</td>
<td>$1 \cdot 10^{-14}$</td>
<td>$1 \cdot 10^{-14}$</td>
<td>$5 \cdot 10^{-15}$</td>
<td>$1 \cdot 10^{-14}$</td>
<td>$1 \cdot 10^{-13}$</td>
<td>$3.7 \cdot 10^{-13}$</td>
</tr>
<tr>
<td>$\phi_{F0}$</td>
<td>[-]</td>
<td>0.8</td>
<td>0.8</td>
<td>0.8</td>
<td>0.8</td>
<td>0.8</td>
<td>0.8</td>
</tr>
</tbody>
</table>
surface and the medullary canal was modelled via a simple diffusion equation:

\[ \frac{\partial c_{\text{cell}}}{\partial t} = D \nabla^2 c_{\text{cell}} \]  

(8.5)

where \( c_{\text{cell}} \) is the current cell concentration and \( D = 0.34 \text{ mm}^2 \text{d}^{-1} \) the diffusion coefficient (Lacroix et al., 2002; Andreykiv et al., 2008).

Fluid flow and shear strain were used as mechanoregulatory stimuli for tissue differentiation (Prendergast et al., 1997; Lacroix and Prendergast, 2002b,a; Isaksson et al., 2006a,b). A scalar stimulus \( S \)

\[ S = \frac{\gamma}{a} + \frac{v}{b} \]  

(8.6)

was calculated where \( \gamma \) was the octahedral shear strain, \( v \) the fluid velocity. The values of the scaling constants were \( a = 0.0375 \) and \( b = 3 \mu \text{m s}^{-1} \) (Huiskes et al., 1997). Differentiation into a certain phenotype was then determined according to

\[
S \begin{cases} 
\geq 3 & \text{fibrous tissue} \\
\in [1;3) & \text{cartilage} \\
\in [0.267;1) & \text{immature bone} \\
\in [0.011;0.267) & \text{mature bone} \\
< 0.011 & \text{resorption}
\end{cases}
\]  

(8.7)

The intervals for \( S \) were established in Huiskes et al. (1997) and later extended to include two bone phases (Lacroix et al., 2002) and bone resorption (Lacroix and Prendergast, 2002a). Mechanical properties of the resulting differentiating tissue were calculated using a rule of mixtures. The following equations use the Young’s modulus as an example. However, the described approach was followed for all material properties. Following the computational smoothing procedure from Lacroix and Prendergast (2000) to avoid numerical instabilities and account for the time delay between stimulus and MSC differentiation, the Young’s modulus of differentiated tissue in the next iteration \( E_{n+1}^{\text{diff}} \) is determined as the average from the Young’s moduli \( E_i \) of the tissue phenotypes during the last ten iterations.

\[ E_{n+1}^{\text{diff}} = \frac{1}{10} \sum_{i=n-9}^{n} E_i \]  

(8.8)

Employing the rule of mixtures again, final mechanical properties (e.g. \( E_{n+1} \)) in an integration point are obtained as a weighted average of the properties of the granulation tissue and the differentiated tissue:

\[ E_{n+1} = \frac{c_{\text{max}} - c_{\text{cell}}}{c_{\text{max}}} E_{\text{gran}} + \frac{c_{\text{cell}}}{c_{\text{max}}} E_{n+1}^{\text{diff}} \]  

(8.9)
where \( c_{\text{cell}} \) is the current and \( c_{\text{max}} \) the maximum cell concentration.

Fibre directions were updated equivalently to Eq. (8.8). The unit vectors designating the preferred directions in the following iteration \( \mathbf{a}_{0,n+1} \) and \( \mathbf{g}_{0,n+1} \) were thus obtained via

\[
\mathbf{a}_{0,n+1} = \frac{\sum_{i=n-9}^{n} \mathbf{a}_{0,i}}{\left| \sum_{i=n-9}^{n} \mathbf{a}_{0,i} \right|} \quad \text{and} \quad \mathbf{g}_{0,n+1} = \frac{\sum_{i=n-9}^{n} \mathbf{g}_{0,i}}{\left| \sum_{i=n-9}^{n} \mathbf{g}_{0,i} \right|} \quad (8.10)
\]

as the average of the predicted unit vectors from the last ten iterations, \( \mathbf{a}_{0,i} \) and \( \mathbf{g}_{0,i} \). Prior to summation the directional sense of the vectors was modified to yield a non-negative dot product.

Fibres were assumed to align between the positive principal directions of a symmetric stimulus tensor \( \mathbf{S} \) (see Fig. 8.2). Consider the spectral decomposition of \( \mathbf{S} \)

\[
\mathbf{S} = \sum_{j=1}^{3} s_j \mathbf{v}_j \otimes \mathbf{v}_j \quad \text{with} \quad s_1 \geq s_2 \geq s_3 \quad (8.11)
\]

where \( s_j \) are the eigenvalues of \( \mathbf{S} \) and \( \mathbf{v}_j \) its eigenvectors. In case of \( \mathbf{S} \) being a material tensor, predicted fibre directions immediately follow from

\[
\mathbf{a}_{0,i} = \frac{s_1 \mathbf{v}_1 + s_2 \mathbf{v}_2 + s_3 \mathbf{v}_3}{\sqrt{s_1^2 + s_2^2 + s_3^2}} \quad \mathbf{g}_{0,i} = \frac{s_1 \mathbf{v}_1 - s_2 \mathbf{v}_2 - s_3 \mathbf{v}_3}{\sqrt{s_1^2 + s_2^2 + s_3^2}} \quad (8.12)
\]

where only tensile eigenvalues \( s_j \) are used. If all principal stresses or stretches are non-tensile, then current fibre directions are kept. If only one eigenvalue is tensile, then both fibre directions coincide with the corresponding eigenvector. The resulting vectors from Eq. (8.12) where then used to update fibre orientation in the next iteration via Eq. (8.10).

In case of \( \mathbf{S} \) being a spatial tensor, fibre directions in the current configuration were driven by the spatial stimulus

\[
\mathbf{a}_i = \frac{s_1 \mathbf{v}_1 + s_2 \mathbf{v}_2 + s_3 \mathbf{v}_3}{\sqrt{s_1^2 + s_2^2 + s_3^2}} \quad \mathbf{g}_i = \frac{s_1 \mathbf{v}_1 - s_2 \mathbf{v}_2 - s_3 \mathbf{v}_3}{\sqrt{s_1^2 + s_2^2 + s_3^2}} \quad (8.13)
\]

However, the update of the fibre directions (Eq. (8.10)) took place in the stress free reference configuration. The predicted fibre angles were therefore pulled back.
and normalised using

\[ a_{0,i} = \frac{F^{-1}a_i}{||F^{-1}a_i||} \quad \text{and} \quad g_{0,i} = \frac{F^{-1}g_i}{||F^{-1}g_i||} \]  

(8.14)

and only then fed into Eq. (8.10).

In this paper three possible phenomenological stimuli for fibre reorientation were examined – the material right Cauchy-Green tensor \( C \) as well as two spatial stress tensors: The solid extra stress \( \sigma_S^E \), which is directly determined from the material model via the solid matrix deformation gradient, and the total stress in the solid \( \sigma_S = \sigma_S^E - \rho \phi_S I \), which also includes the fraction of the pore pressure born by the solid phase.

### 8.2.4. Geometry & discretisation

The experimental study by Cullinane et al. (2002, 2003) was investigated with the extended mechanoregulation theory. An idealised geometry of a rat femur with
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Figure 8.4.: Phenotypes after 24 iterations. Influence of fibre stimulus and material parameters. No bone is predicted to form.

(a) isotropic
(b) $C$, low
(c) $C$, mid
(d) $C$, high
(e) $\sigma_E^S$, low
(f) $\sigma_E^S$, mid
(g) $\sigma_E^S$, high
(h) $\sigma_S$, low
(i) $\sigma_S$, mid
(j) $\sigma_S$, high

A 3 mm fracture gap was created (see Fig. 8.3). A plane strain finite element model was used to simulate $\pm 6^\circ$ bending for 24 days, the time period of loading investigated by Hayward and Morgan (2009).

Fibre angles were measured in Cullinane et al. (2003) where “The superficial zone was determined as the area immediately under the cartilage surface, the deep zone was the area immediately adjacent to the underlying bone, and the intermediate zone was exactly half way between the deep and superficial zones”. Here, fibre angels were averaged in accordingly defined zones (see Fig. 8.3). Two regions of interest were defined in the internal callus: An inner and an outer region as illustrated in Fig. 8.3.

The geometry was discretised using 1036 eight-noded isoparametric elements with biquadratic interpolation of displacements ($\mathbf{u}$) and bilinear interpolation of the
8. Mechanoregulation of Collagen Organisation during Skeletal Tissue Repair

pore pressure \((p)\) (MSC, 2008b). A two-field variational approach was adopted using a mixed \(\mathbf{u}-p\) formulation. The numerical results were obtained via a Newton-Raphson solution strategy (MSC, 2008a). The boundary conditions are shown in Fig. 8.3.

### 8.2.5. Simulations

The following simulations were performed. To assess the distribution of tissue phenotypes compared to previous models that assumed isotropy a simulation without a fibre reinforced constitutive model (parameter set \(E_{\text{iso}}\)) was run. In the following nine simulations the three parameter sets “low”, “mid” and “high” were each combined with each of the three fibre orientation stimuli: (1) the right Cauchy-Green tensor \(\mathbf{C}\) (strain driven remodelling, material tensor); (2) the solid extra stress \(\sigma_{\text{S}}^{E}\) (Cauchy stress driven remodelling, spatial tensor) and (3) the total solid stress \(\sigma_{s}\).

### 8.2.6. Angle averaging

During postprocessing average angles in an area were obtained via the vector summation

\[
\mathbf{a} = \frac{1}{N} \sum_{k=1}^{N} \mathbf{a}_{0,k}
\]

and extracting the resulting angle from \(\mathbf{a}\). This angle was defined in the interval \(\varphi \in (-90^\circ; 90^\circ]\) around the horizontal axis. Note the similarity of equations 8.10 and 8.15 where the difference lies in the normalisation condition. While both yield the same direction for a given set of vectors, Eq. 8.10 always yields a vector of unit length and Eq. 8.15 one of unit length or less. This deviation from unit length (\(||\mathbf{a}|| = 1\) would only occur if all vectors \(\mathbf{a}_{0,k}\) were co-linear) was used to compute the circular standard deviation \(\sigma_{c}\) used in directional statistics (see e.g. Fisher (1993)):

\[
\sigma_{c} = \sqrt{-2 \ln ||\mathbf{a}||}
\]

### 8.3. Results

In all simulations a mixture of cartilage and fibrous tissue was predicted within the fracture callus, with no bone formation. Similar patterns of tissue differentiation were predicted by the classic isotropic model and the anisotropic model with “low”
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Figure 8.5.: Relative influence of stimuli at iteration 24 (octahedral shear strain on x-axis [%], fluid velocity on y-axis [μm s⁻¹], double logarithmic scale). Iteration one is identical for all simulations.

Material parameters (see figures 8.4a to 8.4j). Higher soft tissue stiffness values led to the prediction of less cartilage formation. Area fractions of fibrous tissue were predicted to increase from 55.4% (C, low) to 80.6% (C, high), see fig. 8.9. This was caused by higher fluid velocities within the callus (see figs. 8.5c to 8.5k for comparison).
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Figure 8.6.: Predicted fibre orientations for the three stimuli after 24 days of loading in the internal callus. Figure on left hand side shows the regions defined as the "inner" and "outer" in the internal callus (regions filled with black in figure on the left hand side, separated by the white line).

Figure 8.7.: Predicted fibre orientations for the three stimuli after 24 days of loading in the external callus (region filled in with black in figure on the left hand side).

The predicted fibre angles and circular standard deviations are summarised in table 8.2 for the outer and inner regions, respectively. A visual impression of the fibre architecture can be obtained in Fig. 8.6. The collagen fibre orientation was predicted to change from $\approx 1.5^\circ$ (to the horizontal) in the superficial zone to between $35^\circ$ and $60^\circ$ in the deep zone, depending on the considered stimulus. Circular standard deviations in the regions of interest increased strongly from superficial to deep zones as was observed in Cullinane et al. (2002). Generally, all regulatory stimuli were able to capture this fundamental trend in the fibre architecture, although certain variations were observed that are described below.

The strain driven model predicted a more gradual increase of the fibre angle throughout the depth of the tissue. On the other hand the stress controlled model predicted rapid changes in fibre orientation and also predicted higher angles in the deep zones (see figs. 8.8a to 8.8c). The transition from parallel to perpendicular orientation occurred more towards the lower middle zone when $\sigma_s$ was chosen...
Table 8.2.: Fibre angles [°] and circular standard deviations [-]. Note, that in Cullinane et al. (2002) no distinction was made between outer and inner region.

<table>
<thead>
<tr>
<th>parameter set</th>
<th>stimulus</th>
<th>inner region</th>
<th>outer region</th>
<th>combined</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>SZ</td>
<td>MZ</td>
<td>DZ</td>
</tr>
<tr>
<td>low</td>
<td>C</td>
<td>4.47 ± 2.65</td>
<td>6.55 ± 4.9</td>
<td>53.20 ± 70.9</td>
</tr>
<tr>
<td>mid</td>
<td>C</td>
<td>1.03 (0.099)</td>
<td>7.33 (0.113)</td>
<td>2.28 (0.131)</td>
</tr>
<tr>
<td>high</td>
<td>C</td>
<td>0.52 (0.047)</td>
<td>11.35 (0.137)</td>
<td>1.34 (0.109)</td>
</tr>
<tr>
<td>low</td>
<td>σₜ</td>
<td>2.05 (0.080)</td>
<td>5.92 (0.083)</td>
<td>24.62 (0.259)</td>
</tr>
<tr>
<td>mid</td>
<td>σₜ</td>
<td>1.03 (0.099)</td>
<td>7.33 (0.113)</td>
<td>2.28 (0.131)</td>
</tr>
<tr>
<td>high</td>
<td>σₜ</td>
<td>0.52 (0.047)</td>
<td>11.35 (0.137)</td>
<td>1.34 (0.109)</td>
</tr>
<tr>
<td>low</td>
<td>σₛ</td>
<td>1.37 (0.030)</td>
<td>-1.83 (0.128)</td>
<td>63.41 (0.142)</td>
</tr>
<tr>
<td>mid</td>
<td>σₛ</td>
<td>1.71 (0.017)</td>
<td>9.83 (0.018)</td>
<td>58.08 (0.428)</td>
</tr>
<tr>
<td>high</td>
<td>σₛ</td>
<td>1.52 (0.017)</td>
<td>9.20 (0.045)</td>
<td>49.83 (0.561)</td>
</tr>
<tr>
<td>low</td>
<td>σₛ</td>
<td>0.52 (0.047)</td>
<td>11.35 (0.137)</td>
<td>1.34 (0.109)</td>
</tr>
<tr>
<td>mid</td>
<td>σₛ</td>
<td>1.12 (0.014)</td>
<td>6.29 (0.076)</td>
<td>44.30 (0.344)</td>
</tr>
<tr>
<td>high</td>
<td>σₛ</td>
<td>2.35 (0.025)</td>
<td>13.88 (0.068)</td>
<td>45.16 (0.437)</td>
</tr>
</tbody>
</table>
as the stimulus. For the other stimuli, the reorientation occurred mostly in the deep zone. Total solid stress driven remodelling also predicted a peak fibre angle between middle and deep zone followed by a drop towards the deep zone (see Fig. 8.8b). The other stimuli did not exhibit this pronounced behaviour.

In both the outer and inner region of the internal callus (see Fig. 8.3), the models predicted an architecture with fibres parallel to the transverse plane in the superficial zone. In the middle zone the strain driven algorithm predicted similar angles in inner and outer regions, whereas a the angle changed from -1.8° in the inner region to 9.8° in the outer region for the extra stress driven algorithm. When the total solid stress was used as fibre stimulus the angle doubled from outer to inner region. In the deep zones a large drop in the fibre angle was predicted during strain driven remodelling from 46.4° to 24.6° between the outer and inner region. During stress driven remodelling the trend was not as severe and opposite, with an increase from the outer to the inner region predicted.

Increasing the soft tissue stiffness values from the baseline values led to changes in the predicted fibre angle in deeper regions (see table 8.2). This is most pro-
nounced for strain driven remodelling. Only minimal changes in the predicted fibre angles were observed in the superficial zone retaining the general result of a fibre orientation parallel to the transverse plane for all stimuli. Total solid stress driven remodelling of the fibre architecture was least sensitive to changes in the stiffness. This was the only stimulus to retain high deep zone angles throughout the parameter variation.

The fibre architecture in the repair tissue of the external callus was also predicted (see Fig. 8.7). While all stimuli predict vertical fibre arrangement to some degree, strain driven remodelling predicted a more disorganised tissue. In contrast stress driven remodelling exhibits a highly organised vertical fibre alignment in the direction of maximum tensile stresses during bending.

8.4. Discussion

In this chapter the mechanoregulation algorithm proposed by Prendergast et al. (1997) was extended to predict changes in tissue architecture during differentiation. Towards this end the large strain anisotropic biphasic material model presented in chapter 3 was combined with a rule for collagen fibre organisation depending on the mechanical environment. Switching from an isotropic to a fibre reinforced constitutive model led to a reconsideration of material parameters normally used
8. Mechanoregulation of Collagen Organisation during Skeletal Tissue Repair

in mechanoregulation models. A parameter variation study revealed that a set of material parameters in the range of the soft tissues' equilibrium properties produced tissue differentiation predictions that were similar to those determined by making the assumption of tissue isotropy. The tensile moduli of this set were equal to the moduli used in previous isotropic studies. Of greater importance was the fact that this novel constitutive framework allowed for a fundamental investigation of the role of the local mechanical environment in regulating the architecture of regenerating soft tissue.

The model predictions of tissue differentiation for the non-union of a fracture subject to cyclic bending loads can be compared to histological data from developing neoarthroses (Cullinane et al., 2002). The mechanoregulation algorithm predicted a mixture of cartilaginous and fibrous tissue, generally consistent with experimental findings and previous computational models (Cullinane et al., 2002, 2003; Hayward and Morgan, 2009). However, the bony arcade structures reported by Cullinane et al. (2002) were not predicted. Both strain- and stress-driven remodelling predicted collagen fibre angles similar to those measured throughout superficial, intermediate and deep zones of the neoarthroses, recapitulating the fibre architecture of native articular cartilage (Cullinane et al., 2002). This study demonstrates for the first time that mechanoregulation models can be used to successfully predict both tissue differentiation and organisation during fracture repair. This provides further evidence that the collagen organisation of the repair tissue is regulated by the local mechanical environment.

Predicted fibre orientations were least sensitive to a material parameter variation when based on $\sigma_s$. With this stress type stimulus the lowest deviation in predictions of fibre architecture between the inner and outer regions were observed. However, there was no data available on these deviations in experimental studies (Cullinane et al., 2002).

The external callus is dominated by highly aligned vertical fibres during stress driven remodelling (Fig. 8.7). Those fibres may contribute to a stabilisation of the fracture by supporting tensile stresses during bending. The strain driven algorithm predicted a more disorganised tissue due to lateral strains occurring during compressive loading. Vertical alignment can still be seen, however in a smaller area (Fig. 8.7).

Higher stiffness values for the soft tissues in tension and compression led to an increase in the calculated pore pressure values and gradients. These pressure gradients drive the fluid flow in the biphasic mixture leading to an increase in the
stimulus for differentiation $S$ (Eq. (8.6)), thus leading to more fibrous tissue being predicted by the mechanoregulation algorithm. The parameter set “low” yielded comparable results to the isotropic model predictions. During compression of a biphasic medium lateral tensile strains can occur. Thus, though fibres only bear load during tensile loading in the described material model, depending on their orientation fibres can contribute to the overall apparent stiffness of a compressed piece of tissue (compare chapter 4). The fibres then support fluid pressurisation. If this induced pressure is non-homogeneous, i.e. $\text{grad} \ p \neq \ 0$, it will elevate the fluid flow induced stimuli experienced by the cells (compare Eq. 3.54).

While both stress and strain driven remodelling algorithms have been proposed, one should keep in mind that either are but mathematical, not physical, concepts. Due to their phenomenological nature their validity must be supported by application to various different scenarios and comparison to experimental data (Humphrey, 2001). Though the angles predicted by stress driven remodelling in this study are slightly closer to the measured ones and were influenced less by the parameter variation, this result is not sufficient to make any kind of statement in that direction. Comparison of both stress-type stimuli shows quite similar values in the predicted architecture. This result can not be generalised to all scenarios. In a mechanical environment where the hydrostatic pressure is high compared to the solid matrix extra stress, the total stress in the solid will be quite different from that extra stress and will predict a different fibre architecture.

The employed model had some limitations: The fracture was modelled as a two dimensional structure to reduce calculation times. An idealised geometry was used rather than experiment specific data. These two simplifications are likely contributing to the lack of bone formation predicted in our models compared to that observed in the experimental study (Cullinane et al., 2002). The intrinsic viscoelasticity of the involved soft tissues was neglected. The model did furthermore not include angiogenesis (Geris et al., 2006; Checa and Prendergast, 2009b) because the developing neoarthrosis and not bone formation was the focus of interest. Stem cell dispersal as a combination of proliferation and migration was implemented as a simple diffusive process without accounting for cell death or the biochemical environment (such as growth factors, e.g. Bailón-Plaza and van der Meulen (2001)). The model assumed that all MSCs migrated into the callus from the bone marrow, the periostem and the outer cortical surface and neglects other MSCs that may home into the injured site from alternative sources. Furthermore, it was assumed that all MSCs will respond identically to their mechanical envi-
8. Mechanoregulation of Collagen Organisation during Skeletal Tissue Repair

ronment, whereas in reality the bone marrow cell population in itself is highly heterogeneous. Cellular events and characteristics can be captured more accurately with extended continuum models (Andreykiv et al., 2008; Isaksson et al., 2008b,a) or lattice based approaches including stochastic components (Perez and Prendergast, 2007; Byrne et al., 2007; Checa and Prendergast, 2009a; Khayyeri et al., 2011). Callus growth was not considered, but has been incorporated into other models (Gómez-Benito et al., 2005, 2006; Garcia-Aznar et al., 2007). Tissue damage was not included in the mechanoregulation model, but would be considered likely in regions of high strain. Despite these limitations the model was able to capture the main experimental results and predict the zonal architecture of the developing cartilage. Future tests of the hypotheses made in the model could include attempts to simulate healing under altered loading conditions and fracture gap sizes. Another aspect is related to the time course of the healing: Presently all tissues and components are synthesised at the same rate. However, a composition based approach (e.g. Wilson et al. (2007, 2006c); Loboa et al. (2003)) with cell-specific activities (such as for example demonstrated in Isaksson et al. (2008b)) would be more suitable to capture such effects like age, species and tissue specific synthesis rates. To ease parameter identification a linear fibre model \( (\beta_a = \beta_g = 0) \) was used which does not capture the typical stretch induced stiffening of collagen molecules. Fibre dispersion was not included in our remodelling considerations, as has been done in other remodelling algorithms (Driessen et al. (2008), chapters 5, 6, 7.).

This model can be applied to investigate specifically those healing processes and tissue engineering strategies where recapitulating normal tissue architecture is important. For example chondral and osteochondral defect repair critically depend on achieving a native-like zonal structure so that the tissue can endure in-vivo loads. However, this study was focused on the integration of fibre synthesis and organisation with an existing theory for mechanoregulated tissue differentiation. Evidence was provided that mechanoregulation algorithms can be combined with knowledge from remodelling studies to simultaneously address the differentiation and organisation of regenerating tissues. It is expected that the mechanoregulation algorithms will become more powerful tools by considering not only changes in phenotype during regenerative processes but also tissue architecture.
9 Discussion

9.1. Summary

The foundation of this thesis has been the development of a material model for biphasic media at large strains (chapter 3). It was based on a multiplicative decomposition of the deformation gradient into elastic and viscous parts, a procedure common in finite strain constitutive theories of varying kinds (Lubarda, 2004). All constitutive relations have been derived from the Clausius-Duhem inequality in a thermodynamically consistent manner. The model was set up in a modular fashion such that different material effects like anisotropy, fluid flow dependent and independent viscoelasticity as well as swelling can be integrated or excluded depending on the material of interest or the experimental data available. This allows for the modelling of a wide variety of biological tissues and materials. The model can further be extended to other constitutive relations in a straight-forward manner. The material model was implemented for the two most common nonlinear FE packages, Abaqus and Marc, and has been made available in parts online (Nagel and Kelly, 2012a).

This model was then applied to investigate the relationship between intrinsic and apparent properties of fibre reinforced biomaterials (chapter 4). A comparison between charged and neutral materials revealed pronounced differences in the influence of fibre orientation in both materials such that an intuitive understanding of one material class cannot be directly transferred to the other. These results highlighted the functional significance of the Benninghoff architecture – the proteoglycan-collagen interaction stiffens the deeper cartilage layers due to higher GAG content, less volumetric expansion during swelling (when the cartilage is restrained by the subchondral bone and the vertically aligned fibres) as well as the pre-stress in the collagen network. Without this interaction, the Benninghoff architecture cannot be functional. It further became apparent that the smooth-
ness of the transition between tensile and compressive cartilage properties (e.g. Chahine et al., 2004) stems from both the Benninghoff architecture itself as well as local fibre dispersion. Fibre dispersion was modelled using an ellipsoidal fibre distribution ansatz. Such models introduce not only additional computational overhang but also present with some counter-intuitive aspects in their behaviour. A parameter variation was performed to illustrate the differential effects of various material parameters and facilitate easier initial parameter estimation. A scaling methodology was further introduced to overcome some of the unexpected consequences of a changing collagen architecture on the apparent properties and allow modelling of a constant collagen content.

A phenomenological remodelling framework based on another multiplicative decomposition of the deformation gradient was introduced in chapter 5. The remodelling framework itself was kept general via the introduction of an anisotropy tensor from which several discrete or continuous fibre architectures can be derived depending on the application. Collagen network orientation and stress-free configuration (both anisotropic) were algorithmically uncoupled via the definition of individual evolution equations. Illustrative one dimensional examples revealed complex feedback mechanisms during remodelling between fibre and ground substance properties as well as boundary conditions. A principal model validation was successfully performed on compacting fibrous hydrogels and periosteum. This framework is suitable to capture the evolution of other biological tissues including those of the cardiovascular system (e.g. Soares et al. (2011)).

In chapter 6 the concepts of all previous chapters were combined to investigate the role of structure-function relationships in tissue engineered cartilage. Full bioreactor culture lasting several weeks could be simulated due to modelling simplifications based on the physical behaviour of biphasic tissues under cyclic loads as well as the development of the stimulus configuration concept. It was shown that the traditionally dominant feature investigated in remodelling studies, that is collagen (re)orientation, is insufficient to explain experimentally observed results. If it is combined with a reconfiguration of the collagen recruitment state, the experimentally observed changes in Young’s modulus, Poisson’s ratio and geometry in response to dynamic compression could be predicted. Aside from that, it became apparent that the recruitment configuration has a major impact on construct stiffness due to the modulation of swelling pressures and collagen network pre-stress and should therefore not be neglected. A striking result in this context was a decrease in tissue stiffness despite ongoing net accumulation of collagen. One
Conclusion is that in order to obtain accurate composition-function relationships, ECM architecture needs to be taken into account. Simple correlations might not work or produce a high degree of variability.

Composition based heterogeneity in the material properties and the remodelling framework were then used to predict the development of a Benninghoff architecture in a tibial plateau in response to internal swelling pressures and externally applied joint loading (chapter 7). The introduction of even small defects led to significant alterations in the predicted architecture within the undamaged articular cartilage. In other words, since the Benninghoff architecture is optimised for the healthy state, the altered deformation state due to the presence of a chondral defect leads to excessive strains because the collagen network cannot support the load optimally. These deformations can have negative effects both on the collagen architecture (degradation or direct damage) and the chondrocytes. An investigation of the implantation of tissue engineered cartilage with varying composition revealed that mature implants do not only stabilise the surrounding tissue more effectively but facilitate the establishment of deformation characteristics in the replacement tissue that are more suitable for a recreation or maintenance of the Benninghoff architecture. In the presence of ongoing synthesis and if the immature construct is assumed to have a higher initial capability to remodel some of these differences are alleviated. A general conclusion from this chapter is that a mature implant with appropriate modifications for a good integration with the surrounding tissue seems the most promising strategy for cartilage tissue engineering. The possible issue of insufficient remodelling could be overcome by implanting a tissue and/or scaffold with the proper architecture already in place.

In chapter 8 a fibre reorientation model was combined with a tissue differentiation theory in order to investigate whether the assumptions of a mechanically guided tissue architecture hold during skeletal regeneration. A neoarthrosis was modelled and several driving stimuli considered. All were able to reproduce the experimentally observed fibre angles and based on the experimental data available no one stimulus could be preferred. Simultaneously with tissue architecture, the dominant tissue phenotypes could be predicted based on fluid flow and shear strain. This provides further evidence that the collagen architecture in a large variety of tissues and regenerative events correlates well with the mechanical environment and establishes an “optimal” structure.
9.2. Limitations

Anatomical geometries are complex and vary between individuals. Both the chondral defect as well as the neoarthrosis model were axisymmetric or 2D simplifications of the actual geometries, respectively. The main obstacle here is computational feasibility – aside from the creation and meshing of anatomical models the computational demand during the actual simulation increases significantly and biphasic contact modelling in these models is very challenging. While three-dimensional knee models have been created and are partially available online\(^1\), they are usually combined with simple material models (Donahue et al., 2002; Peña et al., 2007; Shirazi and Shirazi-Adl, 2009) or used to perform analyses of specific invariant states rather than feedback-driven simulations (Mononen et al., 2012). The simulations in this thesis required quite complex material models as well as many iterations to cover week-long experiments while maintaining a sufficiently fine time discretisation. Taken together, three dimensional anatomically accurate models are not a feasible option for this kind of simulation at present. Aside from that, a conceptual understanding requires a build-up of complexity – by taking out the dimension of complicated geometries the interpretation of the initial simulation results on the material level can be aided. The results presented in chapters 8 and 7 are generally expected to hold for more complex geometries. While local quantitative deviations are expected, the general heterogeneity of the mechanical environment is a result of the external loading and the local mechanical properties (e.g. depth-dependent composition). Their interplay determines both the predicted phenotype and the fibre architecture and should not change fundamentally.

The variations in the mechanical properties of both native and engineered tissues are significant. In order to simulate a specific experiment or establish composition-function relationships, sample specific fits are required. In all presented studies average material parameters were used. This is justified as no particular sample from any experiment was modelled but the investigations were conceptually focussed on the effects of tissue architecture. In this case it was considered more important to capture the relevant material effects rather than quantify them exactly for a specific sample.

The recruitment stretch proved to be an important contributor to the apparent material behaviour. Its parameter identification, especially in the context of

\(^1\)https://simtk.org/home/opennknee, Sible et al. (2010)
remodelling, is impaired by some significant conceptual difficulties: It has a di­
rect structural meaning but changing its value immediately affects the apparent
behaviour of the material. This coupling between the recruitment stretch and
the constitutive behaviour means that the recruitment stretch needs to be fit in
conjunction with the other material parameters. This requires detailed location
dependent experimental data on tissue deformations (obtained for example via
digital image correlation), ideally combined with structural information (crimp
and recruitment imaging), overall force data etc. Large scale inverse simulations
can then be used to extract the parameters. Until then, indirect evidence such
as swelling, imaging, stress-strain behaviour and scaffold contraction can allow a
rough parameter estimation.

Recently, collagen recruitment has been measured during deformation in arterial
tissue (Hill et al., 2012). The recruitment stretch was found to be statistically
distributed and significant recruitment occurred at finite strains (40% and above
from the stress-free tissue configuration). Valentín and Humphrey (2009) assumed
deposition stretches of 1.4 for elastin, 1.08 for collagen and 1.2 for smooth muscle
in arterial tissue. Modelling the evolution of the collagen architecture in corneo-
scleral shells, Grytz and Meschke (2010) assumed a homeostatic stretch of 1.001.
Finally, simulating chondrocyte hypertrophy, van Donkelaar and Wilson (2012)
assumed unstrained collagen deposition. The value of recruitment and deposition
stretches in native and engineered cartilage remains speculative. A parameter
variation was therefore performed in chapter 6.

The simulations in this work were intended to investigate phenomena that have
currently not been assessed directly in experiments, such as mechanoregulated re-
modelling of collagen recruitment in engineered cartilage. Some model aspects and
predictions therefore cannot be fully validated at the moment and remain some-
what speculative. While this validation is ultimately needed it also illustrates the
contribution simulations can make by allowing the systematic investigation of hy-
pothesised phenomena relying on physically based models (Kelly, 1998; Oberkampf
et al., 2004). The effect of collagen recruitment on the functionality of engineered
cartilage is a very novel idea. Conceptual frameworks like this represent a first step
towards rationalising and quantifying such phenomena. They can therefore be a
valuable tool in guiding and quantifying future experiments. The role of computer
simulations in mechanobiology has been defined by van der Meulen and Huiskes
(2002) as follows:

"Computational mechanobiologists hypothesize a potential rule and
determine if the outcome of this hypothesis produces realistic tissue structures and morphologies, hence trial-and-error. If the results correspond well, they might be an explanation for the mechanism being modeled. This method of research is common practice and productive in physics, less common in biology (Huiskes, 1995); although theoretical biology is based on this type of approach."

Computer models in general are being increasingly recognised as a pillar of scientific progress in its own right where a "really good dynamic computer model [...] is like a theory that throws off data" (Kelly, 1998).

9.3. Conclusions

- A framework has been established and verified that combines a versatile constitutive model for biological materials with a general remodelling framework for the collagen architecture.

- The functional significance of the Benninghoff architecture can only be explained in the context of swelling induced pre-stress. The mostly vertical fibre architecture in the deeper layers stiffens these regions, while the horizontal alignment in the superficial zone limits tensile strains in this layer caused by the contact of articulating bodies.

- It was shown that the mechanical environment, namely tissue deformation, can potentially explain collagen remodelling in engineered cartilage tissue.

- Remodelling of the collagen recruitment configuration can significantly affect the functionality of tissue engineered cartilage. This mechanism may have to be taken into account when interpreting composition-function relationships.

- Sufficient engineered tissue maturity has been shown to be beneficial for the establishment/maintenance of a Benninghoff structure in the replacement tissue within a chondral defect.

- The simulations further provided evidence that the evolving tissue architecture during skeletal healing is influenced by mechanical stimulation in a manner similar to other soft tissues.
9.4. Future work

The recruitment stretch has been shown to have a major impact on material behaviour and hence the functionality of engineered tissues. Its value in native tissue needs to be further quantified by performing mechanical tests in combination with imaging techniques. For articular cartilage, an overall indirect assessment via tests in varying salt concentrations can be a first step. In tissues with continuing ECM turnover in the sense of a homeostatic (stationary) state the homeostatic fibre stretch will be closely related to the deposition stretch of the ECM molecules (Humphrey, 1999). However, if there is an imbalance between synthesis and degradation, the two might differ and both need to be assessed. This relationship is not only amenable to experimentation but can be investigated using theories based on multigenerational growth (Ateshian and Ricken, 2010). In conjunction with frameworks such as the one presented here this could lead to the derivation of better evolution equations for the constituent fractions, material parameters and the recruitment stretch itself. In this context, collagen turnover \textit{per se}, i.e. its rate under various conditions, in engineered cartilage during bioreactor culture needs to be quantified further. Attention should be given to the formation of cross-links as well.

Only very few studies attempted to assess repair tissue architecture in (osteop)ochondral defects (Hunziker, 2001; Kim et al., 2010). Experiments that allow a quantification of these effects are needed. Until then, simulations remain somewhat speculative but can still produce more insight or pose suitable experimental hypotheses. Based on the final two studies (chapters 7 and 8) a logical progression would be to model osteochondral defect healing (Kelly and Prendergast, 2005, 2006). Tissue differentiation plays an important role in osteochondral defects and the establishment of a stable cartilage phenotype is often hindered by fibrocartilage formation in the superficial layers and an advancing bone front in the deeper layers (Hunziker, 2002; Gotterbarm et al., 2008). What role the lack of an arcade-like repair tissue architecture (Hunziker, 2002) plays in the long term failure of the repair cartilage could be investigated with the presented framework. Location specificity of the repair tissue quality (Buckwalter et al., 2003; Jung et al., 2009) is a further subject but requires the use of 3D models with the challenges described above. Detailed quantitative depth and location dependent data on cartilage architecture before and after repair are not available. Finally the question arises whether tissue repair and specifically the establishment of a native like architecture can be aided
9. Discussion

by a scaffold. Scaffold optimisation for osteochondral repair has been supported by computational modelling (Kelly and Prendergast, 2006).

The development of the cartilage structure during maturation is another interesting topic. Compositional changes (and hence swelling pressures) and increasing joint loading during maturation (Klein et al., 2007) are likely driving the reorganisation of the initially isotropic/horizontal architecture to the Benninghoff arcades. This hypothesis can be tested by implementing suitable evolution equations for the applied load and the biochemical composition into the present framework, possibly combining it with a growth model to capture joint morphogenesis (Heegaard et al., 1999). This development is also of interest for tissue engineers. Based on correlations between hydrostatic pressure and an inhibition of ossification as well as between shear strains and a promotion of ossification, it has been speculated that the establishment of the Benninghoff architecture during maturation may be key to the maintenance of a functional cartilage layer in the adult organism (van Turnhout et al., 2010a). In the light of the present simulations, this mechanism can be supported: The vertical fibrils in the deep zone increase swelling pressures and limit shear at the subchondral bone junction. With respect to osteochondral repair this means that an ossification front progressing into the cartilage layer might be prevented by raising hydrostatic pressures in the deep zone – one way of achieving this may be the establishment of a native architecture in the repair cartilage. These hypotheses can be approached with the present remodelling framework.

The constitutive model itself can be used in a number of ways. Together with biochemical data, changing composition-structure-function relationships with age or as a consequence of ECM accumulation during tissue engineering can be investigated by fitting the model to a series of mechanical tests. Thus, composition specific poroviscoelastic properties can be extracted and related to architectural aspects as well. Tissue engineered constructs with different architectures can further be used to quantify the interplay between structural and mechanical anisotropy (Thomopoulos et al., 2007; Sander et al., 2009; Lake et al., 2011).
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Appendix
A Derivations and Definitions

A.1. Integral theorems

The Green-Gauss-Ostrogradsky or divergence theorem reads for smooth vector \( \mathbf{a}(\mathbf{x}) \) and tensor \( \mathbf{A}(\mathbf{x}) \) fields, as well as scalar fields \( a \), derived by using \( \mathbf{A} = a \mathbf{I} \) and \( \text{div} \, (a \mathbf{I}) = \text{grad} \, a \) that are defined on a volume \( \Omega \) bound by a closed surface \( \Gamma \)

\[
\int_{\partial \Omega} \mathbf{a} \cdot \mathbf{n} \, d\Gamma = \int_{\Omega} \text{div} \, a \, d\Omega \quad (A.1)
\]

\[
\int_{\partial \Omega} \mathbf{A} \mathbf{n} \, d\Gamma = \int_{\Omega} \text{div} \, \mathbf{A} \, d\Omega \quad (A.2)
\]

\[
\int_{\partial \Omega} a \mathbf{n} \, d\Gamma = \int_{\Omega} \text{grad} \, a \, d\Omega \quad (A.3)
\]

Reynold’s transport theorem is useful for establishing the time derivative of an integral over a time-varying region \( \Omega \):

\[
\frac{d}{dt} \int_{\Omega} a(\mathbf{x}, t) \, d\Omega \quad (A.4)
\]

Transforming the integral to the (time-independent) reference configuration allows the differentiation under the integral. Using \( \dot{J} = J \text{div} \, \mathbf{v} \) and the material time
A. Derivations and Definitions

derivative one finds
\[
\frac{d}{dt} \int_{\Omega} a(x, t) d\Omega = \int_{\Omega} \frac{d}{dt} [a(x, t) J] d\Omega_0
\]
\[
= \int_{\Omega} \left[ \frac{\partial a}{\partial t} + \text{div} \, v \right] J d\Omega_0
\]
\[
= \int_{\Omega} \left[ \frac{\partial a}{\partial t} + \text{grad} \, a \cdot v + \text{div} \, v \right] d\Omega
\]
\[
= \int_{\Omega} \left[ \frac{\partial a}{\partial t} + \text{div} \, (a v) \right] d\Omega
\]

Using the divergence theorem an alternative formulation can be obtained:
\[
\frac{d}{dt} \int_{\Omega} a(x, t) d\Omega = \int_{\Omega} \frac{\partial a}{\partial t} d\Omega + \int_{\partial \Omega} a v \cdot n d\Gamma \tag{A.5}
\]

A.2. Entropy inequality for biphasic porous media

Relating to section 3.2.3, the entropy inequality for isothermal processes of a binary mixture is given as the sum of the constituent Clausius-Duhem inequalities (Görke et al., 2009; Ehlers, 2002)
\[
\sigma_S : d_S + \sigma_F : d_F - \rho_S \left( \tilde{\psi}_S \right)'_S - \rho_F \left( \tilde{\psi}_F \right)'_F - \dot{p}_F \cdot w_F \geq 0 \tag{A.6}
\]
Here, $\tilde{\psi}_\alpha$ are mass specific constituent free Helmholtz energy functions and the partial rate of deformation tensors
\[
d_\alpha = \frac{1}{2} \left[ \text{grad} \, v_\alpha + (\text{grad} \, v_\alpha)^T \right] \tag{A.7}
\]
have been used. Introducing an effective momentum production term analogous to the effective stress definition as
\[
\dot{p}_F = \dot{p}_F^E + p \text{grad} \, \phi_F \tag{A.8}
\]
the entropy inequality can be re-written in terms of the effective quantities (Görke et al., 2009) by adding the volume balance multiplied with a Lagrange multiplier

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as a constraint to the entropy inequality:

\[
\sigma_S : l_S + \sigma_F : l_F - \rho_S (\psi_S)' - \rho_F (\psi_F)' - \mathbf{p}_F \cdot \mathbf{w}_F \\
+ p [\text{div} \mathbf{v}_s + \phi_F (\text{div} \mathbf{v}_F - \text{div} \mathbf{v}_s) + \text{grad} \phi_F \cdot \mathbf{w}_F]
\]

\[
= \sigma_S : l_S + \sigma_F : l_F - \rho_S (\psi_S)' - \rho_F (\psi_F)' - \mathbf{p}_F \cdot \mathbf{w}_F \\
+ p [\phi_S \text{div} \mathbf{v}_s + \phi_F \text{div} \mathbf{v}_F + \text{grad} \phi_F \cdot \mathbf{w}_F]
\]

\[
= \sigma_S : l_S + \sigma_F : l_F - \rho_S (\psi_S)' - \rho_F (\psi_F)' - \mathbf{p}_F \cdot \mathbf{w}_F \\
+ p [I : (\phi_S l_s + \phi_F l_F) + \text{grad} \phi_F \cdot \mathbf{w}_F]
\]

\[
= (\sigma_S + p\phi_S I) : l_S + (\sigma_F + p\phi_F I) : l_F - \rho_S (\psi_S)' - \rho_F (\psi_F)' \\
- (\mathbf{p}_F - p\text{grad} \phi_F) \cdot \mathbf{w}_F
\]

\[
= \sigma_S^E : d_S + \sigma_F^E : d_F - \rho_S (\tilde{\psi}_S)' - \mathbf{p}_F^E \cdot \mathbf{w}_F \geq 0 \quad (A.9)
\]

Use was made of the double contraction of a spherical tensor with another tensor only affecting the latter’s diagonal elements. If that tensor is a gradient, this leads to the divergence (trace of the gradient).

We further consider the neglect of dissipative effects due to inner friction in the fluid\(^1\) and hence are left with

\[
\sigma_S^E : d_S - \rho_S (\tilde{\psi}_S)' - \mathbf{p}_F^E \mathbf{w}_F \geq 0 \quad (A.10)
\]

The transformation of the remainder

\[
\sigma_S^E : d_S - \rho_S (\tilde{\psi}_S)' \geq 0 \quad (A.11)
\]

into the reference configuration follows as (without loss of generalisation performed in Cartesian coordinates):

\[
\frac{1}{J_S} \sigma_{ijkl}(v_{i,j} + v_{j,i}) - \rho_S (\tilde{\psi}_S)'
\]

\[
= \frac{1}{J_S} F_{ik} T_{KL} F_{j,k} \frac{1}{2}(v_{i,j} + v_{j,i}) - \frac{\rho_{S0}}{J_S} \dot{\psi}_S
\]

\[
= \frac{1}{2} T_{KL} \left( \frac{\partial x_i}{\partial X_K} \frac{\partial x_i}{\partial X_L} + \frac{\partial x_j}{\partial X_K} \frac{\partial x_j}{\partial X_L} \right) - \rho_{S0} \psi_S
\]

\[
= - \rho_{S0} \psi_S + \frac{1}{2} T_{S}^E : \dot{\mathbf{C}}_S \quad (A.12)
\]

Here, \(\rho_{S0}\) is the partial solid density in the reference configuration, not to be mistaken with the true solid density \(\rho_{SR}\).

\(^{1}\)The fluid then only carries hydrostatic stresses: \(\sigma_F = -p\phi_F \mathbf{I}\) and the extra stress vanishes.
A.3. Elastic tangent moduli in Marc and Abaqus

Marc offers the choice between the Total Lagrangian (TL) and Updated Lagrangian (UL) version of its user material interface hypela2. The most convenient and straightforward way is the TL option, where second Piola-Kirchhoff stresses $T$ and the corresponding material tangent (assuming hyperelasticity)

$$\mathbb{C} = 2 \frac{\partial T}{\partial \mathbb{C}} = 4 \frac{\partial^2 \psi}{\partial \mathbb{C}^2}$$  \hfill (A.13)

In the UL version, the Cauchy stresses $\sigma$ and the spatial tangent $\mathbb{C} = 2 \mathbb{I} = 4 \mathbb{I} (A.13)$

$$c = g \mathbb{I}$$  \hfill (A.14)

are required, where $c$ is the spatial elasticity of the Kirchhoff stress $\tau = J \sigma$. Given the material tangent $\mathbb{C}$ the following push-forward operation can be used

$$c_{abcd} = F_{aA}F_{bB}F_{cC}F_{dD}C_{ABCD}$$  \hfill (A.15)

Abaqus requires Cauchy stresses and a tangent that is related to the Jaumann rate of the Kirchhoff stress tensor. For a thorough derivation, see Stein and Gautam (2008). The required tangent $\tilde{c}$ has the components

$$\tilde{c}_{abcd} = c_{abcd} + \frac{1}{2} \left[ \delta_{ac} \sigma_{bd} + \sigma_{ac} \delta_{bd} + \delta_{ad} \sigma_{bc} + \sigma_{ad} \delta_{bc} \right]$$

$$= \frac{1}{J} \left( c_{abcd} + \frac{1}{2} \left[ \delta_{ac} \tau_{bd} + \tau_{ac} \delta_{bd} + \delta_{ad} \tau_{bc} + \tau_{ad} \delta_{bc} \right] \right)$$  \hfill (A.16)

A.4. Isotropic hyperelasticity

For the constitutive model under consideration the partial derivatives $\frac{\partial \psi}{\partial I_3}$ can be determined as:

$$\frac{\partial \psi_{iso}}{\partial I_1} = C_1 e^{\alpha(I_1 - \ln I_3 - 3)}$$  \hfill (A.17)

$$\frac{\partial \psi_{iso}}{\partial I_3} = 2 D_2 \ln I_3 - C_1 e^{\alpha(I_1 - \ln I_3 - 3)}$$ \hfill (A.18)

The use of the chain rule yields the material elasticity tensor as (Holzapfel, 2008):

$$\mathbb{C} = \delta_1 \mathbb{I} \otimes \mathbb{I} + \delta_2 (\mathbb{I} \otimes \mathbb{C} + \mathbb{C} \otimes \mathbb{I}) + \delta_3 (\mathbb{I} \otimes \mathbb{C}^{-1} + \mathbb{C}^{-1} \otimes \mathbb{I}) +$$

$$+ \delta_4 \mathbb{C} \otimes \mathbb{C} + \delta_5 (\mathbb{C} \otimes \mathbb{C}^{-1} + \mathbb{C}^{-1} \otimes \mathbb{C}) +$$

$$+ \delta_6 \mathbb{C}^{-1} \otimes \mathbb{C}^{-1} + \delta_7 \mathbb{C}^{-1} \otimes \mathbb{C}^{-1} + \delta_8 \frac{\mathbb{I} + \mathbb{I}}{2}$$  \hfill (A.19)
A. Derivations and Definitions

with the definitions of some fourth order tensors

\[
(C^{-1} \otimes C^{-1})_{ABCD} = \frac{1}{2} (C^{-1}_{AC} C^{-1}_{BD} + C^{-1}_{AD} C^{-1}_{BC}) \quad (A.20)
\]

\[
\mathbb{I} = \delta_{ik} \delta_{jl} e_i \otimes e_j \otimes e_k \otimes e_l \quad (A.21)
\]

\[
\mathbb{I} = \delta_{ij} \delta_{kl} e_i \otimes e_j \otimes e_k \otimes e_l \quad (A.22)
\]

\[
\mathbf{I} \otimes \mathbf{I} = \delta_{ij} \delta_{kl} e_i \otimes e_j \otimes e_k \otimes e_l \quad (A.23)
\]

Again dropping the dependence on the third invariant simplifies this relation to

\[
C^{(4)}_i = \delta_1 \mathbf{I} \otimes \mathbf{I} + \delta_3 (\mathbf{I} \otimes C^{-1} + C^{-1} \otimes \mathbf{I}) + \delta_6 C^{-1} \otimes C^{-1} + \delta_7 C^{-1} \otimes C^{-1} \quad (A.24)
\]

with the coefficients

\[
\delta_1 = 4 \frac{\partial^2 \psi}{\partial I_1^2}
\]

\[
\delta_3 = 4I_3 \frac{\partial^2 \psi}{\partial I_1 \partial I_3}
\]

\[
\delta_6 = 4 \left( I_3 \frac{\partial \psi}{\partial I_3} + I_3 \frac{\partial^2 \psi}{\partial I_3^2} \right)
\]

\[
\delta_7 = -4I_3 \frac{\partial \psi}{\partial I_3}
\]

The non-zero second order derivatives of the strain energy densities are:

\[
\frac{\partial^2 \psi_{iso}}{\partial I_1^2} = C_1 \alpha e^{\alpha(I_1 - \ln I_3 - 3)} \quad (A.25)
\]

\[
\frac{\partial^2 \psi_{iso}}{\partial I_3^2} = 2D_2 (1 - \ln I_3) + C_1 (\alpha + 1) e^{\alpha(I_1 - \ln I_3 - 3)} \quad (A.26)
\]

\[
\frac{\partial^2 \psi_{iso}}{\partial I_1 \partial I_3} = -\frac{C_1 \alpha}{I_3} e^{\alpha(I_1 - \ln I_3 - 3)} \quad (A.27)
\]

A.5. Anisotropic hyperelasticity

There is only one non-zero component per family of fibres additionally contributing to the fourth order elasticity tensor \( C \) due to the previous split into \( \psi_{iso} \) and \( \psi_{aniso} \) as well as the fact, that \( \frac{\partial^2 I_1}{\partial C^2} = 0 \). Thus we introduce the factors \( \delta_9' \) as follows

\[
\delta_9' = 4 \frac{\partial^2 \psi_{aniso}}{\partial (I_1)^2} \quad (A.28)
\]
and get the new elasticity tensor as

\[
\mathbf{C} = \delta_1 \mathbf{I} \otimes \mathbf{I} + \delta_3 (\mathbf{I} \otimes \mathbf{C}^{-1} + \mathbf{C}^{-1} \otimes \mathbf{I}) + \delta_0 \mathbf{C}^{-1} \otimes \mathbf{C}^{-1} + \delta_7 \mathbf{C}^{-1} \otimes \mathbf{C}^{-1} + \sum_{i=1}^{n_{6h}} \delta_0^i \mathbf{a}_0^i \otimes \mathbf{a}_0^i \otimes \mathbf{a}_0^i \otimes \mathbf{a}_0^i
\]

(A.29)

The necessary partial derivatives for the various SED formulations are

\[
\frac{\partial \psi_{\text{Fung}}}{\partial \mathbf{I}_4} = C_4^i (\mathbf{I}_4^i - 1) e^{\beta (\mathbf{I}_4^i - 1)^2}
\]

(A.30)

\[
\frac{\partial \psi_{\text{Gent}}}{\partial \mathbf{I}_4} = 2C_4^i J_a^i (\mathbf{I}_4^i - 1)
\]

(A.31)

\[
\frac{\partial \psi_{\text{Ateshian}}}{\partial \mathbf{I}_4} = C_4^i \beta^i (\mathbf{I}_4^i - 1)^{\beta - 1}
\]

(A.32)

\[
\frac{\partial^2 \psi_{\text{Fung}}}{\partial (\mathbf{I}_4)^2} = C_4^i [1 + 2\beta (\mathbf{I}_4^i - 1)^2] e^{\beta (\mathbf{I}_4^i - 1)^2}
\]

(A.33)

\[
\frac{\partial^2 \psi_{\text{Gent}}}{\partial (\mathbf{I}_4)^2} = 2C_4^i J_a^i [J_a^i + (\mathbf{I}_4^i - 1)^2]
\]

(A.34)

\[
\frac{\partial^2 \psi_{\text{Ateshian}}}{\partial (\mathbf{I}_4)^2} = C_4^i \beta^i (\mathbf{I}_4^i - 1)^{\beta - 2}
\]

(A.35)

### A.6. Nonlinear viscoelasticity

#### A.6.1. Useful derivations

With the assumption that the potential \( \bar{\psi}_v \) is an isotropic function of \( \mathbf{\varepsilon}_{ev} \) the following simplification can be used:

\[
\frac{\partial \bar{\psi}_v}{\partial \mathbf{\varepsilon}_{ev}} : (\mathbf{L}_v^T \mathbf{\varepsilon}_{ev} + \mathbf{\varepsilon}_{ev} \mathbf{L}_v) = 2\mathbf{\varepsilon}_{ev} \frac{\partial \bar{\psi}_v}{\partial \mathbf{\varepsilon}_{ev}} : \mathbf{\Delta}_v \quad (A.36)
\]

With the definition

\[
\frac{\partial \bar{\psi}_v}{\partial \mathbf{\varepsilon}_{ev}} =: \mathbf{A} \quad (A.37)
\]

and exploiting the symmetry of \( \mathbf{\varepsilon}_{ev} \) and \( \mathbf{A} \) (both are coaxial and commute due to the isotropy assumption) the left hand side of A.36 can be rewritten:

\[
2A_{ij} [(\mathbf{\varepsilon}_{ev})_{ik}(l_v)_{kj}] = A_{ij}(\mathbf{\varepsilon}_{ev})_{ik}[(l_v)_{kj}+(l_v)_{jk}]
\]
Using
\[ \Delta \varepsilon_v = \frac{1}{2} F_-^{-T} \left[ F_+^T (I - F_-^{-T} F_-^{-1}) F_-^{-1} \right] F_-^{-1} \]
\[ = \frac{1}{2} \left( F_-^{-T} F_-^T + F_-^{-T} F_-^{-1} \right) \]
\[ = \frac{1}{2} (I_+^T + I_-) \quad (A.38) \]
the last line of the derivation in index notation can be rewritten in symbolic notation (again, exploiting symmetries)
\[ 2\varepsilon_{\gamma \kappa} A : \Delta \varepsilon_v \quad (A.39) \]
which concludes the proof of A.36

### A.6.2. Time integration

To implement these constitutive relations into the user material, the evolutional equation 3.115 has to be integrated numerically. For the time integration of eq. 3.115 a generalised implicit single step scheme was used. Consider the ordinary differential equation
\[ \frac{dy}{dt} = \dot{y} = f(t, y) \quad (A.40) \]
It can be discretised with \( \Delta t = t_{n+1} - t_n \) as follows
\[ y_{n+1} = y_n + [\zeta f_{n+1} + (1 - \zeta) f_n] \Delta t \quad (A.41) \]
where
\[ \zeta \in [0, 1] \quad \zeta = \begin{cases} 
0.0 & : \text{Euler forward} \\
1.0 & : \text{Euler backward} \\
0.5 & : \text{Crank - Nicholson} 
\end{cases} \quad (A.42) \]
Applying that scheme to the evolution equation of the viscous right Cauchy-Green tensor
\[ \dot{C}_v = \frac{1}{\eta_v} C_v T_{\alpha \kappa} C \quad (A.43) \]
\[ \text{Alternatively, the Taylor series expansion} \ y_{n+1} = y_n + \dot{y} \Delta t \text{ can be used where} \ \dot{y} \text{ can be approximated by a forward, backward or central difference.} \]

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produces after a quick rearrangement

\[
C_v^{n+1} = C_v^n + \frac{1 - \zeta}{\eta_v^n} \Delta t T_{ov}^n C^n \left( I - \frac{\zeta}{\eta_v^{n+1}} \Delta t T_{ov}^{n+1} C^{n+1} \right) = C_v AB^{-1} \quad (A.44)
\]

\( \eta_v^{n+1}, C_v^{n+1} \) and \( T_{ov}^{n+1} \) are therefore determined iteratively within each FE iteration while \( \eta_v^n, C_v^n \) and \( T_{ov}^n \) are the values from the previous converged increment.

**A.7. Swelling**

For the tangent modulus one needs:

\[
\frac{\partial^2 \psi_{osm}}{\partial I_3^2} = \frac{RT}{2J_S^2} \left[ \frac{c_F^2}{4(J_S - \phi_{S0}) \sqrt{c_{ext}^2 + \frac{c_L^2}{4}}} + \frac{\sqrt{c_{ext}^2 + \frac{c_L^2}{4}}}{J_S} \right] \quad (A.45)
\]

**A.7.1. Material parameter modification**

Swelling pressures constitute a volume-dependent isotropic stress tensor in the current configuration. Thus they support the material against volumetric deformations rather than deviatoric strains.

Consider the 2\textsuperscript{nd} Piola-Kirchhoff stress tensor.

\[
T = 2 \frac{\partial \psi}{\partial I_1} I + 2 \frac{\partial \psi}{\partial I_3} I_S C^{-1} \quad (A.46)
\]

After a push-forward we get with the left Cauchy-Green tensor \( b = FF^T \):

\[
\sigma = 2J^{-1} \frac{\partial \psi}{\partial I_1} b + 2J \frac{\partial \psi}{\partial I_3} I \quad (A.47)
\]

The aim of the material parameter modification now is to achieve the same initial compressive response of the charged and uncharged materials.

The compressive response of a material can be described by the compressive modulus \( K \) which relates the hydrostatic pressure to the volume change at small strains: \( p = K e \). Here, \( e = \text{tr} \epsilon \) and \( p = \frac{1}{3} \text{tr} \sigma \). To determine the compressive modulus, we therefore derive \( \text{tr} \sigma \).

\[
\text{tr} \sigma = 2J^{-1} \frac{\partial \psi}{\partial I_1} \text{tr} b + 6J \frac{\partial \psi}{\partial I_3} \quad (A.48)
\]
A. Derivations and Definitions

Assuming small strains the following Taylor-type approximations will be applied:

\[(1 \pm x)^n \approx 1 \pm nx \quad \ln(x + 1) \approx x \quad (A.49)\]

and therefore

\[\text{tr } b \approx 3 + 2e \quad J \approx e + 1 \quad (A.50)\]

so that finally

\[p \approx \frac{6 + 4e}{3(1 + e)} \frac{\partial \psi}{\partial I_1} \bigg|_{e \ll 1} + 2(e + 1) \frac{\partial \psi}{\partial I_3} \bigg|_{e \ll 1} \quad (A.51)\]

For the standard Neo-Hooke formuilation the partial derivatives at small strains are

\[\frac{\partial \psi}{\partial I_1} = C_1 \quad \frac{\partial \psi}{\partial I_3} \approx \frac{4D_2e - C_1}{2e + 1} \quad (A.52)\]

so that for the hydrostatic pressure in dependence on the volumetric strain

\[p \approx C_1 \frac{6 + 4e}{3(1 + e)} + \frac{2(e + 1)}{2e + 1} (4D_2e - C_1) \quad (A.53)\]

The initial compressive modulus for that material can now be found as

\[K = \frac{\partial p}{\partial e} \bigg|_{e=0} = 8D_2 + \frac{4}{3} C_1 \quad (A.54)\]

Note: Inserting the small strain approximations \(C_1 = \frac{E}{4(1+\nu)}\) and \(D_2 = \frac{E\nu}{8(1+\nu)(1-2\nu)}\) into the above relation yields \(K = \frac{E}{3(1-2\nu)}\) which is the known result from linear elasticity. As a Neo-Hookean material approaches a linear Hookean one at small strains, this result is expected.

If we now look at the combination of Neo-Hookean material with swelling, the third invariant is extended and \(p\) reads at small strains (neglecting higher order terms):

\[p \approx C_1 \frac{6 + 4e}{3(1 + e)} + \frac{2(e + 1)}{2e + 1} (4D_2e - C_1) - 2RT \left[ \sqrt{c_{ext}^2 + \frac{c_{F0}^2}{4(\phi / F_0)^2}} - c_{ext} \right] \quad (A.55)\]

so that the initial compressive modulus is

\[K = \frac{\partial p}{\partial e} \bigg|_{e=0} = 8D_2 + \frac{4}{3} C_1 + \frac{RTc_{F0}^2}{2\phi / F_0 \sqrt{c_{ext}^2 + \frac{c_{F0}^2}{4}}} \quad (A.56)\]

The last term in this equation will be called the osmotic modulus \(\Pi\):

\[\Pi = \frac{\partial (-\Delta \pi)}{\partial e} = \frac{RTc_{F0}^2}{2\phi / F_0 \sqrt{c_{ext}^2 + \frac{c_{F0}^2}{4}}} \quad (A.57)\]
A. Derivations and Definitions

Thus, with the shear modulus unaffected, i.e. $C_{1}^{app} = C_{1}^{intr}$, the modified value $D_{2}^{intr}$ yielding the same initial compressive stiffness is obtained from the relation

$$8D_{2}^{app} + \frac{4}{3}C_{1}^{app} = 8D_{2}^{intr} + \frac{4}{3}C_{1}^{intr} + \Pi$$

(A.58)

as

$$D_{2}^{intr} = D_{2}^{app} - \frac{\Pi}{8}$$

(A.59)

Thus, one can use the apparent biphasic material properties and modify $D_{2}$ when osmotic swelling is included. The value for $c_{ext}$ will be the value of the solution in which material testing took place (solution mostly 0.15 M).

A limitation of this approach is the following: Simulations will begin with a swelling step inducing a strain. Therefore, initially entered material parameters are not the apparent parameters in the free swollen state due to nonlinear effects. This effect grows in significance with the magnitude of swelling strains. It can only be overcome by iteratively adapting the initially entered material parameters until the desired values are reached in the free swelling state.

Finally, relation A.59 will be related to the usual descriptive small strain material parameters $E$, $\nu$ and $H_{A}$. The Lamé constants are related to the Neo-Hookean material parameters at small strains:

$$\mu = 2C_{1} \quad \text{and} \quad \lambda = \frac{E\nu}{(1+\nu)(1-2\nu)} = 8D_{2}$$

(A.60)

Thus we find

$$\mu^{app} = \mu^{intr} \quad \text{and} \quad \lambda^{app} = \lambda^{intr} + \Pi$$

(A.61)

With $\nu = \frac{\lambda}{2(\lambda+\mu)}$ and $E = \frac{\mu(3\lambda+2\mu)}{\lambda+\mu}$ one arrives at

$$E^{app} = \frac{\mu(3\lambda^{intr} + 2\mu + 3\Pi)}{\lambda^{intr} + \mu + \Pi} \quad \text{and} \quad \nu^{app} = \frac{\lambda^{intr} + \Pi}{2(\lambda^{intr} + \mu + \Pi)}$$

(A.62)

The aggregate modulus $H_{A} = \lambda + 2\mu$ follows as

$$H_{A}^{app} = H_{A}^{intr} + \Pi$$

(A.63)

The dependence of the apparent Young’s modulus, Poisson’s ratio and aggregate modulus on the FCD is plotted in Fig. A.1.
A. Derivations and Definitions

![Graphs showing dependence of Young's modulus, Poisson's ratio, and aggregate modulus on FCD and bath salt concentrations.](image)

Figure A.1: Dependence of the apparent Young's modulus, Poisson's ratio and aggregate modulus of an isotropic linear elastic biphasic material on the FCD for various bath salt concentrations and two intrinsic Poisson’s ratios: 0.1 (solid lines) and 0.3 (dashed lines).

A.8. Treatment of rotations

With

\[ \mathbf{\omega} = \begin{pmatrix} \omega_1 \\ \omega_2 \\ \omega_3 \end{pmatrix} \quad \text{and} \quad \dot{\mathbf{\omega}} = \begin{pmatrix} 0 & -\omega_3 & \omega_2 \\ \omega_3 & 0 & -\omega_1 \\ -\omega_2 & \omega_1 & 0 \end{pmatrix} \quad (A.64) \]

\[ \dot{\mathbf{a}} = \mathbf{\omega} \times \mathbf{a}. \]  

If \( \mathbf{\omega} \) is a unit vector it can be shown that

\[ \dot{\mathbf{\omega}} = -\omega^3 \mathbf{\omega}^5 = -\omega^7 = \ldots \quad \text{and} \quad \dot{\mathbf{\omega}}^2 = -\omega^4 = \omega^6 = -\omega^8 = \ldots \quad (A.65) \]

The extraction of the rotation vector is based on a singularity-free algorithm to compute the quaternion \( \mathbf{q} = [q_0, \mathbf{q}] = [\cos(\theta/2), \sin(\theta/2)\mathbf{e}] \), for details see Spurrier (1978); Menzel (2007). The algorithm is as follows:
A. Derivations and Definitions

Algorithm 1 Extraction of the quaternion $\mathbf{q} = [q_0, \mathbf{q}] = [q_0, q_1, q_2, q_3]$ using a singularity-free algorithm (Spurrier, 1978). Note, that $i, j, k$ follows a cyclic permutation of 1, 2, 3.

if $\text{tr} \mathbf{R} > R_{11}, R_{22}, R_{33}$ then
  $q_0 = \frac{1}{2} \sqrt{1 + \text{tr} \mathbf{R}}$
  $q_i = \frac{1}{4q_0}(R_{kj} - R_{jk})$
else
  $R_{(ii)} > R_{(jj)} \geq R_{(kk)}$
  $q_i = \sqrt{\frac{1}{2}R_{(ii)} + \frac{1}{4}(1 - \text{tr} \mathbf{R})}$
  $q_0 = \frac{1}{4q_i}(R_{kj} - R_{jk})$
  $q_i = \frac{1}{4q_i}(R_{li} - R_{il}) \quad l = j, k$
end if

$\theta = 2\arccos q_0$

$e = q/\sin(\theta/2)$

A.9. Extrafibrillar fluid volume fraction

In our notation, Wilson et al. (2007) present for the extrafibrillar fluid volume fraction:

$$\phi_{F,e} = \frac{\tilde{\rho} \mu_{F,e}}{1 - \mu_{F,e} + \tilde{\rho} \mu_{F,e}} \quad (A.66)$$

This appears inaccurate, as the following derivation shows:

$$\phi_{F,e} = \frac{d\Omega_{F,e}}{d\Omega} = \frac{d\Omega_{F,e}}{d\Omega_S + d\Omega_F} \quad (A.67)$$

With the true densities the partial volumes can be substituted by the corresponding partial masses and, after division of both numerator and denominator with the total mass element $dm$, their mass fractions:

$$\phi_{F,e} = \frac{dm_{F,e}}{\tilde{\rho}_{FR} + \tilde{\rho}_S} = \frac{dm_{F,e}}{\tilde{\rho}_{FR} + \rho_{SR}} \quad (A.68)$$

The substitutions $\mu_S = 1 - \mu_F$ and $\tilde{\rho} = \rho_{SR}/\tilde{\rho}_{FR}$ finally yield

$$\phi_{F,e} = \frac{\tilde{\rho} \mu_{F,e}}{1 - \mu_F + \tilde{\rho} \mu_F} \quad (A.69)$$

The maximum deviation of Eqs. A.66 and A.69 with the presented compositional data occurs in the deep zone and is on the order of 15% (not solved iteratively).
B Model Verification and Tests

B.1. Transverse isotropy

Transversely isotropic tension-compression nonlinear fibre reinforcement was verified simulating a uniaxial tensile and compressive test of a homogeneous material. Consider a brick-shaped specimen with edges parallel to the Cartesian coordinate axes. This specimen will be stretched along the 1-direction. For uniaxial tension and compression in the direction of the Cartesian basis vector \( e_1 \) the deformation gradient reads

\[
F_{ij} = \text{diag}(\lambda_1, \lambda_2, \lambda_3) \quad (B.1)
\]

with \( \lambda_i \) the stretches along the coordinate axes / edges of the specimen, so that for example \( \lambda_1 = \frac{L}{L_0} \). The right Cauchy-Green tensor and its inverse follow as

\[
C_{ij} = \text{diag}(\lambda_1^2, \lambda_2^2, \lambda_3^2) \quad \text{and} \quad C_{ij}^{-1} = \text{diag}(\lambda_1^{-2}, \lambda_2^{-2}, \lambda_3^{-2}) \quad (B.2)
\]

The values for the first and third invariant of \( C \) and \( J = \det F \) are then

\[
I_1^C = \lambda_1^2 + \lambda_2^2 + \lambda_3^2 \quad I_3^C = \lambda_1^2 \lambda_2^2 \lambda_3^2 \quad J = \lambda_1 \lambda_2 \lambda_3 \quad (B.3)
\]

The fibre direction was defined parallel to the direction of loading, as would be the case in tendons or ligaments for example:

\[
a_0 \equiv e_1 \quad (B.4)
\]

The Fung-like model for fibre reinforcement was used (Eq. 3.80). Simulations of a cylindrical bar that is compressed and stretched in its axial direction were performed and compared to two analytical solutions. A Neo-Hookean ground phase material was used (\( \alpha = 0 \)). In case 1 the material was highly compressible to show no lateral expansion (\( D_2 = 0 \text{ MPa} \)). The second Piola-Kirchhoff stress in axial direction then followed via

\[
T_{11} = 2 \left[ C_1(1 - \lambda_1^{-2}) + C_4(\lambda_1^2 - 1)e^{\beta_0(\lambda_1^2-1)^2} \right] \quad (B.5)
\]
In case 2 the material was modelled as nearly incompressible with $D_2 = 499.5$ MPa. For the analytical solution the indeterminate pressure term $p_s$ was introduced as follows:

$$T = 2 \left[ C_1 \left( I - C^{-1} \right) + 2D_2 \ln I_3 C^{-1} \right] = p_s \quad \text{(B.6)}$$

Here $C$ shall only contain deviatoric deformations so that $\lambda_2 = \lambda_3 = \lambda_1^{-0.5}$. This solution strategy was motivated by the Flory split of the deformation gradient into purely volumetric and deviatoric parts usually used in incompressible hyperelasticity. Using $T_{22} = 0$ we find for the pressure term

$$p_s = C_1 \left( 1 - \lambda_1^{-1} \right) \quad \text{(B.7)}$$

The axial second Piola-Kirchhoff stress is then found as

$$T_{11} = 2 \left[ C_1 \left( 1 - \lambda_1^{-2} \right) + p_s \lambda_1^{-2} \right] = 2C_1 \left( 1 - \lambda_1^{-3} \right) \quad \text{(B.8)}$$

and with fiber reinforcement

$$T_{11} = 2 \left[ C_1 \left( 1 - \lambda_1^{-3} \right) + C_4 \left( \lambda_1^2 - 1 \right) e^{\beta_a (\lambda_1^2 - 1)^2} \right] \quad \text{(B.10)}$$

Material parameters were arbitrarily chosen to illustrate the effect of tension-compression nonlinearity in principle: $C_1 = 2$ MPa, $\beta_a = 0.5$ as well as $C_4 = 20.0$. Cauchy stresses were determined from second Piola-Kirchhoff stresses via the relation

$$\sigma = J^{-1} F T F^T \quad \text{(B.11)}$$

Verification was successful, showing good agreement between the simulation results and the analytical solution (fig. B.1a). The effect of tension-compression nonlinearity is clearly evident at the transition between tensile and compressive loading ($\lambda_1 = 1$).

The compressible case was also used to demonstrate the influence of fiber orientation. Plane strain simulations were performed of a rectangular prism being compressed and stretched along one of its main axes. Fibers were either oriented parallel, perpendicular or $\pm 45^\circ$ to the direction of loading.

Varying fiber angles from $0^\circ$ over $45^\circ$ to $90^\circ$ demonstrated the expected effect: the highest stresses occurred when the fibers were aligned in the direction of loading, no effect was apparent when they were perpendicular to the loading direction and
an intermediate result was obtained for fiber angles of \( \pm 45^\circ \) (Fig. B.1b). Due to rotation of the fibers lateral contraction occurred despite \( D_2 = 0 \).

![Graphs showing tension compression nonlinearity and anisotropy](image)

Figure B.1.: (a) Tension compression nonlinearity for varying compressibility. (b) Stress-stretch curves for varying fibre alignments. Box shows lateral contraction due to fibre rotation.

### B.2. Viscoelasticity

#### B.2.1. Analytical solution

For simplicity \( D_2 = 0 \). Hence, the equilibrium and overstresses follow as

\[
T_{eq} = 2C_1(I - C^{-1}) \tag{B.12}
\]

\[
T_{ov} = 2C_{1v}(C_{v}^{-1} - C^{-1}) \tag{B.13}
\]

Deformation tensors for uniaxial loading:

\[
C_{ij} = \text{diag}(\lambda^2, 1, 1) \quad C_{ij}^{-1} = \text{diag}(\lambda^{-2}, 1, 1) \tag{B.14}
\]

\[
(C_v)_{ij} = \text{diag}(\lambda_v^2, 1, 1) \quad (C_v^{-1})_{ij} = \text{diag}(\lambda_v^{-2}, 1, 1) \tag{B.15}
\]

Hence, the axial stress components are

\[
T_{eq}^{11} = 2C_1(1 - \lambda^{-2}) \tag{B.16}
\]

\[
T_{ov}^{11} = 2C_{1v}(\lambda_v^{-2} - \lambda^{-2}) \tag{B.17}
\]

The evolution equation

\[
\dot{C}_v = \frac{1}{\eta_v} C_v T_{ov} C \tag{B.18}
\]
B. Model Verification and Tests

can be simplified to
\[ \dot{C}_v = \frac{2C_{1v}}{\eta_v} (C - C_v) \]  
with the one component of relevance
\[ (\lambda_v^2) = \frac{2C_{1v}}{\eta_v} (\lambda^2 - \lambda_v^2) \]
With constant viscosity and constant stretch it can be solved as
\[ \lambda_v^2 = \lambda^2 - (\lambda^2 - \lambda_v^2_0)e^{-\frac{2C_{1v}}{\eta_v}(t-t_0)} \]
Including two Maxwell elements parallel to the equilibrium spring, the total stress will be
\[ T^{11} = 2C_1(1 - \lambda^{-2}) + 2C_{1v1} \left( \frac{1}{\lambda^2 - (\lambda^2 - \lambda_v^2_0)e^{-\frac{2C_{1v}}{\eta_v}(t-t_0)}} - \lambda^{-2} \right) + 2C_{1v2} \left( \frac{1}{\lambda^2 - (\lambda^2 - \lambda_v^2_0)e^{-\frac{2C_{1v2}}{\eta_v2}(t-t_0)}} - \lambda^{-2} \right) \]
The influence of the various viscoelastic parameters was tested. Material parameters for the 5 test simulations are listed in table B.1 Analytically a step load was modelled, while numerically the deformation (unconfined compression) was applied within 0.01 s and subsequently held constant. The sets have the following motivation:

- Set \( C_{1v} \): The spring stiffness of the Maxwell element is varied.
- Set \( \eta_v \): The viscosity of the dashpot of the Maxwell element is varied.
- Set \( \lambda \): The applied compression level is varied.
- Set 2 Maxwell: The influence of two distinct relaxation time scales is modelled, i.e. two Maxwell elements are included and their time constants varied via their respective dashpot viscosities.

<table>
<thead>
<tr>
<th>parameter unit</th>
<th>( C_1 ) [MPa]</th>
<th>( \nu ) [-]</th>
<th>( C_{1v1} ) [MPa]</th>
<th>( C_{1v2} ) [MPa]</th>
<th>( \eta_{v1} ) [MPa s]</th>
<th>( \eta_{v2} ) [MPa s]</th>
<th>( \nu_{v1} ) [-]</th>
<th>( \nu_{v2} ) [-]</th>
<th>( \lambda ) [-]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Set ( C_{1v} )</td>
<td>1.0</td>
<td>0.0</td>
<td>0.5/1.0/2.0</td>
<td>0.0</td>
<td>10.0</td>
<td>-</td>
<td>0.0</td>
<td>0.0</td>
<td>0.9</td>
</tr>
<tr>
<td>Set ( \eta_v )</td>
<td>1.0</td>
<td>0.0</td>
<td>1.0</td>
<td>0.0</td>
<td>2.5/5.0/10.0</td>
<td>-</td>
<td>0.0</td>
<td>0.0</td>
<td>0.9</td>
</tr>
<tr>
<td>Set ( \lambda )</td>
<td>1.0</td>
<td>0.0</td>
<td>1.0</td>
<td>0.0</td>
<td>10.0</td>
<td>-</td>
<td>0.0</td>
<td>0.0</td>
<td>0.9/0.9/0.95</td>
</tr>
<tr>
<td>Set 2 Maxwell</td>
<td>1.0</td>
<td>0.0</td>
<td>1.0</td>
<td>1.0</td>
<td>10.0/25.0</td>
<td>2.5/1.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.9</td>
</tr>
<tr>
<td>Set ( D_{2v} )</td>
<td>1.0</td>
<td>0.1</td>
<td>0.5</td>
<td>0.5</td>
<td>25.0</td>
<td>5.0</td>
<td>0.4</td>
<td>0.4</td>
<td>0.9</td>
</tr>
<tr>
<td>Set rate</td>
<td>1.0</td>
<td>0.0</td>
<td>1.0</td>
<td>0.0</td>
<td>10.0</td>
<td>-</td>
<td>0.0</td>
<td>0.0</td>
<td>0.9</td>
</tr>
</tbody>
</table>
- Set $D_{2v}$: No analytical solution is available for this case. However, the influence of $D_2$ and $D_{2v}$ is investigated numerically. At equilibrium the material has the Poisson's ratio of $\nu = 0.1$ while the viscous overstress relation exhibits a Poisson's ratio of $\nu = 0.4$. A time dependent radial expansion of the plug in unconfined compression is expected.

- Set rate: The loading rate is varied to observe how the peaks behave in relation to the instantaneous-load relaxation curve. Numerical results only.

The influence of the model parameters is illustrated in Fig. B.2. The numerical and analytical solutions coincide. The two relaxation-time model exhibits distinct relaxation behaviours in the short and long term response (Fig. B.2d). Fig. B.2e additionally illustrates the influence of $D_{2v}$. Since the Poisson's ratio of the overstress is higher than the equilibrium value, the material responds with a high degree of lateral expansion during compression and subsequently relaxes to the lateral strain determined by the equilibrium Poisson's ratio.

### B.2.2. Robustness test

As a convergence robustness test a prismatic bar (dimensions 6x1x1) was axially stretched by 100% and subjected to a simultaneous torsional load via a relative axial rotation of its top and bottom faces by 180° within 1 s. The deformation was subsequently allowed to relax. Material parameter sets were as in table B.2.

<table>
<thead>
<tr>
<th>parameter</th>
<th>$C_1$</th>
<th>$\nu$</th>
<th>$C_{1\nu 1}$</th>
<th>$C_{1\nu 2}$</th>
<th>$\eta_{\nu 1}$</th>
<th>$\eta_{\nu 2}$</th>
<th>$\nu_{\nu 1}$</th>
<th>$\nu_{\nu 2}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>unit [MPa]</td>
<td>[-]</td>
<td>[MPa]</td>
<td>[MPa]</td>
<td>[MPa]</td>
<td>[MPas]</td>
<td>[MPas]</td>
<td>[-]</td>
<td>[-]</td>
</tr>
<tr>
<td>Set 3D $\nu = 0$</td>
<td>1.0</td>
<td>0.0</td>
<td>1.0</td>
<td>0.0</td>
<td>20.0</td>
<td>-</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Set 3D $\nu = 0.3$</td>
<td>1.0</td>
<td>0.3</td>
<td>1.0</td>
<td>0.0</td>
<td>20.0</td>
<td>-</td>
<td>0.29°</td>
<td>0.0</td>
</tr>
</tbody>
</table>

Results are depicted in Fig. B.3. With $D_2 = D_{2v} = 0$ moment and force behave similarly. With $D_2 = D_{2v} \neq 0$ the force relaxed faster.
B. Model Verification and Tests

B.3. Ellipsoidal fibre distribution and swelling

B.3.1. Isotropic case

Consider a free swelling sample where the swelling pressure is balanced by an isotropic, i.e. spherical, fibre network and a Neo-Hookean ground matrix. The
B. Model Verification and Tests

(a) final deformation

(b) Set 3D $\nu = 0$

(c) Set 3D $\nu = 0.3$

Figure B.3.: Results for the 3D robustness test. Results show the axial forces and moments normalised by their equilibrium values.

total stress vanishes, i.e.

$$\Delta \pi I = \frac{2}{J} F \left[ C_1 I + (4D_2 \ln J - C_1)C^{-1} + \int_0^{2\pi} \int_0^\pi H(I_4 - 1) \frac{\partial \psi_{\text{aniso}}}{} \sin \phi \, d\phi \, d\theta \right] F^T$$

(B.23)

In free swelling, the swelling pressure has to be balanced by the pressure in the solid matrix.

$$\Delta \pi = \frac{1}{3} \text{tr} \sigma_E$$

(B.24)

The deformation is isotropic, so that

$$F = \lambda I \quad \text{and} \quad J = \lambda^3 \quad \text{and} \quad b = FF^T = \lambda^2 I$$

(B.25)

Using the constitutive model from Eq. 3.82, the pressure balance reads

$$f(\lambda) = 0 = -2RT \left[ \sqrt{c_{\text{ext}}^2 + \frac{c_{F0}^2 \phi_{F0}^2}{4(\lambda^3 - \phi_{S0})^2}} - c_{\text{ext}} \right] + \frac{2C_1}{\lambda} + \frac{4D_2 \ln \lambda^3 - C_1}{\lambda^3} + \frac{2\pi C_4}{3} \beta \frac{(\lambda^2 - 1)^{\beta - 1}}{\lambda}$$

(B.26)

The function $f(\lambda)$ was minimised iteratively using a custom written C++ code for Newton’s method:

$$x_{n+1} = x_n - \frac{f(x_n)}{f'(x_n)} \quad \text{with} \quad f'(x_n) = \frac{f(x_n + \epsilon) - f(x_n - \epsilon)}{2\epsilon}$$

(B.27)
with \( \epsilon = 10^{-6} \) and the termination criterion \( |f(x_n)| - \epsilon < 0 \). A set of parameter variations was performed to illustrate the influence of the solid matrix Young’s modulus \( E \), Poisson’s ratio \( \nu \), fibre parameters \( C_4 \) and \( \beta \), fixed charge density \( c_{F0} \), external salt concentration \( c_{ext} \) and the porosity \( \phi_{F0} \) on the free swelling stretch \( \lambda \). Several parameter combinations were chosen to verify the FE implementation against the results obtained by the C++ code. The material parameters for the simulation sets are listed in Table B.3.

### Table B.3.: Simulation sets for the free swelling verification.

<table>
<thead>
<tr>
<th>parameter</th>
<th>( E ) [MPa]</th>
<th>( \nu )</th>
<th>( C_4 ) [MPa]</th>
<th>( \beta )</th>
<th>( c_{F0} ) [mmEq mm(^{-3})]</th>
<th>( c_{ext} ) [mmol mm(^{-3})]</th>
<th>( \phi_{F0} ) [-]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Set ( E )</td>
<td>0.2 ( [0:0.49] )</td>
<td>0.1</td>
<td>0.0</td>
<td>2.5</td>
<td>0.0002</td>
<td>0.00015</td>
<td>0.8</td>
</tr>
<tr>
<td>Set ( \nu )</td>
<td>1.0</td>
<td>0.0</td>
<td>2.5</td>
<td>0.0002</td>
<td>0.00015</td>
<td>0.8</td>
<td></td>
</tr>
<tr>
<td>Set ( C_4 )</td>
<td>0.1</td>
<td>0.1</td>
<td>0.5</td>
<td>[2.0:4.0]</td>
<td>0.0002</td>
<td>0.00015</td>
<td>0.8</td>
</tr>
<tr>
<td>Set ( \beta )</td>
<td>0.0</td>
<td>0.1</td>
<td>2.0</td>
<td>0.0</td>
<td>[0.0:0.004]</td>
<td>0.00015</td>
<td>0.8</td>
</tr>
<tr>
<td>Set ( c_{F0} )</td>
<td>0.0</td>
<td>0.1</td>
<td>2.0</td>
<td>2.5</td>
<td>0.0002</td>
<td>[0.0:0.002]</td>
<td>0.8</td>
</tr>
<tr>
<td>Set ( c_{ext} )</td>
<td>0.0</td>
<td>0.1</td>
<td>2.0</td>
<td>2.5</td>
<td>0.0002</td>
<td>0.00015</td>
<td>0.8</td>
</tr>
<tr>
<td>Set ( \phi_{F0} )</td>
<td>0.0</td>
<td>0.1</td>
<td>2.0</td>
<td>2.5</td>
<td>0.0002</td>
<td>0.00015</td>
<td>0.8</td>
</tr>
</tbody>
</table>

The results of the parameter variation are shown in Fig. B.4. Note, that due to the power law structure of the constitutive model for the fibrous material an increase in the parameter \( \beta \) leads to a decreased fibre stiffness at small deformations (Fig. B.4d). The derivation of the predicted FE values was less than 0.1% in all cases and was likely due to less stringent convergence criteria in the FE simulations.

### B.3.2. Anisotropic case

For verification of the user material code in the anisotropic case, the Abaqus and Marc implementations were compared to the open source FE package FEBio (version 1.3.0, URL: http://mrl.sci.utah.edu/software/febio) which offers implementations of material models with ellipsoidal fibre distributions described here. Integration of the ellipsoidal fibre distribution can be performed with either 320 or 1280 points in FEBio. The point locations are based on a geodesic dome discretisation where each direction is weighted by its individual area element, whereas the discretisation method employed in our code produces equal area elements on the surface of the unit sphere. The user can choose the resolution as accuracy or practicality demand. Preliminary simulations showed that due to the degree of anisotropy the higher resolution is required in FEBio for a good match in the simulations. For further details on the implementation see e.g. Ateshian et al. (2009). Tension and compression tests were simulated along all three axes.
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![Graphs](image.png)

Figure B.4.: Results for the free swelling test simulations.

of cuboid samples made of either the charged or the neutral material with the fibre architecture $\Xi = \text{diag}(4, 1, 0.25)$. Note, that the definition of the external salt concentration in FEBio differs by a factor of 2 to the one employed in the present notation. Also, when entering the parameters $\xi_i$ into the FEBio input file one needs to factor in the parameter $C_4$ from Eq. 3.86 for a direct comparison.

The following definitions were employed and referred to as the “charged” or the “neutral” materials:

\[
\text{charged: } T = T_{\text{fib}} - \Delta\pi J C^{-1} \\
\text{neutral: } T = T_{\text{NH}} + T_{\text{fib}}
\]  

where “NH” is a Neo Hookean contribution and the fibre stress was calculated as described in section 3.3.3 with the constitutive model 3.82.
The neutral material was given the parameters $C_1 = 1.0 \text{ MPa}$, $\beta = 2.5$, $C_4 = 2.0 \text{ MPa}$. One compressible and one nearly incompressible material were simulated: $\nu_i = [0.1 \ 0.499]^T$. The charged material: $c_F = 0.0002 \text{ mEq mm}^{-3}$, $C_{ext} = 0.00015 \text{ mmol mm}^{-3}$, $T = 298 \text{ K}$, $\phi_F = 0.8$, $\beta = 2.5$, $C_4 = 2.0 \text{ MPa}$.

![Stress strain curves](image)

(a) neutral, $\nu_i = 0.1$

(b) neutral, $\nu_i = 0.499$

(c) charged, tension

(d) charged, compression

Figure B.5.: Stress strain curves for the neutral (a,b) and charged (c,d) materials as computed with MSC Marc, Abaqus and FEBio.

Tensile and compressive stress-strain curves for the charged and neutral materials were for all practical purposes congruent over the entire strain range covered for each implementation (Fig. B.5). The initial stress softening range at early compressive strains applied to the charged material was also recovered by all implementations (Fig. B.5d).

**B.3.3. Integration methodology**

Fibre architectures aligned with the Cartesian axes and various degrees of anisotropy $\xi_k$ were considered such that $\Xi = \text{diag}(\xi_k, 1.0, \xi_k^{-1})$ and $\Phi = \phi$, $\Theta = \theta$. The per-
B. Model Verification and Tests

The performance of the numerical implementation in capturing the average value $\bar{\xi}$

$$\bar{\xi} = \frac{1}{4\pi} \int_0^{2\pi} \int_0^\pi \left[ \frac{(\cos \theta \sin \phi)^2}{\xi_1^2} + \frac{(\sin \theta \sin \phi)^2}{\xi_2^2} + \frac{(\cos \phi)^2}{\xi_3^2} \right] \sin \phi \, d\phi \, d\theta$$

is approximated as

$$\bar{\xi} \approx \frac{1}{N} \sum_{i=1}^{N} \left[ \frac{(\cos \theta \sin \phi)^2}{\xi_1^2} + \frac{(\sin \theta \sin \phi)^2}{\xi_2^2} + \frac{(\cos \phi)^2}{\xi_3^2} \right]^{-\frac{1}{2}}$$

and the peak value $\xi_k$ was evaluated for the following discretisation levels

$$N = [92, 252, 492, 812, 1212, 3612]^T$$

either with an aligned vector cloud or the original vector cloud (Fig. 3.2b) and quantified in terms of the relative error to the reference value as determined by MathCad (PTC Corporation, Needham, MA, USA). The parameter $\xi_k$ was varied from 1 (isotropic material) to 10.

![Graph](image)

Figure B.6.: (a): Relative error of the average (integral) value of a numerically integrated function $\xi(\Phi, \Theta)$; (b): relative error of the maximum value of $\xi(\Phi, \Theta)$ obtained during integration. Results shown for various discretisation levels (92, 252, 492, 812, 1212, 3612 points) as well as aligned and non-aligned integrations. The factor $\xi_k$ determines anisotropy with $\Xi = \text{diag}(\xi_1, 1, \xi_k^{-1})$.

The magnitude of the relative errors in the calculation of the mean value $\bar{\xi}$ of the ellipsoidal distribution increased with increasing anisotropy (Fig. B.6a). Increasing the number of integration points on the unit sphere decreased the mismatch and allowed more accurate integration of more anisotropic fibre distributions. Despite higher errors when high anisotropies were integrated, the error quickly decreased for increasing integration point densities with the prealigned integration method becoming the most accurate. Prealignment of the vector cloud mostly
led to an overprediction of the mean radius rather than an underprediction in the non-aligned case (Fig. B.6a). However, the aligned integration was able to exactly capture the maximum radius of the ellipsoid at all integration resolutions while the relative errors increased quickly with anisotropy for the unaligned integration (Fig. B.6b). Increasing the integration point density decreased the error albeit it still remained significant.

B.4. Remodelling of the anisotropy tensor

Simple shear was modelled such that the shear strain $\gamma = \tan \alpha$ was held at $\gamma = 0.4$ for 1000 s in phase 1, then reversed and held at $\gamma = -0.6$ for a further 1000 s during phase 2. The setup and coordinate system can be seen in Fig. B.7a. The remodelling time constants were set to $\tau = 40$ s. Since $\Xi$ evolves to $C$ the following remodellled anisotropy tensors are expected from Eq. 5.2:

$$\left(\Xi_{1}^{\text{analyt}}\right)_{ij} = \begin{pmatrix} 1 & 0.4 & 0 \\ 0.4 & 1.16 & 0 \\ 0 & 0 & 1 \end{pmatrix} \quad \text{and} \quad \left(\Xi_{2}^{\text{analyt}}\right)_{ij} = \begin{pmatrix} 1 & -0.6 & 0 \\ -0.6 & 1.36 & 0 \\ 0 & 0 & 1 \end{pmatrix}$$

The numerically predicted values were very close:

$$\left(\Xi_{1}^{\text{num}}\right)_{ij} = \begin{pmatrix} 0.99985 & 0.39997 & 0 \\ 0.39997 & 1.160149 & 0 \\ 0 & 0 & 1 \end{pmatrix}$$

$$\left(\Xi_{2}^{\text{num}}\right)_{ij} = \begin{pmatrix} 1.00008 & -0.600024 & 0 \\ -0.600024 & 1.35992 & 0 \\ 0 & 0 & 1 \end{pmatrix}$$

The evolution of the individual in-plane tensor components as well as the principal values is graphed in Fig. B.7b. While the principal values $\xi$ approach the (squared) principal stretch values in an exponential manner, the normal components of $\Xi$ overshoot before they ease out on their final values. The point where $\xi_1$ and $\xi_2$ “switch” due to the ordering definition that $\xi_1 > \xi_2$ is clearly evident in the graph.

B.5. Robustness test remodelling algorithm

The following test were performed to qualitatively illustrate some effects of the presented remodelling algorithm as well as test its robustness during large 3D
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![Diagram](a simple shear)

![Diagram](b evolution of Ξ)

Figure B.7.: (a): Simple shear setup used here. (b): Evolution of the in-plane components of Ξ as well as the in-plane principal values ξ. Thin lines represent the deformation (target) values derived from C.

deforations. Material parameters: $C_1 = 1.0\,\text{MPa}$, $\nu = 0.1$, $\bar{C}_4 = 2.0\,\text{MPa}$, $\beta = 2.5$, ellipsoidal distribution scaled for constant collagen content, remodelling times $\tau = 10\,\text{s}$. Two load cases for a prismatic bar (dimensions 6x1x1 mm$^3$):

- **Loadcase "Torque"**: stretched by 1 mm and twisted by 180° in 1 s, held for 100 s.

- **Loadcase "Cantilever"**: a pressure load is applied to the bar’s top (0.05 MPa) within 1 s, held for 100 s, removed within 1 s and kept unloaded for 150 s.

The algorithm ran robustly without convergence problems.
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Figure B.8.: Results for the 3D robustness test loadcase "Torque". Contour plots depict volume ratio.
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Figure B.9.: Loadcase “Cantilever”. Contours depict displacements.
B.5.1. Loadcase “Torque”

The volume ratio \( J \) was identical for all remodelling cases immediately after load application (Fig. B.8a). With remodelling, the volume ratios became more homogeneous for all remodelling cases and decreased slightly for the rotation only remodelling (Fig. B.8b), increased for remodelling towards \( \lambda_h = 1 \) (Fig. B.8c) and decreased for remodelling towards \( \lambda_h = 1.05 \) (Fig. B.8d). Fibre reorientation “optimises” the fibre reinforced composite material for its specific load case. This is illustrated by increasing reaction forces and moments in the case of only reorientational remodelling, i.e. stiffening (Figs. B.8e and B.8f). If a reconfiguration is allowed, the remodelling of the transition stretch releases fibre stress and the reaction forces decrease. Observe however, the subsequent light increase in reaction force for \( \lambda_h = 1.05 \) (Figs. B.8e and B.8f).

B.5.2. Loadcase “Cantilever”

The displacements are identical for all remodelling cases immediately upon loading (Fig. B.9a). After 100s of sustained pressure loading reorientation leads to an optimisation of the fibre structure and hence decreased displacements slightly (Fig. B.9b). Unloading returned the cantilever into the undeformed configuration (Fig. B.9c). In contrast, remodelling towards \( \lambda_h = 1.02 \) caused fibre stress relaxation during sustained loading and hence more bending after 100s (Fig. B.9d). Upon unloading, the remodelled fibres had caused residual stresses in the cantilever which did therefore not return to the undeformed configuration (Fig. B.9e). Residual stresses reduced with time but were still significant enough to maintain some degree of bending after 150s in the unloaded state (Fig. B.9f).

B.6. Uniaxial compression of a composite rod undergoing thermal expansion

To more easily interpret the results from chapter 4 it might be convenient to set up an analogy from classic (undergraduate level) engineering mechanics. Consider a bimaterial rod with a core made from material 1 that is surrounded by a cylindrical mantle made from material 2 (Fig. B.10a). Core and mantle have the axial rigidities \( (EA)_1 \) and \( (EA)_2 \), respectively. Both are of initial (stress free) length \( l_0 \) and bonding between both members is ideal. The core material has a coefficient
of thermal expansion $\alpha_1$.

![Diagram](image)

**Figure B.10.** (a) Thermal analogy. A composite rod with a core material representing the proteoglycans surrounded by a cylindrical mantle representing the collagen. The core is heated up and expands ("swells"). The mantle is assumed to be able to bear tensile loads only. (b) Normalized compressive force $F_c/[(EA)_1]$ for the composite rod. At $\lambda \leq 1.0$ the load contribution of the mantle vanishes. A qualitatively similar tension-compression non-linear behaviour is seen in cartilage at small compressive strains.

The core will represent the proteoglycans in the analogy. Swelling will be modelled by heating up the core by a temperature difference $\Delta T$. The mantle represents the collagenous network and will be stretched passively by the heated up core material, from which it is ideally insulated. In further analogy to collagen fibrils we assume that $(EA)_2 > 0$ only for tensile loads, while $(EA)_2 = 0$ for compressive loads.

If the core would not be restrained by the surrounding cylinder it would expand to a length $l_r$ of

$$l_r = (1 + \alpha_1 \Delta T)l_0$$  \hspace{1cm} (B.31)

However, due to the tensile stresses building up in the mantle in response to the expansion of the core, an equilibrium length $l_{eq}$ will be established depending on the axial rigidities involved:

$$l_{eq} = \left( 1 + \frac{\alpha_1 \Delta T (EA)_1}{(EA)_1 + (EA)_2} \right) l_0$$ \hspace{1cm} (B.32)

At this length the rod experiences a compressive stress in the core and a tensile state of stress in the mantle although no external mechanical loads are applied.
This is in analogy to the pre-stress in the collagen network in a free swelling cartilage sample, which restrains the proteoglycans to a fraction of their volume in free solution.

We now compress the rod to a length \( l_c < l_{eq} \). The force \( F_c \) required for that is given as the sum of the forces in the core and the mantle as

\[
F_c = \begin{cases} 
\left( \frac{l_c - l_0}{l_0} - \alpha_1 \Delta T \right) (EA)_1 + \frac{l_c - l_0}{l_0} (EA)_2 & \text{if } l_c > l_0 \\
\left( \frac{l_c - l_0}{l_0} - \alpha_1 \Delta T \right) (EA)_1 & \text{if } l_c \leq l_0 
\end{cases}
\]

(B.33)

where we used the assumption of the mantle only bearing tensile loads.

To show the behavior of the analogy we assumed the following parameters for illustration: \( 3(EA)_1 = (EA)_2 \) and \( \alpha_1 \Delta T = 0.4 \), so that the "free swelling" state is at 10\% strain:

\[
\lambda = \frac{l_{eq}}{l_0} = 1.1
\]

(B.34)

From that state (\( \lambda = 1.1 \)) the assembly will be compressed to \( \lambda = 0.9 \). The normalised force \( F_c/[(EA)_1] \) is plotted in Fig. B.10b. The tension compression non-linearity is clearly visible at \( \lambda = 1 \). This is qualitatively analogous to the compression behaviour of cartilage observed at small strains. Note, that the stretch is given here with respect to the stress free state prior to heating. If the expanded state after application of \( \Delta T \) would be chosen as reference – as would be the case when loading a cartilage sample, where the free swelling state is taken as the reference configuration – all considered stretches would measured to be \( \leq 1 \).

**B.7. Modulating the collagen network structure via the swelling properties during in vitro culture – A pilot study**

**B.7.1. Introduction**

Recent studies have found enhanced mechanical and biochemical properties of tissue engineered cartilage when the culture medium osmolarity was increased (Sampat et al., 2012). Another study hypothesised that rapid GAG synthesis constitutes an impediment to collagen synthesis and showed improved outcomes.
when chondroitinase ABC was applied at discrete time points during culture (Bian et al., 2009). This latter study also speculated that "by reducing the prestress due to osmotic swelling, temporary suppression of GAG content by CABC and agarase may have also created a more conducive environment for the elaboration and organization of the collagen network leading to better tensile properties." (Bian et al., 2009, p. 2070). In this section, the influence of culture medium osmolarity and proteoglycan depletion on the collagen network in free swelling hydrogels is briefly illustrated.

B. Model Verification and Tests

B.7.2. Methods

General considerations

ECM synthesis was modelled using a bilinear model with FCD reaching its target value within 42 days and collagen reaching its target value within 256 days (compare chapter 6). Agarose properties remained constant. The time constant for remodelling the collagen network was assumed to scale linearly with the collagen content (cf. chapter 7) according to

\[
\tau(t) = \frac{C_4(t)}{C_4(t = \infty)} \tau_0
\]

(B.35)

This constituted a low remodelling activity. The deposition stretch was assumed to be 1.001. All material parameters were as listed in table 6.2. The collagen network was allowed to remodel its stress-free configuration as described in chapters 5 and 6.

Culture medium osmolarity

During 50 days of simulated free swelling culture, the culture medium osmolarity was set to either 0.15 M (physiological), 0.2 M (hyperosmolarity) or 0.1 M (hypoosmolarity). To evaluate constructs, the amount of swelling, osmotic pressure and fibre stretch in physiological saline were compared between the different culture conditions.

Proteoglycan depletion

Culture medium osmolarity was maintained at physiological concentrations. The FCD was decreased to 10% at days 36 and 59 ("2 treatments"), at day 36 only
(“treatment”) or not at all (“no treatment”). Subsequent synthesis continued unimpaired. The simulation was continued until day 105 (Bian et al., 2009).

**Solution procedure**

The set of equations governing this remodelling problem were implemented in C++ which allowed for a rapid solution of longer time intervals. The governing stress balance equations were solved iteratively in each time increment (compare appendix B.3).

**B.7.3. Results**

Figure B.11.: FS volume (fold increase with respect to day 0 dimensions), swelling pressure, fibre stretch and recruitment stretch depending on the culture medium osmolarity during 50 days of culture.

**Culture medium osmolarity**

With increasing culture medium osmolarity, the free swelling volume in physiological solution decreased (Fig. B.11a). This was due to earlier fibre recruitment in the
hyperosmolar samples (Fig. B.11d). This earlier recruitment under physiological conditions led to higher fibre pre-stresses, higher osmotic pressures (Fig. B.11b) and higher fibre stretches (Fig. B.11c) in the samples cultured in hyperosmolar medium compared to physiological or hypoosmolar culture conditions. These effects combined will lead to higher construct stiffnesses (see chapter 6).

In summary, culturing tissue engineered articular cartilage in high osmolarity medium during ECM synthesis is predicted to lead to a more compact and pre-stressed collagen network as well as a higher swelling pressure in the tissue under physiological salt concentrations and hence improve construct functionality.

**Proteoglycan depletion**

With each treatment of chondroitinase ABC the FCD and therefore swelling pressure was reduced drastically (Fig. B.12b) and hence the FS volume dropped.
B. Model Verification and Tests

suddenly and recovered slowly thereafter due to the synthesis of new PG (Fig. B.12a). At day 105, the FS volume of the treated samples was lower than that of the untreated samples (Fig. B.12a) corresponding to higher swelling pressures in the treated samples (Fig. B.12b). The decreased sample volume after PG depletion not only caused a drop in fibre stretch (Fig. B.12c) but also caused the recruitment stretch to increase slower than in untreated samples to the effect that at day 105 treated samples exhibited lower recruitment stretches than untreated ones (Fig. B.12d).

In summary, proteoglycan depletion is predicted to lead to a more compact and pre-stressed collagen network as well as a higher swelling pressure in tissue engineered cartilage and hence contribute to improved construct functionality.