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Corroboration of computational models for mechanoregulated stem cell differentiation

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Do computational models contribute to progress in mechanobiology? Jacobs and Kelly (in Advances on Modelling in Tissue Engineering, p. 1–14, 2011) suggest that they do, but at the same time propose a limitation in the form of the 'paradox of validation', whereby the information needed to validate mechanoregulation theories obviates the need for them in the first place. In this article, the corroboration of theories describing mechanoregulation of tissue differentiation is reviewed. Considering the falsifiability of computational models derived using the theories as a measure of their predictive power, it is shown that the predictive power of some theories is poor and that models based on these theories fall into the 'paradox of validation'. Week theories for any phenomenon would succumb to such a paradox. We argue that mechanobiology needs theories that can have more potentially falsifying experiments and that perhaps the discipline does suffer from theories that are a priori designed to minimise falsifiability. However, several theories do have predictive power beyond the data used to validate them, so a paradox of validation should disappear as the subject develops.

Keywords: mechanobiology; corroboration; computer model; K.R. Popper

Introduction

One central concept in mechanobiology is that the differentiation pathways of mesenchymal stem cells (MSCs) in the musculoskeletal system is modulated by mechanical stimulation (Carter and Beaupré 2001; Minguell et al. 2001; Estes et al. 2004). Computational models have been developed to serve as predictive tools for tissue remodelling and differentiation. These models encapsulate a fundamental understanding of the role of mechanical forces in biology so that the tissue differentiation and remodelling can be predicted in clinical design applications; for example, in the design of orthopaedic implants or tissue engineering constructs. Furthermore, the ability of computational models to incorporate a high degree of complexity has made simulations another way of experimentation; this holds out great potential for better understanding fundamental interactions in mechanobiology (Huiskes 1995).

The mechanical forces that act on the skeleton generate a host of different mechanical or biophysical stimuli within the tissues themselves. Several theories have been proposed for mechanoregulation of MSC differentiation (Pauwels 1960; Perren 1979; Carter et al. 1988; Prendergast et al. 1997; Claes and Heigele 1999; Gomez-Benito et al. 2005). These theories are often used in computer simulations together with some assumptions about cell activities and material properties. Experiments can be used to corroborate certain aspects within the model; for example, modelling the process of cell migration as random walk. However, the mechanoregulation aspect of the models needs to be tested using computer models that enable determination of the mechanical environment. Tests of the early mechanobiological models were analyses at a single time-point of fracture healing. The mechanical stimuli (distributions of stresses and strains) calculated in a simplified fracture domain were compared with the fracture healing patterns from animal experiments (Carter et al. 1988). Later, these models developed into simulations of the differentiation process that consider biphasic tissues (Prendergast et al. 1997; Lacroix et al. 2002), anisotropic materials (Nagel and Kelly 2010), cell phenotype-specific activities (Kelly and Prendergast 2005; Isaksson et al. 2008), stochasticity in cellular activities (Perez and Prendergast 2007), osseointegration (Andreykiv et al. 2008; Liu and Niebur 2008; Swider et al. 2011) and the effect of angiogenesis (Geris et al. 2008a; Checa and Prendergast 2009). Recent mechanobiological simulations can predict many aspects of fracture healing (Geris et al. 2006; Isaksson et al. 2008; Geris et al. 2009; Byrne et al. 2011), distraction osteogenesis (Loboa et al. 2005; Isaksson et al. 2007; Hayward and Morgan 2009; Reina-Romo et al. 2009), scaffold tissue engineering (Kelly and Prendergast 2006; Byrne et al. 2007; Sandino et al. 2010) and even capture aspects of animal variability (Checa et al. 2011; Khayyeri et al. 2011). However, despite published advances in this field of computational mechanobiology, there were, and

still are, great scepticism and criticism about the capacities and the use of such simulations in mechanobiology.

We argue that computer models play an essential role in mechanobiology and give insights in to mechanobiological processes that would be impossible to get from experiments alone; it was partly inspired by the work of Jacobs and Kelly (2011), who argue for a 'paradox of validation' whereby the value of mechanoregulation theories is questioned as the data needed to validate them are said to obviate the need for the model in the first place.

Computer simulation in mechanobiology

Computational mechanobiologists want to create models of the adaptive responses of biological tissues to mechanical stimulation; biological processes of particular interest are bone remodelling and tissue differentiation. In considering such models, it is useful to define the word *simulation*. According to Knuth (1973), simulations imitate the behaviour of a system, usually by means of an iterative computerised process. Simulations track the behaviour of the system over time and do not have to converge to a tissue equilibrium state but can be interrupted at any time-point chosen by the modeller. The predictions made by computer simulations can be compared with *in vivo* and *in vitro* studies. Kitano (2002) writes, '*any inconsistency at this stage means that the assumptions that represent our knowledge on the system under consideration are at best incomplete*'. Models that have been corroborated can then be used to test hypothesis with *in silico* experiments and explore questions that are impossible to investigate with *in vitro* and *in vivo* experiments.

Mechanobiological computer simulations of tissue differentiation are built up from algorithms that model individual cell activities, such as migration, proliferation, apoptosis, differentiation and capillary formation. Other components of the simulation depict material behaviour and matrix synthesis. These components of the total simulation can be tested in their own right by comparing with *in vitro* experiments. When all components are put together to simulate a mechanobiological process on the organ level, the predictions made allow the model to be corroborated with *in vivo* animal experiments (Figure 1).

Testing scientific theories with falsifying hypotheses

One of the most well-known perspectives on the scientific method was proposed by Karl R. Popper in his book *Logic of Scientific Discovery*. Popper claimed that the empirical sciences are a system of theories which are composed of *universal* and *singular* statements (Popper 1959). He defined singular statements as describing a specific event and defined universal statements as hypotheses with 'the character of natural laws'. Singular statements are

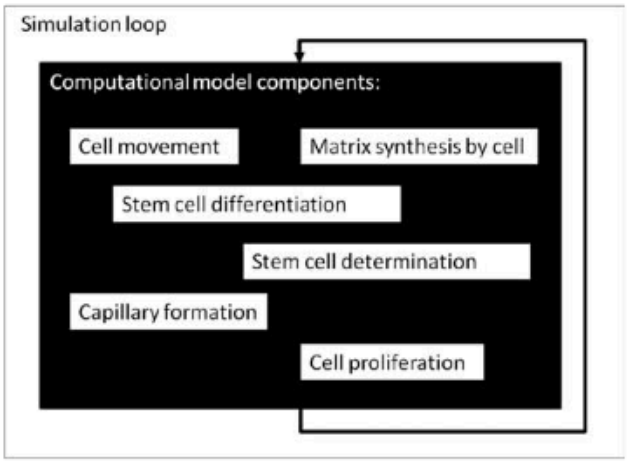

Figure 1. Illustration of a mechanobiological computer model with its different components and corroboration methods. The white boxes are algorithms that describe specific cell activities, which can be validated by *in vitro* cell culture experiments. The black box is the computational model containing all the algorithms regarding cell activities and material behaviour. The grey box represents the simulation of a mechanobiological process, which is a combined interaction between all the components in the black box. The predictions made by this type of simulation can be corroborated with *in vivo* experiments. Adapted from Prendergast (2008).

distinguishable from universal statements as they always contain individual names, whereas universal statements contain universal names, i.e. can be thought of as 'all-statements'. For example, 'all ravens are black' is a universal statement, whereas 'this raven is not black' is a singular statement.

Universal statements, in turn, can be divided into three groups, *numerical universal statements, strictly universal statements* and *strictly existential statements*. It is the distinction between the strictly universal statements and the strictly existential statements that is the most important one. Popper argued that strictly existential statements, also known as 'there-are statements' cannot be falsified (only verified if found that something exists). On the other hand, strictly universal statements are falsifiable if a single contradictory statement (called *falsifying hypothesis*) can be found. The more useful the theories, the more potentially falsifying hypotheses it has or as Popper expressed it, '*not for nothing do we call the laws of nature "laws": the more they prohibit the more they say*'. According to Popper, a theory is only empirical if it is *falsifiable*, i.e. if it can be refuted by observation; so, if we accept Popper's perspective, our mechanobiological theories should be formed as strictly universal statements.

Biomechanical theories have previously been analysed by Prendergast (2001), where it was argued that the lack of potentially falsifying hypotheses in biomechanical theories impedes the emergence of new theories with greater predictive power. It was proposed that significant progress

would be made if the theories of mechanobiology were created such that they could be subject to more rigorous testing. The theories would either be upheld after rigorous tests using computational and experimental methods, or be rejected and replaced with better theories (Prendergast 2001). However, theories set up to be so general as to preclude falsification inhibit this natural scientific process.

Current mechanoregulation theories

Several mechanoregulation theories that propose different mechanical stimuli to modulate the process of stem cell differentiation into musculoskeletal tissues have been introduced in orthopaedic research (Pauwels 1960; Carter et al. 1988; Prendergast et al. 1997; Claes and Heigele 1999; Gomez-Benito et al. 2005), and computational investigations have claimed to have partly corroborated many of these theories (Lacroix and Prendergast 2002a, 2002b; Isaksson et al. 2006b; Hayward and Morgan 2009; Checa and Prendergast 2010). However, some of these theories do not propose boundaries of mechanical stimuli for the differentiation of a tissue phenotype but suggest that mechanical stimuli in terms of hydrostatic pressure and compressive strain can affect the differentiation process (Pauwels 1960; Carter et al. 1988). Despite being highly novel when first presented and forming the impetus for many studies, this type of mechanoregulation theory cannot easily be rejected but can be only correlated with the tissue differentiation patterns observed *in vivo*, as illustrated by the authors in the original study (Carter et al. 1988). They proposed an 'osteogenic index', which must be calibrated with experimental data for each specific anatomical site and species to quantify the boundaries of mechanical stimuli that guide tissue differentiation. This calibration precludes hypotheses testing using the theory because each implementation needs to establish the empirical parameters used for that simulation to facilitate corroboration of the hypothesis of the study – making it very hard to find potentially falsifying hypotheses. In fact, it could seem that the mechanoregulation theory of Carter et al. (1988) is in analogy to a singular statement rather than a universal statement.

Other researchers have proposed that deviatoric strain is the primary mechanical stimulus that determines stem cell fate (Gomez-Benito et al. 2005). This theory is implemented with a highly integrated set of differential equations that depend on cell maturity and the degree of ossification, making the model quite complex. Nonetheless, it is important to distinguish between the performance of the computational model (being a collection of equations or algorithms for cell activities, matrix synthesis and material properties) used to simulate the mechanobiological process and the mechanoregulation theory for stem cell differentiation itself. Several mechanoregulation theories are formed such that many potentially falsifying experiments can be found, which can be used for testing mechanobiological hypotheses (see Table 1).

Reviewing this and other work, Jacobs and Kelly (2011) presented the concept of 'the paradox of validation' for computational models in mechanobiology. They stated that '*The paradox is that the experimental data collected to validate the model may actually obviate the need for the model*'. This paradox can hold true in mechanobiology if computational models have little predictive power. However, the more predictive power the model has, the more potentially falsifying hypotheses can be found; therefore, we believe, the paradox of validation can be avoided if we are clear about avoiding theories that do not admit potentially falsifying hypotheses.

Adopting the idea of potentially falsifying hypotheses means that our hypotheses can be either falsified or corroborated. In engineering, however, we often must use theories that are only 'partly' corroborated or 'qualitatively' corroborated, meaning that they capture some of the main phenomena observed in the system but not all potentially relevant aspects. Engineers may be quite aware of the theories' limitations but do not want to reject the theory just yet, because changes in the computational model can be made for better predictions. Clearly this can lead to a downward spiral into the 'paradox of validation'.

Corroborating biomechanical hypotheses

We believe that the theories proposed for mechanoregulated stem cell differentiation are different in their degree of corroborability. Although some mechanoregulation theories have been able to capture some of the main concepts (Isaksson et al. 2006a; Liu and Niebur 2008; Hayward and Morgan 2009), later studies, using the same theories, actually failed to capture the mechanobiological process observed in experiments when applied on a different tissue differentiation scenarios (Geris et al. 2003; Isaksson et al. 2006b; Geris et al. 2008b); thus, none of the theories can be considered as accepted as a 'natural law'. It is noteworthy that some of the early mechanoregulation theories (e.g. Pauwels mechanoregulation theory) were formed at a time when computational tools to test hypotheses did not exist.

Many current computational models do not consider animal variability in response to loading, despite reports of variability in animal experiments. This is a significant limitation as variability is an inherent part of a biological system and not due merely to experimental error. In fact variability between the animals is so great that some group of results match the simulation outcomes, whereas the other groups do not and show a completely different differentiation pathway (Tagil and Aspenberg 1999; de Rooij et al. 2001; Bishop et al. 2006) making corroboration of mechanoregulation theories impossible with deter-

Table 1. Examples of the well-known mechanoregulation theories and the relationship between their falsifiability and their predictive power.

Theory	Comments on falsifiability (number and 'severity' of potential falsifying hypotheses)
Pauwels theory of hydrostatic pressure and strain (Z. Anat. Entwickl. Gesch., 1960)	• Lack of quantitative information about the mechanical stimuli almost preclude any falsifying hypotheses • Some degree of independence (different ossification sites and species) • Lack of information on constitutive behaviour of tissues and of the loading regime prevented rigorous tests when theory was proposed
Perren's interfragementary strain theory (Clin. Orthop. Rel. Res., 1979)	• Many potential falsifiable experiments based on gap sizes • Some degree of independence (different bones and species) • Quantitative thresholds of mechanical stimulus for the differentiation of different cell phenotypes supports falsifiability • Computational models and experimental models possible • The formulation of the theory, based on fracture gap sizes, restricts the mechanoregulation theory to *in vivo* bone fracture experiments only
Carter and colleague's osteogenic index theory of endochondral ossification (J. Orthop. Res., 1988)	• Dependent model parameter '*k*' which must be calibrated with experimental data combined with the lack of quantitative information about the mechanical stimuli almost preclude any falsifiable experiments • Some degree of independence (different ossification sites, and species) • Simulations not carried out; single time-point analyses only
Claes and Heigele's theory of hydrostatic pressure and strain (J. Biomech., 1999)	• Many potential falsifying experiments • Some degree of independence (different ossification scenarios, different bones and species) • Quantitative thresholds of mechanical stimulus for the differentiation of different cell phenotypes supports falsifiability • Simulations not carried out, single time-point analyses only • Computational models and experimental models • Formulation of the theory is relatively general, enabling corroboration with *in vivo* and *in vitro* experiments
Prendergast et al.'s theory of fluid flow and shear strain (J. Biomech., 1997)	• Many potentially falsifying experiments • Some degree of independence (different ossification scenarios, different bones and species) • Quantitative thresholds of mechanical stimulus for the differentiation of different cell phenotypes supports falsifiability • Computational and experimental models • Formulation of the theory is relatively general, enabling corroboration with *in vivo* and *in vitro* experiments
Gomez-Benito et al.'s theory of deviatoric strain (J. Theor. Biol., 2005)	• Some potential of falsifying experiments on bone regeneration. The model has many dependent biological parameters making it difficult to find potentially falsifying hypotheses for the mechanoregulation theory. The theory for stem cell differentiation depends not only on mechanical stimuli but also on maturation time, degree of calcification and cell-phenotype densities • Some degree of independence (different ossification scenarios, different bones and species) • Quantitative thresholds of mechanical stimulus for the differentiation of different cell phenotypes supports falsifiability • Computational models and experimental models; the model has many dependent variables • The mechanoregulation theory is coupled with bioregulatory networks, making corroboration of the mechanoregulation theory possible with only *in vivo* bone regeneration/formation experiments
Geris et al's theory of fluid flow (Biomech. Model. Mechanobiol., 2010)	• Many potential of falsifying experiments on bone regeneration • Some degree of independence (different ossification scenarios, different bones and species) • Quantitative thresholds of mechanical stimulus for the differentiation of different cell phenotypes supports falsifiability • Computational models and experimental models • The theory does not describe the differentiation of chondrocytes and fibroblasts based on mechanical stimulation, but assumes their differentiation to be based on biological factors and indirectly affected by mechanical stimulus (through angiogenesis and osteogenesis), thus, the mechanoregulation theory to only be corroborated with *in vivo* bone regeneration/formation experiments

phenomena beyond which they were formed for. Current theories have mainly been tested in simulations of fracture healing, distraction osteogenesis and bone implant interface integration, but we suggest that the predictive capacities of mechanoregulation theories should be tested in also the following scenarios to improve corroborability:

- *Well-controlled bone chambers*: Designing chambers with various geometries and with well-controlled mechanical loading environments reduce large parts of the environmental variability that exists in fracture healing and distraction osteogenesis experiments. Chamber experiments could become powerful in combination with microcomputed tomography imaging tools that can provide a 3D image of the tissues inside the chambers.
- *Embryonic bone development*: The endochondral ossification process during embryonic bone development has much in common with that during fracture healing. Mechanical forces have shown to have to significantly influence the ossification process (Landis et al. 2000; Nowlan et al. 2010), and it is likely that the process during bone development is guided by the same mechanical cues as those postnatal.
- *Evolutionary algorithms*: Simulating aspects of bone evolution that could have been dependent on mechanoregulation, such as cross-sectional bone growth (Van der Meulen et al. 1993; Nowlan and Prendergast 2005; Nowlan et al. 2011), can give better insights about the performance of the mechanoregulation theories. Do the theories converge to something realistic?
- *Biomaterials*: Simulations of tissue differentiation when cells are seeded on biomaterials and stimulated in a controlled fashion are suitable for corroborating or falsifying mechanoregulation theories. The outcomes of the simulations can be compared with those of bioreactor experiments or *in vivo* experiments. However, using biomaterials simulations also introduces new degrees of freedom in the computational model, in terms of scaffold material properties, cell perfusion, seeding and attachment, fluid profile, etc.
- *Tendon simulations*: Existing mechanoregulation theories often propose biophysical stimulation that promotes fibrous tissue formation under relatively high mechanical stresses and strains, which can be similar to those required for tendon formation. Simulating the process of tendon healing would be interesting, as it is a mechanoregulated stem cell differentiation scenario where bone and cartilage are not expected, but it is a process that could be modulated by biophysical stimuli proposed by mechanoregulation algorithms.
- *Simulations of different species*: Identical experiments on different species could give us better insights in how mechanobiological rules are similar or dissimilar between different species, depending on their size, metabolism, etc. Existing studies (although they are seldom identical and from the same laboratory, Isaksson (2012) indicate that biophysical stimulation that guides the process are the same but that the degree the cells' respond to stimulation can be different because tissue differentiation rates are variable between species; see results from Lienau et al. (2005) and Schwarz et al. (2011). The predictive capacities of mechanoregulation theories can be tested in simulations of different species for improved quantitative corroboration and give insights of how (if) rules of mechanoregulation can be translated from animal to clinical investigations.
- *Variability simulations*: It is important to consider the inter-animal variability that is reported in mechanobiological experiments in also mechanobiological tissue differentiation simulations. Mechanobiological theories can be further corroborated if they simulate similar variability to that found in the *in vivo* experiments. In the opinion of Huiskes (1997), this level of corroboration requires precise evaluations of experimental patterns, and if repeated predictions are within a margin of quantitative precision, the model can be considered as 'corroborated' and be used to replace other experimentation.

We hope to have demonstrated the clear role of computer simulations as predictive tools in mechanobiological research. A well-developed and corroborated computational model can be used to test hypotheses that are impossible to test with experiments. In more practical applications, computer models that make good predictions can, for example, be used at preclinical testing of implants and optimising biomaterials (Byrne et al. 2007; Sandino et al. 2008), they can be used to perform patient-specific simulations (Galibarov et al. 2010; Byrne et al. 2011; Prendergast et al. 2011; Vaananen et al. 2011) towards patient-specific treatments and help reducing the risks of failure in clinical practises. These would be difficult to achieve with purely experimental techniques. Towards these goals, we hope to have emphasised the significance of forming theories and provoked some thought about finding potentially falsifying hypotheses. It might be useful to move on from existing theories with low falsifiability and continue to develop our mechanoregulation theories such that they have more potentially falsifying hypotheses to avoid falling into the 'paradox of validation'.

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