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A comparative study of shear stresses in collagen-GAG and calcium phosphate scaffolds in bone tissue-engineering bioreactors

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A comparative study of shear stresses in collagen-GAG and calcium phosphate scaffolds in bone tissue-engineering bioreactors

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Abstract

The increasing demand for bone grafts combined with their limited availability and potential risks has led to much new research in bone tissue engineering. Current strategies of bone tissue engineering commonly utilize cell-seeded scaffolds and flow perfusion bioreactors to stimulate the cells to produce bone tissue suitable for implantation into the patient's body. The aim of this study was to quantify and compare the wall shear stresses in two bone tissue engineering scaffold types (collagen-GAG and calcium phosphate) exposed to fluid flow in a perfusion bioreactor. Based on μCT images, 3D numerical CFD models of the two scaffold types were developed to calculate the wall shear stresses within the scaffolds. For a given flow rate (normalized by the cross-sectional area of the scaffolds), shear stress is 2.8-fold higher in the collagen-GAG than the calcium-phosphate scaffold. This is due to the differences in scaffold geometry, particularly the pore size (collagen-GAG pore size ~96μm and calcium phosphate pore size ~350μm). The numerically obtained results were compared to an analytical method which is widely used by experimentalists to determine perfusion flow rates in bioreactors. Our CFD simulations revealed that the cells in both scaffold types are exposed to a wide range of wall shear stresses throughout the scaffolds, and that the analytical method predicts shear stresses 12% to 21% greater than those predicted by the CFD method. The study has demonstrated that the wall shear stresses in calcium phosphate scaffolds (745.2mPa) are approximately 40 times higher than in collagen-GAG scaffolds (19.4mPa) when flow rates are applied which have been experimentally used to stimulate the release of PGE_2. These findings indicate the importance of using accurate computational

models to estimate shear stress and determine experimental conditions in perfusion bioreactors for tissue engineering.

Introduction

According to Laurencin *et al.* 500,000 bone replacement procedures were performed in the United States in 2005 of which 90% used either autografts or allografts [1]. Both autografts and allografts have substantial drawbacks. Therefore, much recent focus of bone graft research is on bone tissue engineering, where cells (taken from the patient's bone marrow) are seeded onto a biological scaffold and growth factors are used to produce mineralized tissue *in vitro.* Alternatively, a bioreactor can be used to perfuse the cell-seeded scaffold with culture medium containing osteogenic growth factors. The fluid flow also provides biophysical stimulation which may enhance extracellular matrix deposition and mineralization of the construct to form bone tissue.

The scaffold biomaterial plays a key role in tissue engineering and the optimal scaffold has to combine a set of essential requirements such as biocompatibility, mechanical strength, and a highly porous microstructure and a pore size that allows cell migration and re-organization [2]. A wide variety of scaffold biomaterials has been studied over the past two decades including naturally occurring polymers (e.g. collagen, fibrin, and gelatin), synthetic polymers (e.g. polylactic acid (PLA) and polyglycolic acid (PGA)), and porous ceramics (e.g. bioglass® and calcium phosphate structures and naturally occurring ceramics, such as coral) [3,4].

Previous work demonstrates that collagen-glycosaminoglycan (CG) [5] and calcium phosphate [6] scaffolds show promising results for bone tissue

engineering. Lyophilised CG scaffolds are characterized by very high porosity (~99%), a high level of pore interconnectivity, excellent biodegradability and biocompatibility. They can be produced with a wide range of homogeneous pore sizes [7,8]. A disadvantage of the CG scaffold for bone tissue engineering is that it has relatively poor mechanical properties. However, recent investigations indicate that cross-linking methods can substantially improve the mechanical stability of CG scaffolds [9]. Among the positive features of calcium phosphate scaffolds are their trabecular bone-like morphology and a composition similar to bone mineral which results in excellent biocompatibility [4]. However, calcium phosphate scaffolds are brittle [4,10,11] and have a relatively low porosity of 47% to 85% [12] which limits cell penetration, nutrient delivery, removal of metabolic waste products and subsequent vascularisation of new tissue [4].

The mechanical stimulation caused by the fluid flow of the culture medium in a bioreactor plays a critical role in anabolic bone cell activity. Wall stress acting on osteoblastic cells activates bone formation activity *in vitro* [13,14]. The applied wall shear stress needs to be in a physiologically relevant range. *In vivo*, bone cells experience estimated shear stresses of 0.8Pa to 3.0Pa for the range of routine physical activity [15]. The effect of physiological shear stresses in 2D cultures (i.e., parallel plate flow chambers) has been analysed in various investigations [13,14,16,17]. These studies showed an increase of bone formation markers, such as nitric oxide (NO) [14], prostaglandin E_2 (PGE_2) [13,14], and osteopontin (OPN) [16,17] due to the mechanical stimulation of osteoblasts caused by the flow perfusion. In 2D experiments using parallel plate flow

chambers, all cells see approximately the same shear stress, which is easily estimated by simple analytical methods because of the regular geometry of the flow chamber. However, shear stress calculations are more complicated in 3D experiments because of complex scaffold geometries and an irregular flow environment. In order to enable physiologically relevant shear stresses to be accurately applied to cells in bioreactors, a computational approach can be used to estimate the mechanical environment.

The calculation of the flow conditions within perfused highly irregular 3D geometries is non-trivial. In general there are two approaches to determine the shear stresses values: (i) analytical methods [18,19,20] and (ii) numerical models [21,22]. Analytical methods are based on crude assumptions about the scaffold geometry. Consequently, they offer only an estimation of the flow conditions, are inherently inaccurate and do not provide a shear stress distribution, whereas numerical approaches based on μCT images of the scaffolds can produce accurate solutions, but are time consuming and computationally expensive.

Recently, two studies demonstrated the potential of using computational fluid dynamics for investigating the flow conditions within 3D scaffolds for tissue engineering. Cioffi et al. [21] used a μCT based model to characterize the local fluid dynamics to which chondrocytes are exposed in a perfusion bioreactor for cartilage tissue engineering. They demonstrated that computational modeling can be successfully used to quantify the shear stress which acts on cells seeded on porous polyesterurethane scaffolds (with a pore diameter of

100μm and a porosity of 77%) in bioreactor experiments. Porter et al. [22] utilized the Lattice-Boltzmann method [23] to simulate the flow conditions within perfused cell-seeded scaffolds. They used a μCT scan of human trabecular bone (pore diameter of 645μm) to define the scaffold microstructure for the simulation. They demonstrated that scaffold pore size, porosity, and dimensions and the bioreactor architecture affect the wall shear stress. Their results indicate that the experimental input flow rate alone cannot be used as a basis for comparison of biological outcomes; in addition to the flow rate, the contribution of scaffold architectural parameters must be considered.

In this study we used 3D CFD models to investigate the fluid dynamics in CG and calcium phosphate scaffolds, which have been used in flow perfusion experiments [5,6]. These experiments demonstrated the potential of fluid shear to stimulate bone cell activity in osteoblastic cell-seeded CG scaffolds [5] and calcium phosphate scaffolds [6] in perfusion bioreactors. ~~Both of these studies were previously published in *Tissue Engineering*.~~ In both studies, PGE_2 release by osteoblastic cells was stimulated by fluid flow shear. The boundary conditions and parameters of the models presented here were chosen to simulate the perfusion bioreactor experiments reported previously for CG [5] and calcium phosphate scaffolds [6]. It is important to note different flow rates were used to stimulate the release of PGE_2 in those experiments. A maximum input flow rate of 1ml/min was used in the CG study [5], which resulted in the stimulation of a PGE_2 value of ~3.5fg/DNA(pg), which was approximately a 20-fold increase over the no-flow control group. In the calcium phosphate scaffold study, the much higher maximum input flow rate of 40ml/min caused

a PGE_2 level of ~2fg/DNA(pg), which was approximately a 5-fold increase over the no-flow control group[6]. In the current study, CFD models of flow through the CG and calcium phosphate 3D scaffolds were used to provide accurate estimations of the shear stress that cells seeded on the scaffolds will experience. ~~Accurate models are important for designing and interpreting results from experimental studies.~~ These models were used to address the following questions: (i) What is the distribution of flow parameters (fluid velocity and shear stress) in 3D scaffolds? (ii) How does the geometry (e.g., pore size and porosity) of two commonly used bone scaffolds (CG and calcium phosphate) affect the distribution of flow parameters? (iii) How good is the agreement between the results obtained using the analytical method reported by Goldstein [18] and the CFD simulations?

Methods

Analytical and computational approaches were used to estimate the shear stresses that bone cells seeded on CG and calcium phosphate scaffolds are exposed to in perfusion bioreactors. The first method used the analytical approach according to Goldstein et al. [18]. The second method was a 3D computational fluid dynamics (CFD) model based on micro-computed tomography (μCT) images of the two scaffolds.

The analytical method [18] is based on the assumption that the scaffold can be represented as a bundle of parallel circular pipes. The diameter of each pipe, d_{pipe}, is equal to the average pore diameter, $d_{pore} = 2*r_{pore}$, of the scaffold. The average fluid velocity inside the scaffold, u_{scaf}, can be estimated by

$$u_{scaf} = Q/(\pi*r_{chamber}^{2}*\Phi), \quad (1)$$

where Q is the inlet flow rate applied to the bioreactor, $r_{chamber}$ is the radius of the bioreactor's scaffold chamber and Φ is the porosity of the scaffold. In the case of laminar fluid flow, the flow profile inside the pipes is parabolic: $u(r) = u_{max}*(1-r^2/r_{pipe}^2)$ and the shear stress τ is given by

$$\tau = \mu*du(r)/dr, \quad (2)$$

where μ is the dynamic viscosity of the culture medium. Calculating the gradient, $du(r)/dr$, at the pipe wall, $r = -r_{pore}$, and using the relation $u_{max} = 2*u_{avg} = 2*u_{scaf}$ we obtain for the wall shear stress τ

$$\tau = 8*\mu*u_{scaf}/d_{pore}. \quad (3)$$

In the analytical calculations presented in this study, the scaffold's porosities were obtained from the µCT scans and a dynamic viscosity of 0.001Pa·s was used, which is in the range of commonly used culture media for flow perfusion bioreactor experiments in tissue engineering [24]. In order to determine the analytical estimates for the CG scaffold a fluid velocity of 235µm/s and an average pore size of 96µm was used, which corresponds to the experimental settings of Jaasma and O'Brien [5]. The applied fluid velocity, u_{scaf}, was calculated by $u_{scaf} = Q/A_{chamber}$ = 1ml/min / ($r_{chamber}^2*\pi$) = 1.667x10^{-8}m^3/s / [(0.00475m)$^2*\pi$] = 0.000235m/s, where an input flow rate Q=1ml/min and a chamber radius $r_{chamber}$=4.75mm was used. The analytical results of the calcium phosphate scaffold were obtained by using an fluid velocity of 24.89mm/s and an average pore size of 350µm corresponding to the experimental values of Vance et al. [6]. The applied fluid velocity u_{scaf} was calculated by $u_{scaf} = Q/A_{chamber}$ = 40ml/min / ($r_{chamber}^2*\pi$) = 6.667x10^{-7}m^3/s / [(0.00292m)$^2*\pi$] = 0.02489m/s, where an input flow rate Q = 40ml/min and a scaffold chamber radius $r_{chamber}$ = 2.92mm was used. In order to improve the accuracy of the analytical estimation, the effective porosity $\Phi' = 1 - V_{scaf\_material}/(h_{scaf}*A_{chamber})$ of the scaffold was used instead of the scaffold's real porosity $\Phi = 1 - V_{scaf\_material}/V_{scaf\_entire}$, where h_{scaf} is the height of the scaffold, $A_{chamber}$ is the area of the bioreactor's scaffold chamber, $V_{scaf\_material}$ is the total volume of the scaffold material, and V_{scaf_entire} is the entire volume of the scaffold (volume of the scaffold material and of the scaffold's interstice).

Figure 1(a-d) show µCT images of the CG and calcium phosphate scaffolds. 1(a) and (b) show 3D views and Figure 1 (c) and (d) cross sections of the scaffolds. The images (resolution = 12µm) of the radio-absorbent calcium phosphate scaffolds were taken on a Scanco Medical 40 Micro CT system with 70kVp X-ray source and 112µA. The µCT scans (resolution = 5µm) of the nearly radio-translucent CG scaffolds were performed by SCANCO Medical AG in Bassersdorf/Switzerland using a special filter technology to increase the contrast of the CG material. The key parameters of the scaffolds are listed in Table 1. Both CFD models are based on µCT images. The µCT grey scale raw data were filtered using a Gaussian rank filter and a threshold procedure was performed to transform the grey scale representation of the scaffolds to black-and-white geometries. Hexahedral meshes of the scaffold's interstices were created after applying a marching-cube-like smoothing process to the structure.

The huge demand on the mesh resolution (caused by the low inter-struts-distance) and the high interstice volume of the CG scaffold prevented us from being able to perform CFD simulations of the entire CG scaffold including the bioreactor's scaffold chamber, the inlet pipe and the outlet pipe. Instead simulations of three randomly chosen sub-volumes with a size of 640µm x 640µm x 480µm were performed (see Figure 2). In order to avoid boundary, artifacts only results of an inner cube of 320µm x 320µm x 320µm have been used for further investigations [21]. An inlet area of a length of 100µm was added on top of the scaffold to allow the entering fluid flow to distribute freely.

The meshes of the CG scaffolds contained approximately 1.7 million hexahedral elements with a mean side length of 4.7μm. A constant inlet velocity of 235μm/s (corresponding to the maximum inlet flow rate of 1ml/min used by Jaasma and O'Brien [5]) was applied. A FEM deformation simulation of the scaffold was performed which showed that the relative deformation of the scaffold caused by the fluid flow is less than 0.2%, which leads to negligible changes in the flow conditions within the scaffold. It has been established that bone cells are more sensitive to fluid shear stress than substrate-strain loading [25,26], with *in vitro* substrate strains above 1.0% required to stimulate bone cells to release signaling molecules [14,27]. In this study, the deformation of the scaffold was neglected and the scaffold walls were assumed to be rigid and impermeable.

Given the differences in porosity, it was possible to simulate the entire calcium phosphate scaffolds including the scaffold chamber and part of the inlet and outlet pipes (see Figure 3). Three simulations were performed using the μCT scans of three different calcium phosphate scaffolds. The meshes of each CFD model contained approximately 8 million hexahedral elements with a mean side length of 23.8μm. A constant inlet velocity of 53.8mm/s was applied at the inlet pipe (corresponding to the maximum inlet flow rate of 40ml/min used by Vance et al. [6]). Calcium phosphate is a rigid and brittle material. Hence, in the CFD model the walls of calcium phosphate scaffold were assumed to be rigid and impermeable.

The bioreactor designs of Jaasma and O'Brien [5] and Vance et al. [6] have many similarities. Both concepts used a programmable syringe pump to perfuse culture medium through the inlet pipe into the scaffold chamber where the fluid perfused the cell-seeded scaffold. The culture medium exited the scaffold chamber through an outlet pipe and was stored in a medium reservoir. The main difference of the two designs concerned the scaffold chamber (see Figure 4). Jaasma and O'Brien [5] used an O-ring to ensure that the whole culture medium flowed through and did not by-pass the CG scaffold. Vance et al. [6], however, used a different approach. They chose a diameter for the scaffold chamber that resulted in a gap of approximately 350μm between the chamber wall and the scaffold. The size of the gap was chosen to be equal to the mean pore diameter in order to create similar flow conditions in the outer region and the center of the calcium phosphate scaffold. The design difference had two effects on our CFD models: (i) it was necessary to simulate the entire calcium phosphate scaffold, including the scaffold chamber, in order to determine whether the flow conditions in the outer region of the scaffold are equal to the center of the scaffold, (ii) it was reasonable to simulate only sub-volumes of the CG scaffolds, because the O-rings guaranteed that no culture medium flowed around the scaffold. This minimizes boundary effects between the scaffold and the chamber wall and creates uniform flow conditions over the whole volume of the CG scaffold.

For both scaffold types the following standard CFD parameters and settings were used: laminar flow, incompressible Newtonian fluid with a viscosity of 0.001Pa·s, no-slip scaffold and bioreactor walls, constant velocity inlet, and

zero pressure outlet. The CFD simulations were performed using the laminar flow solver *icoFoam* of the open source CFD tool box *OpenFOAM*. A mesh resolution study was undertaken to ensure a sufficient mesh density. Starting from coarse CFD meshes, the number of mesh elements was gradually increased and the maximum change in the fluid velocity and the wall shear stress with each increase was determined. The mesh density was increased until the maximum change in the fluid velocity was below 1.0%, and the maximum change in the wall shear stress was below 1.5% of the previous value.

The CG and the calcium phosphate scaffolds were simulated on a 64bit Linux computer cluster using 8 CPUs in parallel. The simulation of one sub-volume of the CG scaffold took approximately 13 days. However, approximately 3 days were required to perform the simulation of one calcium phosphate scaffold.

Results

Table 2 shows the analytical results for the three sub-volumes of the CG scaffold using an average scaffold pore size of 96μm, a dynamic viscosity of 0.001Pa·s, and an fluid velocity of 235μm/s - corresponding to the experimental settings of Jaasma and O'Brien [5]. The analytically estimated mean fluid velocity inside the CG scaffold was 0.260mm/s and resulted in an estimated wall shear stress of 21.7mPa.

Table 3 shows the analytical results for the three different calcium phosphate scaffolds using an average pore size of 350μm, a dynamic viscosity of 0.001Pa·s, and a fluid velocity of 24.89mm/s - corresponding to the experimental settings of Vance et al. [6]. The mean average fluid velocity in the calcium phosphate scaffolds is approximately 160 times higher than in the CG scaffolds (on average 39.5mm/s compared to 0.260mm/s). The higher fluid velocity for calcium phosphate scaffolds is caused by a combination of the much higher inlet flow rate (contributes a 106-fold increase) and the lower porosity of the calcium phosphate scaffold (contributes a 1.6-fold increase). The estimated wall shear stress experienced by cells in the calcium phosphate scaffolds is 903.4mPa, which is 41.6 times higher than cells experienced in the CG scaffold.

Figure 5 shows the distributions of the magnitude of the fluid velocity within the scaffolds using CFD simulations. The distributions do not include the inlet and outlet areas of the simulation volumes. Figure 5(a) depicts the distributions of the three simulated sub-volumes of the CG scaffold. The mean

and maximum fluid velocity (averaged over all three sub-volumes) was 0.296mm/s and 1.316mm/s, respectively. Figure 5(b) shows the distributions of the three calcium phosphate scaffolds. On average, the mean fluid velocity inside these scaffolds was 47.9mm/s and the maximum fluid velocity was 248.5mm/s. The fluid velocity distributions of both scaffold types feature a plateau-like shape from very low velocities up to approximately the mean fluid velocity and a rapid decay for velocities higher than the mean fluid velocity.

Figure 6 depicts the wall shear stress distributions of (a) CG and (b) calcium phosphate scaffolds. The wall shear stress acting on CG scaffolds ranges from 0 to 90mPa, exhibiting a mean wall shear stress value of 19.4mPa (averaged over all three simulated sub-volumes). The calcium phosphate scaffolds experienced much higher wall shear stresses (mean value of 745.2mPa and maximum value of 3107mPa) as shown in Figure 5b. Both distributions clearly show that cells throughout the scaffolds are not subjected to the same wall shear stress values, but experience a wide range of shear stresses.

Table 4 compares the results from the analytical method and the CFD simulations. As shown in Table 4 the analytical approach predicts lower mean fluid velocities within the scaffolds, however, higher mean wall shear stress values. Furthermore, it can be seen that agreement between analytical and CFD approaches is better for CG than for the calcium phosphate scaffold.

Discussion

In this study we developed CFD models of two scaffold types (CG and calcium phosphate), which have been used previously for bone tissue engineering applications, in order to determine the wall shear stresses to which cells seeded on these scaffolds are exposed to during flow perfusion bioreactor experiments. The aim of the study was to investigate the effect of the geometry of the two scaffold types on the flow conditions and how the numerical results compare to results obtained by the simple analytical method reported by Goldstein [18].

The CFD simulations and their comparison with the analytical method revealed three important findings: (i) cells seeded on scaffolds are exposed to a wide range of wall shear stresses, (ii) the simple analytical method (compared to the CFD models) predicts higher wall shear stresses, and (iii) the fluid velocities and the wall shear stresses within scaffolds can vary greatly between different types of scaffolds because of differences in scaffold micro-structural geometries. All three findings are of importance for planning and performing bioreactor experiments for tissue engineering.

The highly irregular geometry of the scaffolds resulted in a complex fluid dynamics environment that causes a wide range of shear stress values (Figure 6). Although the analytical method suggests that all cells are exposed to the same shear stress value (by providing the mean shear stress value only), the CFD models show cells are exposed to a wide distribution of wall shear stresses. Hence, cells at different locations within the scaffold will be

exposed to different magnitudes of fluid shear. The whole range of shear stress values was evenly distributed over the entire CG scaffold. High shear stresses occurred on locations where the culture medium had to pass through narrow channels and orifices, which resulted in an increased flow velocity and thereby an increased wall shear stress. In the calcium phosphate scaffold the highest wall shear stress values occurred at a ring-like region where the scaffold touched the bottom of the bioreactor chamber creating a narrow flow path which increased the wall shear stress.

The analytical method reported by Goldstein [18] which is widely used by experimentalists [5,6] to calculate the wall shear stresses and to determine the bioreactor's input flow rates, predicts shear stresses approximately 12% to 21% higher than the CFD simulations for the scaffolds investigated here. If this method is used to design experiments, this difference might result in bioreactor studies where the input flow rates are too low, leading to a lack of osteogenic stimulation or a stimulation of osteogenic activity that is sub-optimal. Better agreement to the numerical results might be found by using the analytical method reported by Wang et al. [20]. However, Wang's approach requires the non-trivial measurement of the scaffold's Darcy permeability, which introduces a new source of inaccuracy.

Both methods (CFD model and analytical estimation) revealed that the fluid velocity and the wall shear stress largely differed between the two types of scaffold. In order to compare the results with respect to the applied fluid flow, the commonly reported input flow rate, Q, is inadequate due to its dependency

on the cross-sectional area of the scaffolds. Instead, we propose a normalized input flow rate $q_{norm} = Q/A_{scaf}$ is used, which eliminates the influence of the scaffold size. The mean velocity within the calcium phosphate scaffold was approximately 160 times higher than in the CG scaffold (CG: $u_{avg} \approx$ 0.296mm/s, calcium phosphate: $u_{avg} \approx$ 47.9mm/s) mainly caused by the approximately 100-fold higher input flow rate (CG: q_{norm} = 0.235mm/s, calcium phosphate: q_{norm} = 24.9mm/s) and the higher porosity of the CG scaffold. The mean wall shear stress was approximately 38 times higher in calcium phosphate scaffolds than in CG scaffolds (CG: ~20mPa, calcium phosphate: ~750mPa). The lower percentage difference (between CG and calcium phosphate scaffolds) in wall shear stress than in fluid velocity is likely due to the larger pore size and the less complex geometry of the calcium phosphate scaffolds.

In order to obtain the same mean wall shear stress within both scaffold types a 2.8-fold higher input flow rate (normalized by the cross-sectional area of the scaffolds) is required in the calcium phosphate scaffold than the CG scaffold. Despite the lower porosity of the calcium phosphate scaffold, a larger input flow rate is required because of the less complex pore structure and the larger pore size of the calcium phosphate scaffold. However, the input flow rates and estimated mean wall shear stresses may not be sufficient for explaining biological outcomes in different scaffold/bioreactor systems. For example, lower shear stresses produced greater PGE_2 releases from osteoblasts in CG scaffolds [5] than from osteoblasts exposed to higher shear stresses in calcium phosphate scaffolds [6]. ~~In this study we used, for both scaffold types, input flow rates which were sufficient in recent experiments to detect increases in PGE_2 released by~~

~~osteoblastic cells [5,6].~~ The differences in wall shear stresses used to stimulate PGE_2 release in the different scaffolds may be explained by the manner by which cells attach to the scaffold [28]. In scaffolds with large pores (e.g. calcium phosphate scaffolds with a pore size of ~350µm) the cells are flatly attached to scaffold wall [6], whereas cells seeded on scaffolds with smaller pores (e.g. CG with a pore size of ~96µm) can also bridge between two struts of the scaffold [29,30]. Bridging cells are more easily deformed than flatly attached cells and therefore experience greater mechanical stimulation [28]. Therefore, the greater PGE_2 release in the experiment with CG scaffolds may be due in part to greater cell deformation.

In the analytical method, the wall shear stress is determined by assuming that the scaffold is made out of a bundle of parallel circular pipes. These pipes are perfused by the culture medium with a mean fluid velocity u_{scaf}, which is estimated by Equation 1. In bioreactor designs where the culture medium can also flow around the scaffold (as in Figure 4(b)) the accuracy of estimation of the wall shear stress using the analytical method can be significantly improved if the radius of scaffold chamber is used, as in Equation 1, instead of using the scaffold's radius [6]. Using the radius of the scaffold erroneously implies that the fluid has to flow through a smaller cross-section and thereby causes a higher mean fluid velocity and a higher wall shear stress. By using the chamber radius instead of the scaffold radius, the difference in the estimated wall shear stress relative to the wall shear stress obtained by the CFD simulation was reduced from 48.3% (τ_{analyt}=1105.1mPa) to 22.2% (τ_{analyt}=903.4mPa).

The following limitations should be considered in the interpretation of this work. (i) The CFD models did not account for molecular transport which can affect mechanotransduction responses [31]. (ii) The results of the CFD models of the calcium phosphate scaffolds are likely more accurate than that of the CG scaffold for two reasons: Firstly, the CFD model of the calcium phosphate scaffolds contained the whole flow path including the bioreactor's scaffold chamber and part of the inlet and outlet pipe, whereas the model of the CG scaffolds only included a sub-volume of the entire scaffold and did not include the bioreactor geometry. However, the effect on the results is considered to be negligible as the bioreactor utilized O-rings to prevent fluid from flowing around the scaffold [32] (see Figure 4). Secondly, the quality of the μCT images of the radio-absorbent calcium phosphate scaffolds was substantially better than of the nearly radio-translucent CG scaffolds. The minimum pixel size of 5μm of the used μCT (which is in the range of the dimensions of the micro-structures of the CG scaffold), combined with the poor radio-opaque properties of CG resulted in a CFD model with a scaffold porosity of approximately 90.5% compared to a porosity of 99% obtained by physical measurement. (iii) The CFD simulations provide more insight about the physical conditions within the scaffolds than the analytical approach because the CFD simulations provide the distributions of velocities and shear stresses, whereas the analytical approach only provides mean values. However, the absolute and relative accuracy of both methods can only be evaluated by comparing the theoretical results with data obtained by accurate experimental measurements. The measurement of local flow conditions within highly irregular micro-structures is non-trivial and was not performed in this study.

In conclusion this study shows that the flow conditions within two scaffolds which have been used for bone tissue engineering applications are highly irregular due to the complex scaffold geometries. These irregular flow conditions lead to a wide range of wall shear stresses experienced by cells seeded on these scaffolds. In order to subject the majority of the cells to physiologically relevant wall shear stresses, the experimental setup (in particular the determination of the input flow rate) must be chosen carefully. The widely used analytical method reported by Goldstein et al. [18] predicts higher wall shear stresses than the CFD approach and only yields a mean value. Therefore, this analytical method is of limited use for setting up bioreactor experiments. Our study also showed that the wall shear stress used to stimulate PGE_2 release from osteoblastic cells in calcium phosphate scaffolds is much higher than in CG scaffolds. This might be due to the smaller pore size of the CG scaffolds which allows cells to not only attach flatly on the scaffold wall but also to bridge between two struts of the scaffold, which likely causes greater cell deformation than in cells seeded in scaffolds with larger pore sizes such as the calcium phosphate scaffolds studied here [28]. Our findings suggest that computational models can be of great value for planning 3D bioreactor experiential conditions and interpreting results on mechanically induced changes in bone cell metabolism. However, because of the variability in scaffold and bioreactor geometries, custom CFD models need to be developed for specific experimental configurations.

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References

[1] Laurencin, C., Khan, Y., and El-Amin, S. F. Bone graft substitutes. Expert Review of Medical Devices 3, 49, 2006

[2] Stevens, B., Yang, Y., Mohandas, A., Stucker, B., and Nguyen, K. T. A review of materials, fabrication methods, and strategies used to enhance bone regeneration in engineered bone tissue. Journal of Biomedical Materials Research Part B: Applied Biomaterials, 17937408, 2007

[3] Hutmacher, D. W., Schantz, J. T., Lam, C. X. F., Tan, K. C., and Lim, T. C. State of the art and future directions of scaffold-based bone engineering from a biomaterials perspective. Journal of Tissue Engineering and Regenerative Medicine 1, 245, 2007

[4] Rezwan, K., Chen, Q. Z., Blaker, J. J., and Boccaccini, A. R. Biodegradable and bioactive porous polymer/inorganic composite scaffolds for bone tissue engineering. Biomaterials 27, 3413, 2006

[5] Jaasma, M. J., and O'Brien, F. J. Mechanical stimulation of osteoblasts by steady and dynamic fluid flow. Tissue Engineering, accepted, 2008

[6] Vance, J., Galley, D., Liu, D. F., and Donahue, S. W. Mechanical Stimulation of MC3T3 Osteoblastic Cells in a Bone Tissue-Engineering Bioreactor Enhances Prostaglandin E_2 Release. Tissue Engineering 11,1832, 2005

[7] O'Brien, F. J., Harley, B. A., Yannas, I. V., and Gibson, L. Influence of freezing rate on pore structure in freeze-dried collagen-GAG scaffolds. Biomaterials 25,1077, 2004

[8] O'Brien, F. J., Harley, B. A., Yannas, I. V., and Gibson, L. The effect of pore size on cell adhesion in collagen-GAG scaffolds. Biomaterials 26(4), 433, 2005

[9] Haugh, M. G., Jaasma, M. J., and O'Brien, F. J. The Effect of Dehydrothermal Treatment on the Mechanical and Structural Properties of Collagen-GAG scaffolds. Journal of Biomedical Materials Research Part A, accepted, 2008

[10] Karageorgiou, V., and Kaplan, D. Porosity of 3D biomaterial scaffolds and osteogenesis. Biomaterials 26, 5474, 2005

[11] Russias, J., Saiz, E., Deville, S., Gryn, K., Liu, G., Nalla, R. K., and Tomsia, A. P. Fabrication and in vitro characterization of three-dimensional organic/inorganic scaffolds by robocasting. Journal of Biomedical Materials Research Part A 83(2), 434, 2007

[12] Baksh, D. A comparison of 3-dimensional calcium phosphate scaffolds for candidate bone tissue engineering constructs [Master Thesis]. University of Toronto, Toronto, Canada, 1999

[13] Bakker, A. D., Joldersma, M., Klein-Nulend, J., and Burger, E. H. Interactive effects of PTH and mechanical stress on nitric oxide and PGE_2 production by primary mouse osteoblastic cells. Am. J. Physiol. Endocrinol. Metab. 285, E608, 2003

[14] Smalt, R., Mitchell, F. T., Howard, R. L., and Chambers, T. J. Induction of NO and prostaglandin E_2 in osteoblasts by wall-shear stress but not mechanical strain. Am. J. Physiol. 273, E751, 1997

[15] Weinbaum, S., Cowin, S. C., and Zeng, Y. A model for the excitation of osteocytes by mechanical loading-induced bone fluid shear stresses. J. Biomech. 27, 339, 1994

[16] You, J., Reilly, G. C., Zhen, X., Yellowley, C. E., Chen, Q., Donahue, H. J., and Jacobs, C. R. Osteopontin Gene Regulation by Oscillatory Fluid Flow via Intracellular Calcium Mobilization and Activation of Mitogen-activated Protein Kinase in MC3T3-E1 Osteoblasts. J. Biol. Chem. 276 (16), 13365, 2001

[17] Owan, I., Burr, D. B., Turner, C. H., Qiu, J., Tu, Y., Onyia, J. E., and Duncan, R. L. Mechanotransduction in bone: osteoblasts are more responsive to fluid forces than mechanical strain. Am J Physiol. 273, C810, 1997

[18] Goldstein, A. S., Juarez, T. M., Helmke, C. D., Custin, M. C., and Mikos, A. G. Effect of convection on osteoblastic cell growth and function in biodegradable polymer foam scaffolds. Biomaterials 22, 1279, 2001

[19] Botchwey, E. A., Pollack, S. R., El-Amin, S., Levine, E. M., Tuan, R. S., and Laurencin, C. T. Human osteoblast-like cells in three-dimensional culture with fluid flow. Biorheology 40, 299, 2003

[20] Wang, S., and Tarbell, J. M. Effect of Fluid Flow on Smooth Muscle Cells in a 3-Dimensional Collagen Gel Model. Arterioscler. Thromb. Vasc. Biol. 20, 2220, 2000

[21] Cioffi, M., Boschetti, F., Raimondi, M. T., and Dubini, G. Modeling Evaluation of the Fluid-Dynamic Microenvironment in Tissue-Engineered Constructs: A Micro-CT Based Model. Biotechnology and Bioengineering 93(3), 500, 2005

[22] Porter, B., Zauel, R., Stockman, H., Guldberg, R., and Fyhrie, D. 3-D computational modeling of media flow through scaffolds in a perfusion bioreactor. J Biomech 38(3), 543, 2005

[23] Martys, N., and Chen, H. Simulation of multicomponent fluids in complex three-dimensional geometries by the Lattice Boltzmann method. Physics Review E53, 743, 1996

[24] Donahue, T. L., Haut, T. R., Yellowley, C. E., Donahue, H. J., and Jacobs, C. R. Mechanosensitivity of bone cells to oscillating fluid flow induced shear stress may be modulated by chemotransport. J Biomech 36, 1363, 2003

[25] Wang, Y., McNamara, L. M., Schaffler, M. B., and Weinbaum, S. A model for the role of integrins in flow induced mechanotranduction in osteocytes. PNAS 104(40), 15941, 2007

[26] McGarry, J. G., Klein-Nulend, J., Mullender, M. G., and Prendergast, P. J. A comparison of strain and fluid shear stress in stimulating bone cell responses – a computational and experimental study. FASEB Journal 19(3), 482, 2005

[27] You, J., Yellowley, C. E., Donahue, H. J., Zhang, Y., Chen, Q., and Jacobs, C. R. Substrate Deformation Levels Associated With Routine Physical Activity Are Less Stimulatory to Bone Cells Relative to Loading-Induced Oscillatory Fluid Flow. J Biomechanical Engineering 122, 387, 2000

[28] Jungreuthmayer, C., Jaasma, M. J., Al-Munajjed, A. A., Zanghellini, J., Kelly, D. J., and O'Brien, F. J. 3D CFD-deformation simulation of a cell-seeded collagen-GAG scaffold in a flow perfusion bioreactor. Medical Engineering and Physics, submitted, 2008

[29] McMahon, L. The effect of cyclic tensile loading and growth factors on the chondrogenic differentiation of bone-marrow derived mesenchymal stem cells in a collagen-glycosaminoglycan scaffold [PhD thesis]. Trinity College Dublin, Dublin, Ireland, 2007

[30] Freyman, T. M., Yannas, I. V., Pek, Y-S., Yokoo, R., and Gibson, L. J. Micromechanics of Fibroblast Contraction of a Collagen-GAG Matrix. Experimental Cell Research 269, 140, 2001

[31] Burger, J. H., and Klein-Nulend, J. Responses of bone cells to biomechanical forces in vitro. Adv Dent Res 13, 93, 1999

[32] Jaasma, M. J., Plunkett, N. A., and O'Brien, F. J. Design and validation of a dynamic flow perfusion bioreactor for use with compliant tissue engineering scaffolds. J Biotechnology 133, 490, 2008

Table 1

Key parameters of the investigated CG and calcium phosphate scaffolds.

Table 2

The analytical results of the fluid velocity (u_{analyt}) and the wall shear stress (τ_{analyt}) for the CG scaffold using an fluid velocity of 235µm/s, an average pore size of 96µm, and a dynamic viscosity of 0.001Pa·s corresponding to the experimental settings of Jaasma and O'Brien [5].

Table 3

The analytical results of the calcium phosphate scaffold for the fluid velocity (u_{analyt}) and the wall shear stress (τ_{analyt}) using an fluid velocity of 24.89mm/s, an average pore size of 350µm, and a dynamic viscosity of 0.001Pa·s corresponding to the experimental settings of Vance et al. [6].

Table 4

A comparison of the results from the analytical method and the CFD simulations. The displayed results are values averaged over the 3 samples of each scaffold type. E_{rel} indicates the error of the analytical approach relative to the CFD model.

Figure 1

3D views of the CG (a) and calcium phosphate scaffold (b). (c) and (d) show cross-sections of the CG and the calcium phosphate scaffold, respectively.

Figure 2

Three randomly chosen sub-volumes (640µm x 640µm x 480µm) were used to determine the flow conditions of the CG scaffold.

Figure 3

Three entire scaffolds including the scaffold chamber plus part of the inlet and outlet pipes were used to simulate the flow conditions of calcium phosphate scaffolds.

Figure 4

Sketches of the bioreactor's scaffold chambers according to Jaasma et al. [5,31] using CG scaffolds (a) and Vance et al. [6] using calcium phosphate scaffolds (b).

Figure 5

The distribution of the fluid velocity within (a) the CG and (b) the calcium phosphate scaffolds obtained from the CFD simulation. The distributions do not include the inlet and outlet areas of the simulation volume. The input flow rate of (a) was 1ml/min and of (b) was 40ml/min corresponding to recent experiments [5,6].

Figure 6

The distribution of the wall shear stress of the (a) CG and (b) the calcium phosphate scaffolds calculated using CFD models. In order to avoid boundary artefacts for the CG scaffolds only the data of inner cubes of 320µm x 320µm x 320µm have been used for the calculation of the shear stress distribution. The input flow rate of (a) was 1ml/min and of (b) was 40ml/min corresponding to recent experiments [5,6].

	CG	Calcium phosphate
avg. pore size [µm]	96	350
avg. porosity [%] (measured)	99	60
avg. porosity [%] (CFD)	90.5	58.5
scaffold diameter [mm]	9.5	5.0
scaffold height [mm]	3.5	3.9
scaffold volume [mm^3]	248.10	76.58
scaffold's interstice volume [mm^3] (measured)	245.61	49.78
scaffold's interstice volume [mm^3] (CFD)	223.78	44.80
bioreactor’s chamber diameter [mm]	9.5	5.83

	Φ[%]	u_{analyt}[mm/s]	τ_{analyt}[mPa]
Sub-volume 1	92.4	0.254	21.2
Sub-volume 2	89.6	0.262	21.9
Sub-volume 3	89.4	0.263	21.9

	Φ'[%]	u_{analyt}[mm/s]	τ_{analyt}[mPa]
Scaffold 1	67.11	37.09	847.8
Scaffold 2	60.45	41.17	941.0
Scaffold 3	61.74	40.31	921.4

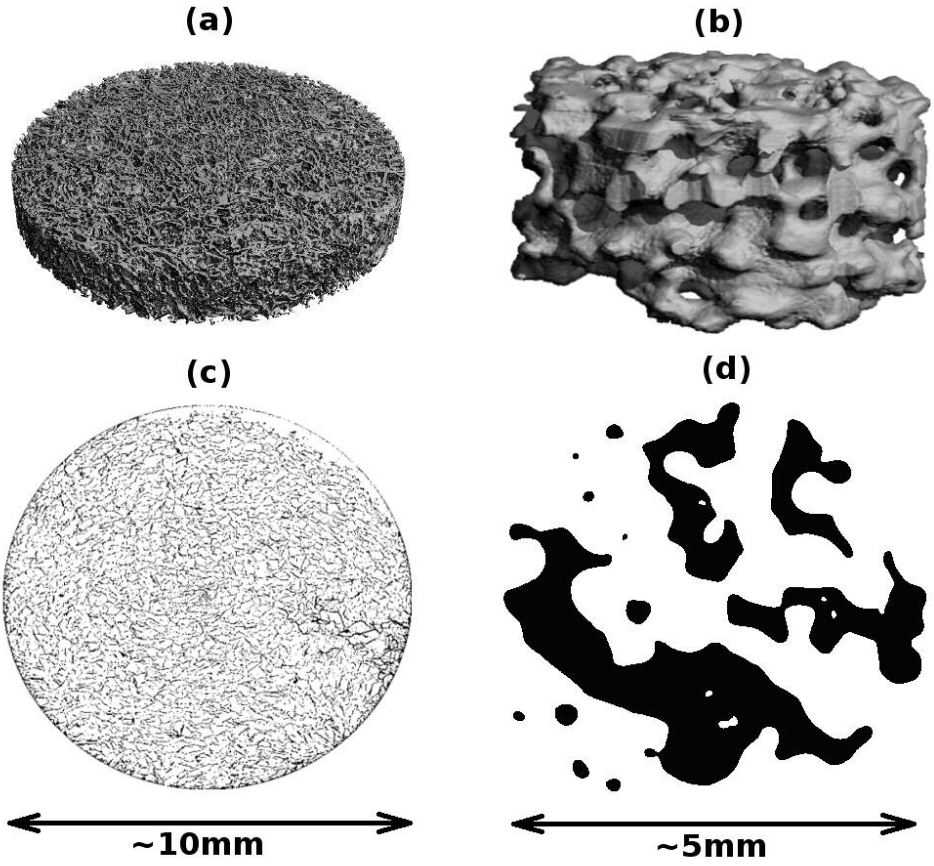


Figure 1
361x321mm (72 x 72 DPI)

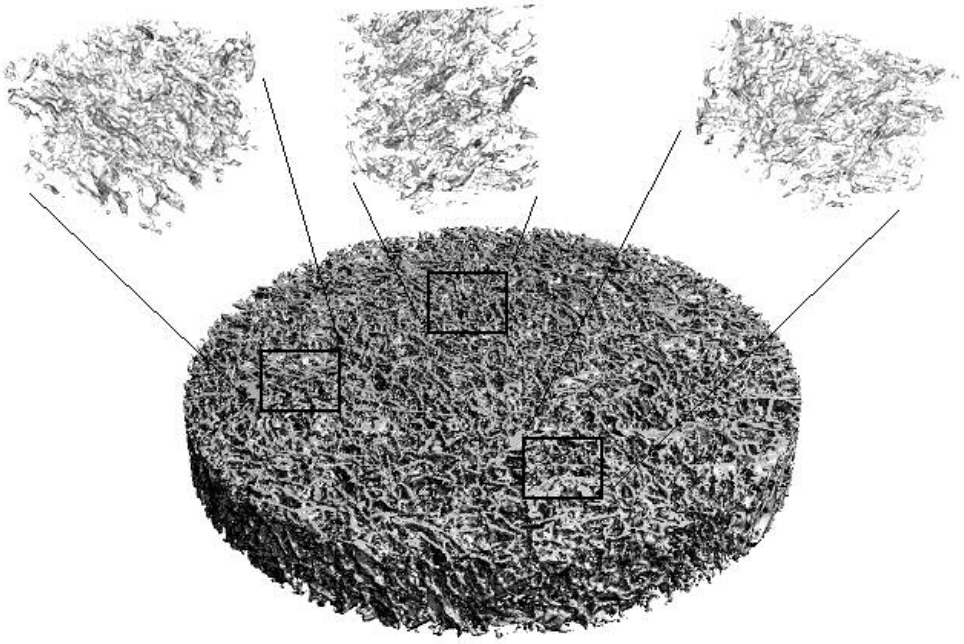


Figure 2

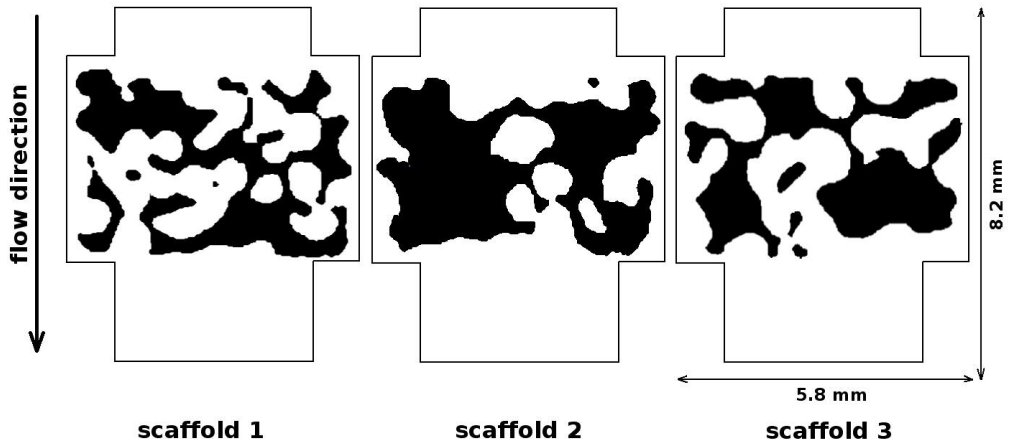


Figure 3

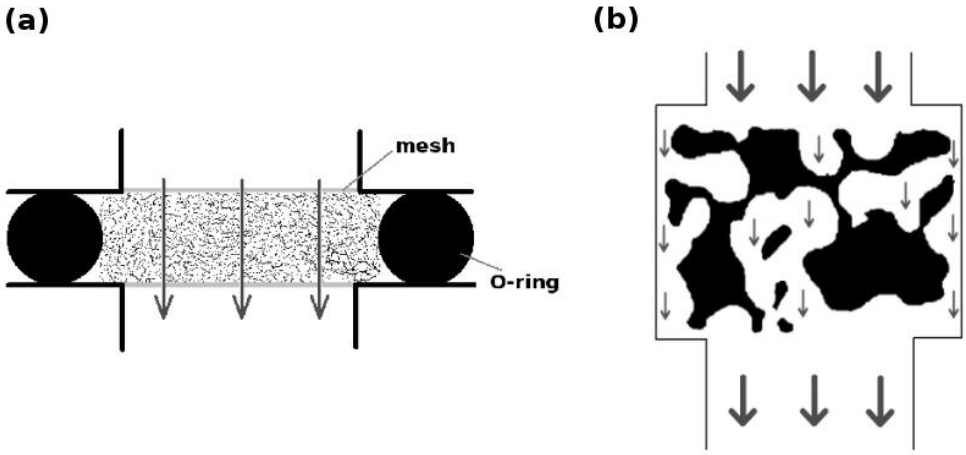
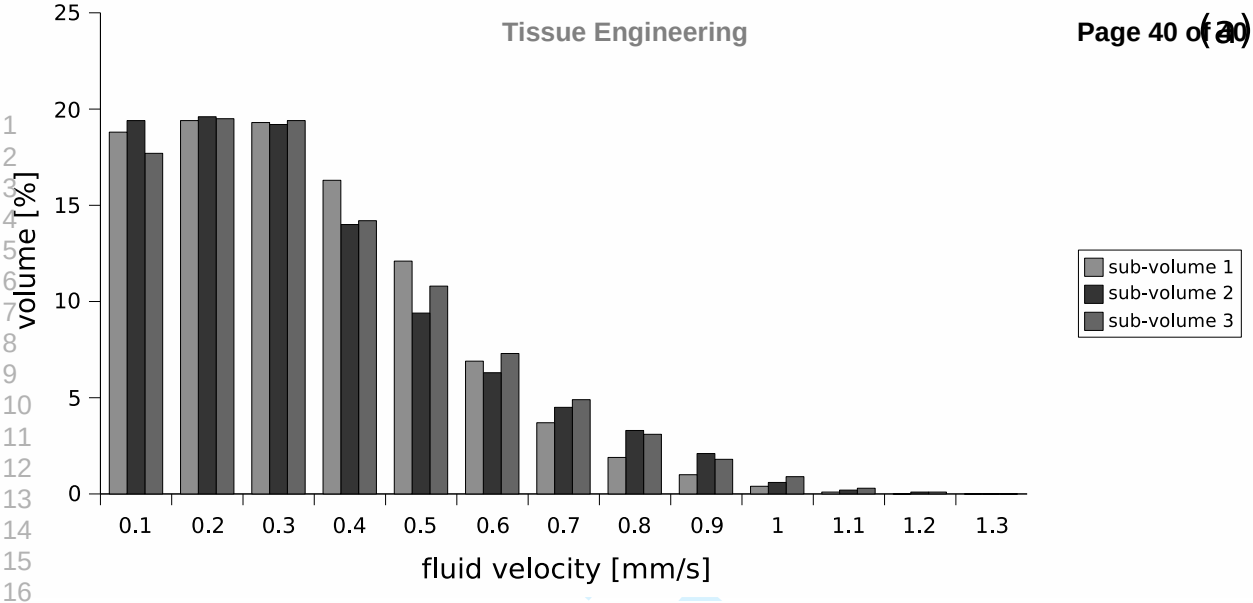
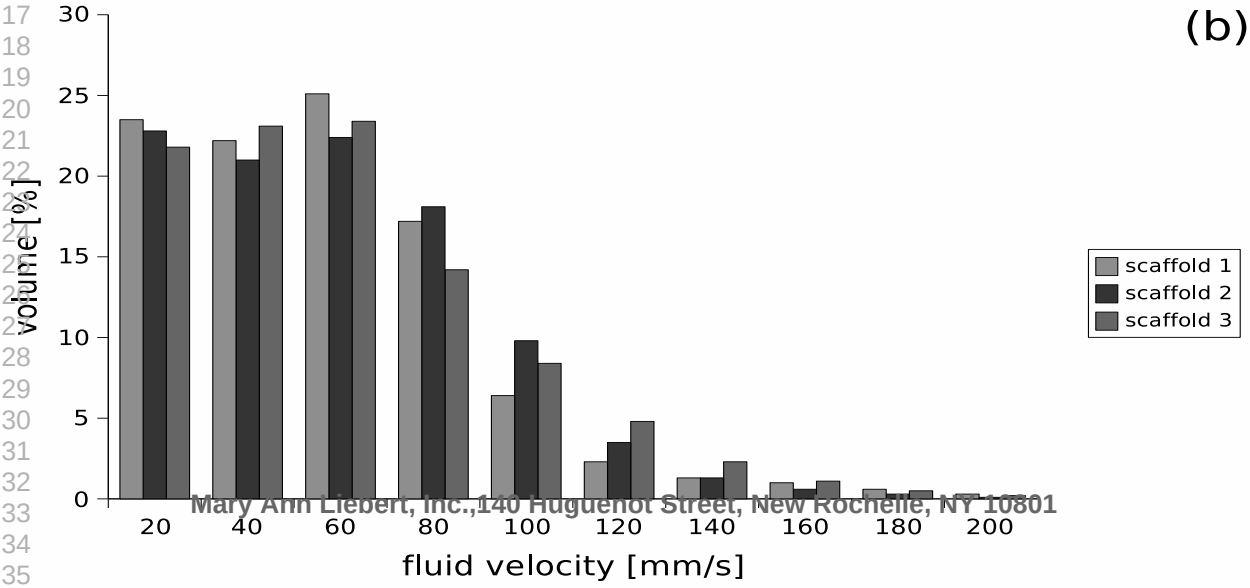


Figure 4

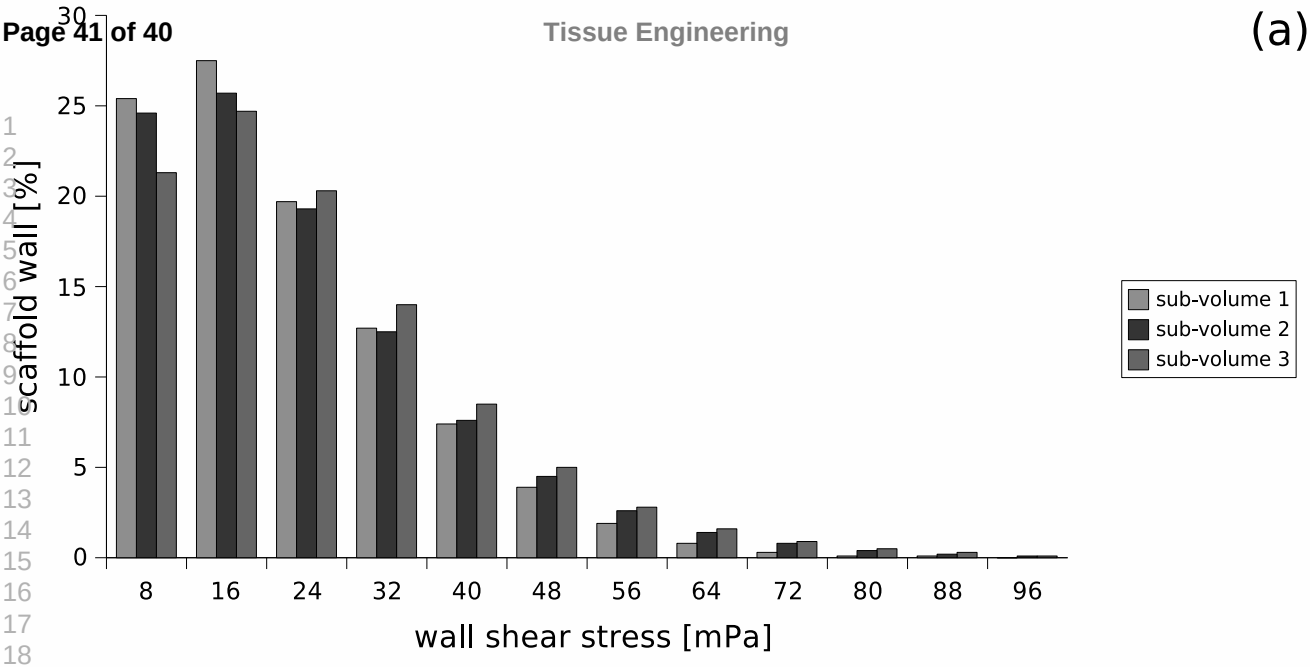
(a)



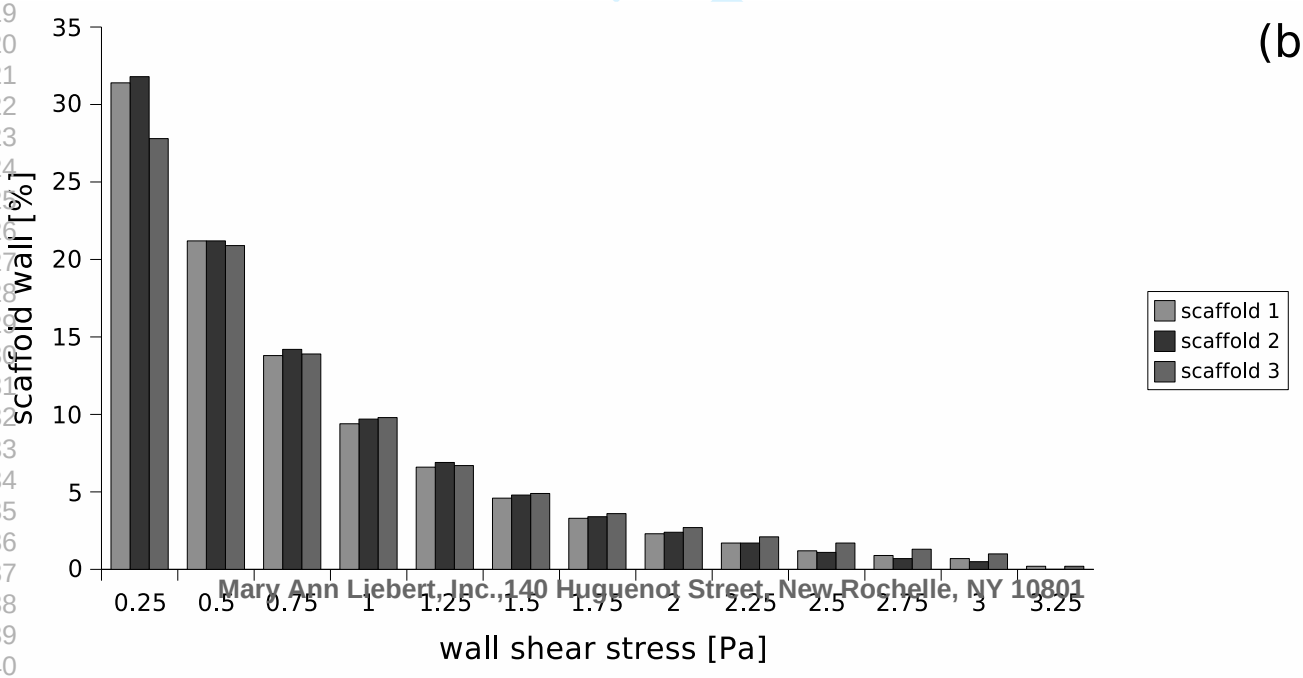
(b)



(a)


scaffold wall [%]
30
25
20
15
10
5
0
8
16
24
32
40
48
56
64
72
80
88
96
wall shear stress [mPa]
sub-volume 1
sub-volume 2
sub-volume 3

(b)


scaffold wall [%]
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30
25
20
15
10
5
0
0.25
0.5
0.75
1
1.25
1.5
1.75
2
2.25
2.5
2.75
3
3.25
wall shear stress [Pa]
scaffold 1
scaffold 2
scaffold 3