Cyclic Deformation-Induced Solute Transport in Tissue Scaffolds with Computer Designed, Interconnected, Pore Networks: Experiments and Simulations

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Abstract—Nutrient supply and waste removal in porous tissue engineering scaffolds decrease from the periphery to the center, leading to limited depth of ingrowth of new tissue into the scaffold. However, as many tissues experience cyclic physiological strains, this may provide a mechanism to enhance solute transport *in vivo* before vascularization of the scaffold. The hypothesis of this study was that pore cross-sectional geometry and interconnectivity are of major importance for the effectiveness of cyclic deformation-induced solute transport. Transparent elastic polyurethane scaffolds, with computer-programmed design of pore networks in the form of interconnected channels, were fabricated using a 3D printing and injection molding technique. The scaffold pores were loaded with a colored tracer for optical contrast, cyclically compressed with deformations of 10 and 15% of the original undeformed height at 1.0 Hz. Digital imaging was used to quantify the spatial distribution of the tracer concentration within the pores. Numerical simulations of a fluid–structure interaction model of deformation-induced solute transport were compared to the experimental data. The results of experiments and modeling agreed well and showed that pore interconnectivity heavily influences deformation-induced solute transport. Pore cross-sectional geometry appears to be of less relative importance in interconnected pore networks. Validated computer models of solute transport can be used to design optimal scaffold pore geometries that will enhance the convective transport of nutrients inside the scaffold and the removal of waste, thus improving the cell survivability deep inside the scaffold.

Keywords—Computational modeling, Cyclic strain, Imaging, Solid freeform fabrication, Tissue engineering.

INTRODUCTION

Cell-seeded tissue scaffolds of clinically relevant sizes suffer from gradients of tissue quality beyond the ‘diffusion distance’ ($>100\text{--}200\ \mu\text{m}$), including inhomogeneities in cell differentiation and proliferation from the scaffold surface toward the center of the construct.^{20,29} These gradients most likely emerge as a result of the difficulty to supply oxygen and nutrients to cells deep inside the scaffold, as well as to remove their waste products. Perfusion of the construct by application of bioreactors may mitigate the tissue gradients in thick scaffolds *in vitro*,^{5,28} however, strategies to provide engineered tissues with nutrients after implantation *in vivo* are imperative to sustain cells until sufficient vascularization is achieved.³⁶

With the exception of dermal tissue,^{21,22} most tissues of interest to tissue engineers have thicknesses beyond the diffusion distance and thus diffusion alone is insufficient to supply scaffolds of clinically relevant sizes.³ For instance, human articular knee cartilage has a thickness in the range of 1.6–3.5 mm.¹ The end-diastolic wall thickness of the left ventricle is generally about 1 cm in humans.³⁵ The wall thickness of small arteries depends on the diameter; a carotid artery with lumen diameter of $\sim 7\text{ mm}$ has a wall thickness of about 0.7 mm.²⁴ Upon implantation of a scaffold with thickness beyond $100\text{--}200\ \mu\text{m}$, convective transport needs to be established to more rapidly transport nutrients from the scaffold surface to its center. The repetitive mechanical strain experienced by many tissues *in vivo* may be a potential approach to achieve enhanced solute transport in scaffolds replacing such tissues. Cyclic loading of porous, deformable (engineered) tissues induces a repeated movement of the fluid inside the pores, thereby increasing the dispersion of solutes dissolved in the fluid.^{6,25} There are strong

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indications that cyclic strain increases the development of bone tissue, smooth muscle and cartilage,^{9,10,14} and enhanced nutrient transport is likely a contributing factor.

Both scaffold deformation and fluid flow are functions of the scaffold-pore geometry. Since technologies such as solid freeform fabrication (SFF) are emerging in tissue engineering,^{8,16,19} it becomes possible to precisely fabricate scaffolds based on computer-aided design (CAD) data with predictable transport properties. Such predictions require a validated, three-dimensional numerical model of deformation-induced solute convection, based on the detailed geometry of the porous scaffold. Previously published models of deformation-induced solute transport have focused on transport in cartilage and scaffolds for cartilage tissue engineering.^{7,23,31} These models indeed predict that cyclic loading could increase solute transport rates and depths, but unfortunately, the current models are limited to two dimensions and describe the mixture of solid matrix and fluid phase at a macroscopic level without a detailed geometrical representation of the pore system. Moreover, validation by experimental data is often lacking.

To overcome these limitations, the aim of this study was to quantify and model solute transport during harmonic compression of deformable porous scaffolds with computer designed pore networks. Polyurethane scaffolds were manufactured based on a three-dimensional (3D) printing and injection molding technique. The scaffold pores were loaded with a tracer, cyclically compressed while immersed in water, and solute transport was quantified with an optical imaging technique. These data were then compared to

simulation results of a three-dimensional numerical model describing the same experiment.

MATERIALS AND METHODS

Scaffold Fabrication

Polyurethane scaffolds were created using a combined 3D printing and injection molding technique as follows. CAD models of injection molds were designed using SolidWorks (SolidWorks Corp., Concord, MA). The molds were constructed such that a cubic scaffold of $5 \times 5 \times 5 \text{ mm}^3$ with eight channels with circular or elliptical cross-sections could be generated (Fig. 1). The models were saved in the stereolithography (STL) file format, and converted to 2D sliced data files using the software Catalyst EX (Stratasys, Eden Prairie, MN). The 3D printer, Dimension Elite SST (Stratasys, Eden Prairie, MN), was used to create the injection molds by printing the 2D sliced data files layer-by-layer with a build material (Acrylonitrile Butadiene Styrene, ABS) and a water soluble support material at a resolution of $254 \mu\text{m}$. After printing, the support material was dissolved by immersing the molds into a hot water/soap bath for $\sim 5 \text{ h}$ and the molds were then cleaned with water and dried at room temperature.

To prepare the polyurethane, 8.4 mL of prepolymer F-15 part A was mixed with 20 mL of curing agent F-15 part B (BJB Enterprises Inc., Tustin, CA). To facilitate removal of air bubbles, 3 drops of anti-foaming agent AF-4 (BJB Enterprises Inc., Tustin, CA) were added to the mixture. The mixture was then stirred, degassed under vacuum for 5 min and injected into the ABS molds with a plastic syringe.

FIGURE 1. Dimensions of the scaffolds generated by 3D printing and injection molding. Scaffolds with channels with circular cross-section (a) and elliptic cross-section (b) were fabricated. The direction of cyclic strain is indicated. Dimensions are in mm.

The polyurethane was allowed to cure for at least 48 h at room temperature. The ABS/polyurethane combination was then immersed in methylene chloride (Sigma–Aldrich, St. Louis, MO) for 24 h, which dissolved the ABS. The ABS-methylene chloride mixture was then completely removed from the pores with water and soap, leaving behind the transparent porous polyurethane. Finally, the scaffolds were dried at ambient temperature.

Experimental Setup

Figure 2 shows a schematic diagram of the experimental setup. Experiments were carried out at room temperature. The polyurethane scaffolds were glued with a transparent adhesive to the bottom of a transparent fluid reservoir placed underneath the pusher rod of a custom-made compression device. The compression device consisted of a spring-loaded piston connected to an eccentric circular cam driven by a motor. This system was used to apply a sinusoidal deformation with amplitude of 10 or 15% of the scaffold height and frequency of 1.0 Hz. A diluted solution of Brilliant Blue food coloring (Tone Brothers Inc., Ankeny, IA) in water (0.015 mL/mL) was used as a tracer and injected into the scaffold pores. Water at 25 °C was then slowly added to the fluid reservoir surrounding the scaffold, such that the tracer inside the scaffold pores remained undisturbed. The compression device was then switched on to apply cyclic deformation to the scaffold and thereby transporting the tracer from

the scaffold pores into the surrounding fluid reservoir. This process was imaged by a digital camera, consisting of a 1/5 in. CMOS image sensor array ($8 \times 8 \mu\text{m}^2$ pixels) with adjustable focus (Logitech, Fremont, CA), mounted underneath the transparent fluid reservoir, recording 24-bit color images of 352×288 pixels at a rate of 15 frames per second. Because of the transparency of the polyurethane, these images showed the blue tracer present in the four intersecting channels of the lower half of the scaffold. During imaging, a white cover was placed over the experimental setup so that white diffuse light illuminated the tracer inside the pores of the polyurethane scaffold.

Image Analysis

The brightness of the three-color images consisted of integers between 0 and 255 for the red, green and blue (R , G and B) levels for each pixel. Images were selected when the scaffold was in the uncompressed condition after 0, 5, 10, 20, and 50 successive compression cycles. Since the red value R was sensitive to the concentration of the tracer (higher tracer concentration resulted in reduced R values), it was used in the quantitative image analysis described below.

The image at $t = 0$ s was used to segment the pore system using a threshold value for R . The reflectance ρ was determined as the normalized R value (i.e., $\rho = R/255$). According to the Kubelka–Munk (K–M) theory of photon transport,¹⁵ the ratio of absorption to scatter (K/S) of an opaque layer inside a translucent medium can be calculated from the reflectance as follows:

$$\frac{K}{S} = \frac{(1 - \rho)^2}{2\rho} \quad (1)$$

Subsequently, the Beer–Lambert law was used to relate the ratio of absorption to scatter to the concentration c times the thickness h of the layer:

$$\frac{K}{S} = \frac{k}{s} \cdot c \cdot h, \quad (2)$$

with $\frac{k}{s}$ the unit absorption-to-scatter ratio. Thus, from Eqs. (1) and (2) the following proportional relationship can be obtained:

$$c \cdot h \propto \frac{(1 - \rho)^2}{2\rho}. \quad (3)$$

The average concentration $\bar{c}$ in a layer with volume V and thickness h is then calculated as the area integral of $c \cdot h$ divided by the volume:

$$\bar{c} = \frac{1}{V} \int_A (c \cdot h) dA, \quad (4)$$

FIGURE 2. Schematic diagram of the experimental setup.

where A is the area of the layer observed on the two-dimensional image. Therefore the average concentration $\bar{c}$ is proportional to the area average of the K–M values:

$$\bar{c} \propto \frac{1}{A} \int_A \frac{(1-\rho)^2}{2\rho} dA \quad (5)$$

In other words, application of the K–M theory to the image data enables calculation of the average concentration of tracer in the observed lower channels of the scaffold, relative to the average concentration of the tracer at $t = 0$ s.

Model Analysis

A computational model was developed and validated using the experiments. The model consisted of partial differential equations described below in component notation. The deformation of the scaffold was considered a dynamic small-strain problem with a linear Hooke's law as constitutive equation. Neglecting external body forces on the scaffold material, the equations describing change in momentum for the scaffold material s were given by

$$\begin{aligned} \frac{\partial \sigma_x}{\partial x} + \frac{\partial \tau_{xy}}{\partial y} + \frac{\partial \tau_{xz}}{\partial z} &= \rho_s \frac{\partial^2 u_s}{\partial t^2} \\ \frac{\partial \sigma_y}{\partial y} + \frac{\partial \tau_{xy}}{\partial x} + \frac{\partial \tau_{yz}}{\partial z} &= \rho_s \frac{\partial^2 v_s}{\partial t^2} \\ \frac{\partial \sigma_z}{\partial z} + \frac{\partial \tau_{xz}}{\partial x} + \frac{\partial \tau_{yz}}{\partial y} &= \rho_s \frac{\partial^2 w_s}{\partial t^2} \end{aligned} \quad (6)$$

where σ_x , σ_y and σ_z are the normal stresses, and τ_{xy} , τ_{xz} and τ_{yz} are the shear stresses. Furthermore, ρ_s is the density of the scaffold material, and u_s , v_s and w_s are the displacements of a material point in the deforming scaffold. Assuming small strains, the following relations between strains and displacements were used:

$$\begin{aligned} \varepsilon_x &= \frac{\partial u_s}{\partial x} & \gamma_{xy} &= \left(\frac{\partial v_s}{\partial x} + \frac{\partial u_s}{\partial y} \right) \\ \varepsilon_y &= \frac{\partial v_s}{\partial y} & \gamma_{xz} &= \left(\frac{\partial w_s}{\partial x} + \frac{\partial u_s}{\partial z} \right) \\ \varepsilon_z &= \frac{\partial w_s}{\partial z} & \gamma_{yz} &= \left(\frac{\partial w_s}{\partial y} + \frac{\partial v_s}{\partial z} \right) \end{aligned} \quad (7)$$

Here ε_x , ε_y and ε_z are the normal strains, and γ_{xy} , γ_{xz} and γ_{yz} are the engineering shear strains. Assuming linear, isotropic elastic behavior, the relation between stress and strain in the scaffold was described by Hooke's law:

$$\begin{bmatrix} \sigma_x \\ \sigma_y \\ \sigma_z \\ \tau_{yz} \\ \tau_{xz} \\ \tau_{xy} \end{bmatrix} = \frac{E}{(1+\nu)(1-2\nu)} \times \begin{bmatrix} 1-\nu & \nu & \nu & 0 & 0 & 0 \\ \nu & 1-\nu & \nu & 0 & 0 & 0 \\ \nu & \nu & 1-\nu & 0 & 0 & 0 \\ 0 & 0 & 0 & \frac{1}{2}-\nu & 0 & 0 \\ 0 & 0 & 0 & 0 & \frac{1}{2}-\nu & 0 \\ 0 & 0 & 0 & 0 & 0 & \frac{1}{2}-\nu \end{bmatrix} \begin{bmatrix} \varepsilon_x \\ \varepsilon_y \\ \varepsilon_z \\ \gamma_{yz} \\ \gamma_{xz} \\ \gamma_{xy} \end{bmatrix} \quad (8)$$

where E is the Young's modulus and ν is the Poisson's ratio of the scaffold material.

Fluid pressure and velocity of the fluid phase are described by the Navier–Stokes equations. Assuming an incompressible fluid, the continuity equation is given by:

$$\frac{\partial u_f}{\partial x} + \frac{\partial v_f}{\partial y} + \frac{\partial w_f}{\partial z} = 0, \quad (9)$$

where u_f , v_f and w_f are the fluid velocity components. Furthermore, we modeled laminar flow of a Newtonian fluid with viscosity μ . Neglecting natural convection due to gravitation, the momentum balance of the Navier–Stokes equations is given by:

$$\begin{aligned} \rho_f \left(\frac{\partial u_f}{\partial t} + u_f \frac{\partial u_f}{\partial x} + v_f \frac{\partial u_f}{\partial y} + w_f \frac{\partial u_f}{\partial z} \right) &= -\frac{\partial p}{\partial x} + \mu \left(\frac{\partial^2 u_f}{\partial x^2} + \frac{\partial^2 u_f}{\partial y^2} + \frac{\partial^2 u_f}{\partial z^2} \right) \\ \rho_f \left(\frac{\partial v_f}{\partial t} + u_f \frac{\partial v_f}{\partial x} + v_f \frac{\partial v_f}{\partial y} + w_f \frac{\partial v_f}{\partial z} \right) &= -\frac{\partial p}{\partial y} + \mu \left(\frac{\partial^2 v_f}{\partial x^2} + \frac{\partial^2 v_f}{\partial y^2} + \frac{\partial^2 v_f}{\partial z^2} \right) \\ \rho_f \left(\frac{\partial w_f}{\partial t} + u_f \frac{\partial w_f}{\partial x} + v_f \frac{\partial w_f}{\partial y} + w_f \frac{\partial w_f}{\partial z} \right) &= -\frac{\partial p}{\partial z} + \mu \left(\frac{\partial^2 w_f}{\partial x^2} + \frac{\partial^2 w_f}{\partial y^2} + \frac{\partial^2 w_f}{\partial z^2} \right) \end{aligned} \quad (10)$$

where p is the fluid pressure, and ρ_f is the density of the fluid. Finally, solute transport was modeled by the scalar convection-diffusion equation:

$$\frac{\partial c}{\partial t} + u_f \frac{\partial c}{\partial x} + v_f \frac{\partial c}{\partial y} + w_f \frac{\partial c}{\partial z} = D \left(\frac{\partial^2 c}{\partial x^2} + \frac{\partial^2 c}{\partial y^2} + \frac{\partial^2 c}{\partial z^2} \right) \quad (11)$$

where c is the concentration of tracer in the fluid and D the diffusion coefficient.

FIGURE 3. (a) Model symmetry planes. (b) Schematic diagram of the model boundary conditions.

The modeled scaffold had the same dimensions as in the CAD file used to print the injection molds. To reduce computational time, this geometry was divided into eight symmetrical parts and only one part was modeled (Fig. 3a). To take into account the fluid surrounding the scaffold, a fluid domain with a length of 5 mm was created with an interface to the top and bottom channel openings.

The solid phase (scaffold) was subjected to the following boundary conditions (Fig. 3b). The displacement of the bottom face of the scaffold was set to zero in all directions (fixed support). The top face of the scaffold was cyclically compressed such that the vertical displacement w_s followed a cosine function:

$$w_s = -A \left[\frac{1}{2} - \frac{1}{2} \cos(2\pi ft) \right], \quad (12)$$

where A is the compression amplitude and f is the compression frequency. At the two symmetry planes,

the displacement perpendicular to the symmetry plane was set to zero. The velocity of the fluid at the channel wall was set equal to the velocity of scaffold material at the channel wall surface. The solute flux transverse to the channel wall was set to zero, i.e., it was assumed that the scaffold material did not absorb the tracer. The pressure at the side of the fluid reservoir farthest from the channel opening was set to zero above ambient pressure. The solute concentration was also set to zero at this boundary. At the upper and lower side faces of the fluid reservoir, no-slip wall boundary conditions were implemented. At the symmetry planes, the fluid velocity and the solute gradient normal to the symmetry plane were assumed to be zero. At $t = 0$, the scaffold was assumed to be at rest and the fluid pressure and velocity were set to zero in the entire fluid domain. The concentration of solute was expressed relative to the initial concentration, and was set to zero in the fluid reservoir and to 1.0 inside the pores. Both

FIGURE 4. (a) Computational mesh of the fluid domain for the scaffold with circular channels. (b) Computational mesh including the solid domain.

TABLE 1. Description and values of parameters used in the computational model.

Parameter	Value	Description
E	1.65 MPa	Young's modulus scaffold material
ν	0.4995	Poisson's ratio scaffold material
ρ_s	1.0 g cm^{-3}	Density scaffold material
ρ_f	1.0 g cm^{-3}	Fluid density (water at 25°C)
μ	$0.89 \times 10^{-3} \text{ Pa s}$	Fluid viscosity (water at 25°C)
D	$1 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$	Tracer diffusion coefficient
f	1.0 Hz	Compression frequency
A	0, 0.5, or 0.75 mm	Compression amplitude

solid and fluid phases were meshed with tetrahedral elements (Fig. 4).

Model parameters are summarized in Table 1. The Young's modulus and density of the polyurethane were obtained from the manufacturer. The scaffold material was assumed to be incompressible, and to avoid numerical instability during the inversion of the stiffness matrix, a value of 0.4995 was used for the Poisson's ratio in the numerical calculations. The modeled fluid was water at 25°C with a density of 1.0 g cm^{-3} and a viscosity of $0.89 \times 10^{-3} \text{ Pa s}$. For the diffusion coefficient of the blue tracer in water, a value of $1 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$ was used.²

After validation of the model, a hypothetical scenario was simulated to predict whether 15% cyclic deformation of the scaffold with intersecting circular channels at 1.0 Hz would allow transporting sufficient oxygen (O_2) to supply cells attached to the channel walls. In this particular simulation, the modeled solute was O_2 with a diffusion coefficient of $2.4 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$.¹⁷ The surrounding fluid reservoir was omitted in this simulation, and the O_2 concentration at the channel openings was fixed at $130 \mu\text{mol L}^{-1}$ (the O_2

concentration in arterial blood). A source boundary condition was added to the convection-diffusion equation at the channel wall to simulate O_2 consumption. For simplicity, O_2 consumption was assumed to be proportional to O_2 concentration. Maximum O_2 consumption rate at $130 \mu\text{mol L}^{-1}$ was calculated based on an average of $5 \text{ nmol O}_2 \text{ min}^{-1} (10^6 \text{ cells})^{-1}$.²⁷ Cells with area $100 \mu\text{m}^2$ were assumed to cover the entire channel wall, yielding a cell density of $4 \times 10^7 \text{ cells mL}^{-1}$ of pore.

The software Ansys Workbench (Ansys Inc., Canonsburg, PA) was used to obtain numerical solutions. The dynamic solid deformation problem was solved using the Finite Element Method (FEM), whereas the Navier-Stokes and scalar transport equations were solved using the Finite Volume Method (FVM). At each time step, the solid deformation problem was solved first, and the mesh displacement at the fluid-solid interface was then transferred to the fluid solver as a moving boundary condition in the fluid flow problem. Three-dimensional 10-node tetrahedral elements with quadratic displacement behavior were used to discretize the scaffold geometry (Fig. 4). The discretized system of equations was solved using a Preconditioned Conjugate Gradient solver in combination with a Newmark time integration method. The fluid domain was discretized using 3D tetrahedral elements with linear shape functions and integration points at the center of each surface. The deformation of control volumes was accounted for by modifying the conservation equations using the Leibnitz integration rule and the fluid node displacements were computed by solving a Poisson's equation. An implicit second-order backward Euler method was used as a transient scheme. A high-resolution advection scheme was used, which varied between first and second order advection schemes throughout the domain to be as close to

second order accurate as possible, while enforcing a boundedness criterion. The solver advanced to the next time step when the root mean square values of the equation residuals were below 1×10^{-4} , or when a maximum of 15 coefficient loop iterations was reached. A fixed time step of $t = 0.025$ s was found to be sufficiently small to obtain stable numerical results.

Statistical Analysis

The tracer concentrations measured during the experiments were statistically evaluated with one-way analysis of variance (ANOVA). The mean tracer concentrations in the groups subjected to diffusion only, 10% cyclic deformation, or 15% cyclic deformation, were compared using Tukey's honestly significant difference criterion (*: deformation vs. diffusion, $p < 0.05$; #: 15% deformation vs. 10% deformation, $p < 0.05$). The same procedure was followed to evaluate statistical differences between the mean tracer concentrations in the circular and elliptic channels at 15% cyclic deformation (* $p < 0.05$).

RESULTS

The injection mold was designed using CAD software (Fig. 5a) and printed using the 3D printer (Fig. 5b). After injection and curing of the polyurethane, the ABS mold was dissolved, rendering the final scaffolds of $5 \times 5 \times 5$ mm³ (Fig. 5c), with circular channels of 1.0 mm diameter, or with elliptical channels of 1.4 mm (major axis) and 0.6 mm (minor axis).

To verify the applicability of the K–M theory to estimate the average tracer concentration, a dilution series of the blue tracer was prepared. The different concentrations of tracer were infused inside the polyurethane scaffolds, images were recorded, and the relative average concentration was calculated from the

images based on the K–M theory. This resulted in a proportional relationship with slope of 1.015 and $R^2 = 0.9902$ (Fig. 6), meaning that this method can be used to estimate the average tracer concentration in the tracer experiments.

Figure 7 shows the original experimental images, the images after conversion by the K–M theory to calculate the tracer concentration, and the simulation results of the computational model as a function of time. The results shown were taken from the experiments with 15% cyclic deformation at 1.0 Hz. It should be noted that the model concentration images were taken in a plane in the middle of the scaffold channels and that these images were mirrored and rotated to obtain the full domain.

FIGURE 6. (a) Proportional relationship between relative tracer concentration and K–M values. (b) Individual measurements at 0, 25, 50, 75, and 100% of the maximal tracer concentration.

FIGURE 5. (a) Initial CAD design of the injection mold. (b) ABS mold after printing. (c) Final polyurethane scaffold (to highlight the channels, tracer was injected inside the channels).

FIGURE 7. Original images (top) images after K–M conversion (middle) and model simulations (bottom) of the scaffold with circular channels at different time points. A total of 15% cyclic strain at 1.0 Hz was applied. In the K–M and model images, dark blue means low tracer concentration, while red means high tracer concentration.

The K–M values were used to quantify the average solute concentration in the lower four channels relative to the average concentration at $t = 0$ s (Fig. 8). Figure 8A shows that at all time points beyond $t = 0$, application of cyclic deformation (bottom two data sets (b) and (c)) yielded a significantly lower tracer concentration in the scaffold pores as compared to diffusion only (upper data set (a)) in the scaffold with circular channels. Moreover, for all time points beyond $t = 0$, the difference between 10 and 15% deformation was also statistically significant. The measurements showed only a small difference between the tracer concentrations in the scaffolds with circular and elliptic channels when cyclically compressed with amplitude of 15%, yielding a statistically significant difference at $t = 5$ s only (Fig. 8B).

The simulation results are also shown in Fig. 8 with dashed lines. The simulated tracer concentrations in the lower four channels agreed well with the measured tracer concentrations. The percentage of tracer removed from the pores was calculated at the end of the test time ($t = 50$ s) and compared to the value obtained from the model (Fig. 9). These results showed an increased efficiency of cyclic pumping as compared to diffusive transport alone. As expected, increasing the strain amplitude also increased the solute transport. Interestingly, transport in the scaffold with elliptical channels seemed to be slightly lower

than transport in the scaffold with circular channels, although this difference was not significant. The simulations of the fluid–structure interaction model agreed quantitatively with the experimental data and followed the same trend (Figs. 8 and 9), proving that simulation can be an effective tool for the rapid design of deformable scaffolds.

The validated model was then used to predict the effect of O_2 consumption by cells attached to the channel walls, as described in [Materials and Methods](#) section. Average oxygen concentration decreased initially due to O_2 depletion at the pore walls, but reached a steady state value at $\sim 80 \mu\text{mol L}^{-1}$ after 60 s due to inward transport by cyclic deformation with amplitude of 15% at 1.0 Hz (Fig. 10a). At $t = 60$ s, the spatial O_2 profile showed a gradient from high to low concentration from the channel openings toward the scaffold center, but the O_2 level remained above $30 \mu\text{mol L}^{-1}$ everywhere inside the channel.

DISCUSSION

Solute transport in cyclically deformed tissue scaffolds with computer-designed pore geometries was quantified using an optical imaging method. A computational model of deformation-induced solute convection was also developed based on the

FIGURE 8. (A) Comparison of data from 5 experiments (means and SD error bars) with model simulations (dashed lines) for the scaffold with circular channels during (a) diffusion only and, (b) 10% and (c) 15% cyclic strain (*: cyclic strain vs. diffusion, $p < 0.05$; #: 15% vs. 10% strain, $p < 0.05$). (B) Data and model for the scaffolds with circular and elliptical channels during 15% cyclic strain (*: elliptic vs. circular, $p < 0.05$).

three-dimensional geometries of the scaffolds. The simulation results agreed well with the experimental data. This provided confidence that similar simulations of more complex pore labyrinths should provide meaningful results for realistic *in vivo* scaffolds, when experimental results are hard to obtain.

For experimental purposes, scaffolds with intersecting channels of both circular and elliptic cross-sections were generated, because it was expected that the channels with elliptic cross-sections with semi-minor axis in the strain direction would yield a greater volume change under the same strain amplitude, and thus generate a higher average transport rate of the solute. Interestingly, both the experimental data and the model results demonstrate that both scaffold pore geometries exhibit similar rates of solute transport.

FIGURE 9. Percentage of tracer removed from the pores after 50 s for the four different cases. Experimental results (black, with error bars) are compared to the model (white).

Hence, it appears that channel intersections play a more important role in cyclic-deformation induced solute transport than pore cross-sectional geometries. The importance of interconnections in scaffold pore systems is evident from comparison to a simulation of a scaffold with a single channel in the middle with a 1.0 mm diameter cylindrical cross-section (Fig. 11a). Application of the same deformation amplitude of 15% at 1.0 Hz yielded a lower solute transport rate as compared to the scaffold with intersecting circular channels. This can be explained by the increased ratio of opening area to pore volume due to the intersections (0.24 mm^{-1} for the intersecting circular channels vs. 0.20 mm^{-1} for the single channel scaffold). The model was also used to predict the effect of joining the two channel layers with additional circular channels that were not physically connected to the boundaries of the scaffold (Fig. 11b). Hence, these additional channels only increased the scaffold pore volume, but not the opening area, thereby creating an area of *stagnant* solute transport inside the channel that connected the two layers, and thus yielding a solute transport rate even lower than in a single channel scaffold (Fig. 11a).

Many tissues undergo certain forms of cyclic loading *in vivo*. Strain data measured in humans are scarce, but emerging imaging techniques such as ultrasound and magnetic resonance enable the measurements. For instance, longitudinal strain in the interventricular septum of the heart of a healthy person can be as large as 20% during peak systole.³⁴ Peak deformations in knee cartilage during single-leg lunges range from 22 to 30%.¹ Strains of up to 25% were measured in the wall of the left anterior descending coronary artery in a human.¹⁸ Strain in the vastus lateralis tendon during

FIGURE 10. (a) Model prediction of the average oxygen (O_2) concentration inside the scaffold with circular channel upon cyclic deformation with 15% strain at 1.0 Hz. Cellular O_2 consumption is taken into account by a source term at the pore walls. (b) Spatial O_2 distribution at $t = 60$ s. A concentration gradient was predicted from the scaffold surface toward the center, but the O_2 level remained above $30 \mu\text{mol L}^{-1}$.

FIGURE 11. (a) Percentage of tracer removed, as simulated for a scaffold with a single circular channel, a scaffold with two layers of intersecting circular channels, and a scaffold where the two layers of intersecting channels are connected. (b) Connection of the layers of intersecting channels results in slow solute transport in the channels connecting the two layers. Darker gray represents higher solute concentration (linear scale).

maximal isometric contraction was measured to be 8%.³³ Strains of these magnitudes could induce significant solute convection in scaffolds replacing such

tissues, provided that the scaffold pore geometry allows for such solute convection.

Lack of oxygen supply to cells populating the scaffold and the accompanied hypoxia-induced lethal acidosis limits the sustainable cell density inside the scaffold.⁴ We used our validated model of cyclic deformation-induced solute transport to predict whether oxygen transport during cyclic compression would be sufficient to supply cells lining the scaffold-pore interface. Although simplifying assumptions about, e.g., the relation between cellular respiration and O_2 level were made, the model predicted that cyclic deformation-induced transport could prevent O_2 from being entirely depleted anywhere in the scaffold pore labyrinth. The average steady state O_2 level within the entire pore remained high at $\sim 80 \mu\text{mol L}^{-1}$, while the range was $30\text{--}130 \mu\text{mol L}^{-1}$ throughout the pore. Thus, our model predicts that cyclic compression of the scaffold should at the least be capable of providing sufficient O_2 supply to the cells attached to the surface of the scaffold pores in the time period between implantation of the scaffold and establishment of blood supply by vascularization.

Potential Limitations

The smallest pore dimension that potentially could be achieved by our method, $254 \mu\text{m}$, is governed by the resolution of the 3D printer. Optimal scaffold pore diameters are dependent on cell type, but have been suggested to range from 100 to $500 \mu\text{m}$.¹¹ It should be noted that these dimensions are based on scaffolds made with non-SFF fabrication techniques; interestingly, scaffolds fabricated with SFF techniques have been shown to sustain similar rates of cell ingrowth

independent of pore diameter in scaffolds with pores greater than 1000 μm in diameter.⁸

Blurring of the recorded images occurred due to the non-ideal point spread function of the imaging system. It can be observed in the images (e.g., Fig. 7) that image blurring affects the visualization of the spatial distribution of the tracer. However, since blurring is a linear convolution with the point spread function, it can be assumed that its effect on the quantification of the *average* tracer concentration relative to $\bar{c}$ at $t = 0$ is minimal.

Quantification of the concentration and distribution of a tracer with digital image sensors is emerging,³² e.g., to determine tracer transport through soils²⁶ or to measure ink concentrations in a printing process.³⁰ Disadvantage of optical imaging methods is the need for a thin or transparent specimen. We have previously shown that deformation-induced transport in opaque scaffolds can be quantified using X-ray micro-imaging.²⁵ Imaging of solute transport through more complex pore systems requires a tomographic imaging method, such as micro-CT.^{12,13}

In conclusion, cyclic compression increases solute convection by cyclic fluid motion and subsequent spreading of solutes dissolved in the fluid. The efficiency of the cyclic pumping can be increased by designing an intersecting channel network with sufficient connections to the scaffold-tissue boundary, so that exchange of solute and waste products can take place. Interestingly, the shape of the pore cross-sections does not seem to have a significant influence on deformation-induced solute transport in scaffolds with intersecting channels. On the other hand, we have shown that adding pore channels which do not directly communicate with the scaffold surroundings are ineffective, as they create regions of stagnant solute transport. Thus, careful design and fabrication of deformable porous tissue scaffolds may be a strategy through which solutes can be transported beyond the diffusion limit after implantation of constructs with clinically relevant thicknesses. Our proposed experimental and modeling techniques could aid such scaffold design.

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