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Multiscale Computational Model of Fluid Flow and Matrix Deformation in Decellularized Liver

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Abstract

Currently little is known about the biomechanical environment in decellularized tissue. The goal of this research is to quantify the mechanical microenvironment in decellularized liver, for varying organ-scale perfusion conditions, using a combined experimental/computational approach. Needle-guided ultra-miniature pressure sensors were inserted into liver tissue to measure parenchymal fluid pressure ex-situ in portal vein-perfused native (n=5) and decellularized (n=7) ferret liver, for flow rates from 3–12 mL/min. Pressures were also recorded at the inlet near the portal vein cannula to estimate total vascular resistance of the specimens. Experimental results were fit to a multiscale computational model to simulate perfusion conditions inside native versus decellularized livers for four experimental flow rates. The multiscale model consists of two parts: an organ-scale electrical analog model of liver hemodynamics and a tissue-scale model that predicts pore fluid pressure, pore fluid velocity, and solid matrix stress and deformation throughout the 3D hepatic lobule. Distinct models were created for native versus decellularized liver. Results show that vascular resistance decreases by 82% as a result of decellularization. The hydraulic conductivity of the decellularized liver lobule, a measure of tissue permeability, was 5.6 times that of native liver. For the four flow rates studied, mean fluid pressures in the decellularized lobule were 0.6 to 2.4 mmHg, mean fluid velocities were 211 to 767 $\mu\text{m/s}$, and average solid matrix principal strains were 1.7 to 6.1%. In future this modeling platform can be used to guide the optimization of perfusion seeding and conditioning strategies for decellularized scaffolds in liver bioengineering.

Graphical abstract

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Keywords

regenerative medicine; tissue engineering; biomechanics; liver decellularization

1. INTRODUCTION

According to the U.S. Centers for Disease Control and Prevention, chronic liver disease and cirrhosis were the 12th leading cause of death in the United States in 2010 [1]. Currently, the only definitive treatment available for end stage liver disease is liver transplantation. Only 5,921 out of 15,027 patients on the waiting list received a transplant in 2013 due to the severe shortage of transplantable donor livers [2]. Therefore, there is a large demand to develop alternatives to liver transplantation. Tissue engineering strategies such as organ decellularization and recellularization techniques are attractive because they have the potential to regenerate a transplantable whole organ [3–6]. The method includes elimination of cellular components to create an acellular scaffold that retains the liver's original extracellular matrix (ECM) architecture and vascular network [5]. While the potential benefits of this approach are well recognized, a critical challenge involves identifying optimal seeding and conditioning strategies to regenerate functional bioengineered liver from the seeded scaffold construct [3,4,7].

Whole organ decellularized scaffolds may be perfused through their intrinsic vasculature in order to seed the scaffold with cells and to maintain viability of the seeded cells. As in native liver, the perfusion of a decellularized scaffold produces mechanical forces across multiple length scales. The flow of perfused fluid through the scaffold will exert pressures and shear stresses in each branch of the vascular network and produce interstitial flow and ECM tension in the parenchymal space. Evidence is beginning to emerge that providing appropriate biomechanical conditions inside seeded decellularized scaffolds is important for successful generation of viable tissue. For example, it has been suggested that fluid flow rate through the cannulated portal vein influences the self-organization of hepatic and endothelial cells to their respective niches in perfused seeded decellularized scaffold constructs [7]. In addition, fundamental mechanosensitivity studies have shown that many liver cell types, including hepatic stem and progenitor cells, hepatocytes, cholangiocytes, endothelial cells, stellate cells, and portal fibroblasts, actively detect and respond to mechanical stimuli such as shear stress, hydrostatic pressure, and ECM stiffness [8–12].

There is increasing interest in using *in silico* experiments to optimize experimental conditions in tissue engineering studies and help speed the development of functional tissue engineered constructs ready for clinical use [13]. However, computational modeling of decellularized organ perfusion has rarely been studied [14]. Very little is known about the

biomechanical environment inside perfused decellularized tissue, or how this biomechanical environment may differ from that of perfused native tissue [15]. The goal of this study was to quantify the macroscopic and microscopic fluid flow environment within perfused decellularized liver, for multiple inlet flow rates, using a combined experimental/computational approach. The resulting multi-scale model of decellularized liver perfusion, reported here, enables investigators to use known organ-scale hemodynamic inputs (such as flow rate through the cannulated portal vein) to generate tissue-scale predictions of the distribution of parenchymal fluid pressures, velocities, and extracellular matrix tensions in the decellularized hepatic lobule. An equivalent multi-scale model was created of native liver perfusion for comparison with the acellular case. Simulations were validated wherever possible through direct comparison to experimental measurements from native and decellularized livers perfused at known flow rates. Understanding how changes in controllable macroscopic input conditions may impact the downstream mechanical microenvironment can aid future efforts to optimize seeding and conditioning strategies to generate functional bioengineered liver.

2. MATERIALS AND METHODS

2.1 Experiments

Data from two experiments were used in this study. In the first experiment, needle-guided ultra-miniature pressure sensors (3.5F) were inserted into liver tissue to measure parenchymal fluid pressure (PFP) *ex situ* in portal vein-perfused native (n=5) and decellularized (n=7) ferret liver (ages 3–6 weeks), for flow rates of 3, 6, 9, and 12 mL/min. *In situ* measurements of liver PFP in Sprague-Dawley rats (n=5) were also recorded under physiological flow conditions, in accordance with and IACUC approved protocol. PFP is the hydrostatic pressure due to the presence of fluid in the liver interstitium [9,15]. Ferret liver specimens consisted of the dissected superior right lobe (SRL) together with the inferior right lobe (IRL), as well as the portal vein which served as the perfusion inlet for the specimen. Together, the SRL and IRL make up the “right lobe”, which is known to contain a functional vascular network due to the lobular nature of ferret liver anatomy [15], similar to rat liver anatomy [16]. Ferrets ages 3–6 weeks were found to have similar body weight and liver weight as the rats used in the study [15]. Ferret and rat livers both consist of seven lobes, which can be denoted as the right median, left median, left lateral, anterior caudate, posterior caudate, superior right (SRL) and inferior right (IRL) lobes [16]. Complete details of the PFP experiments have been published elsewhere [15].

In the second experiment, which has not been previously reported, livers from cadaveric ferrets were obtained in accordance with an IACUC approved protocol and decellularized using established methods [5]. Specimens consisted of the combined SRL and IRL and portal vein inlet, as described above, and were perfused at matched flow rates (3,6,9,12 mL/min). The total pressure drop, $P_{in} - P_{out}$, was measured across the entire perfused liver specimen for each flow rate, to estimate the total resistance across the specimen’s vascular network and its possible dependence on perfusion flow rate. Pressures in 5 liver specimens were recorded: 2 native (weight = 6.94g, 5.45g) and 3 decellularized (weight = 3.57g, 4.08g, 3.93g). Normal saline solution was pumped through the portal vein inlet using a peristaltic

pump and pulse dampener to create steady flow (Fig. 1). An ultra-miniature pressure sensor (3.5F, Millar SPR-524, ADInstruments, Colorado Springs, CO) was inserted through a port near the portal vein inlet cannula to measure P_{in} . Fluid was allowed to escape freely from the tissue and from the inferior vena cava, such that the baseline pressure P_{out} was taken as 0 mmHg. Reference pressure measurements were also recorded at the same inlet port location, but without the liver attached, to account for resistance due to hardware such as tubing and connectors. The pressure gradient values $P = P_{in} - P_{ref}$ were divided by volume flow rate Q to calculate net resistances R according to the equation $R = P/Q$.

2.2 Overview of Computational Modeling Approach

This study adopted a two-tiered computational modeling strategy to simulate decellularized liver perfusion (Fig. S.19). In the first tier of the model, flow within the vascular channels was simulated using electrical analog models of native and decellularized liver hemodynamics. Briefly, in an electrical analog model vascular circuit components throughout the organ are replaced by electric circuit components, with flow analogous to current and pressure analogous to voltage [17]. The effective resistance for each generation of vessels is related to the number of vessels per generation, vessel length, radius, and medium viscosity [17]. In the present work, resistance values were optimized to experimental PFP and then validated by comparing total model resistance with the measured pressure drop across the native and decellularized livers, from inlet to outlet, for each perfusion rate. Figures S.20 and S.21 highlight the distinct data sets used for model optimization versus validation.

The electrical analog models were then used to predict pressure and flow in each blood vessel generation in the hepatic vascular tree, for native and decellularized liver, based on known flow rate input conditions in the portal vein (PV) inlet. These predictions served as boundary conditions for the second tier of the model. The second tier consisted of two three-dimensional poroviscoelastic finite element models of the hepatic lobule, one native and one acellular. These lobule-scale continuum models simulate microscopic fluid flow characteristics within the liver parenchymal space. The terminal and pre-terminal portal veins (tPV and pre-tPV) at the lobule boundaries were used as fluid inlets, with pressure in each inlet vessel based on generation-specific pressures predicted by the validated electrical analog model for a given perfusion flow rate. The fluid outlet was the central vein (CV). Hydraulic conductivities for native and decellularized liver were calculated from tissue void ratios, obtained from electron micrograph image analysis. Distributions of ECM displacement, pore fluid pressure, and pore fluid velocity were then calculated throughout the 3D hepatic lobule models, for perfused native and decellularized liver.

The modeling approach included certain assumptions involving similarity between ferret and rat liver hemodynamics. First, for the right lobe ex vivo perfusion conditions studied in this work, it was assumed that the relative distribution of flow among the lobes of the rat liver (SRL, IRL) is similar to the relative distribution of flow among the lobes of the ferret liver (SRL, IRL). Second, it was assumed that portal flow per gram of liver mass in ferrets is similar to that in rats, which have reported values in the range of 0.72–1.035 mL/(min · g) [18–19]. This assumption was supported by the observation that portal flow rates normalized

by liver mass have similar values across several species, including mouse (0.77 mL/(min · g) [19]), monkey (1.09 mL/(min · g) [19]), dog (0.72–1.19 mL/(min · g) [19,20]), and human (0.60–0.90 mL/(min · g) [19,21]). This assumption was used to determine the relevant range of experimental flow rates (3, 6, 9, 12 mL/min) applied to the right lobe (SRL and IRL) specimens. Using a representative right lobe mass of 6.8 g (native ferret liver), the expected physiological portal flow rate through the right lobe was estimated to be in the range of 4.9 to 8.1 mL/min. The experimental flow rates (3, 6, 9, 12 mL/min) were chosen to include values above and below these limits. Similar flow rates from 0 to 20 mL/min were used in a recent study by Uzarski et al. for experimental perfusion of the right lobe of native and decellularized rat liver specimens [18].

2.3 Organ Scale Model of Liver Hemodynamics

2.3.1 Model Development—In the electrical analog model of liver hemodynamics, blood vessels throughout the organ were categorized as generations (Fig. 2) based on branching topology previously obtained from microCT images of rat liver vascular corrosion casts as reported by Debbaut et al. [16]. The electric circuit analog components were applied to each of the generations under the following assumptions. The perfusion fluid (saline) was considered to be Newtonian and flow was assumed to be laminar. Saline was used in the experiments in order to provide similar viscosity to cell culture media, which is the most common perfusate fluid in liver bioengineering studies involving decellularized scaffolds. Fluid viscosity was taken as 6.53×10^{-4} Pa s, and density was taken as 1004.56 kg/m³. The viscosity change due to vessel diameter change was assumed to be negligible. Under the ex vivo steady perfusion flow condition used in the experiments (analogous to direct current), contributions due to the inertia of the blood, vessel wall compliance, and vessel wall viscoelasticity can be neglected so that only the longitudinal steady flow resistance of the blood vessels, R_s , was considered [17]. With these assumptions, the net vascular resistance of a given blood vessel generation can be expressed as:

$$R_s = \frac{8\eta l}{k\pi r^4} \left[\frac{Ns}{m^5} \right] \quad (\text{Eq. 1})$$

where η is the dynamic viscosity of perfusion fluid [Pa s], l is the mean blood vessel length for a given generation [m], k is the number of blood vessels of the generation, and r is the mean blood vessel radius of the generation [m] [16]. The intact rat liver contains seven lobes. Each lobe model consists of two vascular networks connected in parallel, the hepatic artery (HA) tree and portal vein (PV) tree, which are further connected in series with hepatic vein (HV) tree. Within a given vascular tree, successive blood vessel generations are connected in series. Model code was implemented in Matlab. With this hemodynamic model, fluid pressure and flow rate can be predicted for each blood vessel generation throughout the entire hepatic vascular network (Fig. 2). In addition, vascular resistance values can be tuned to simulate the effects of decellularization on overall liver hemodynamics.

2.3.1 Model Applied to Ex Vivo Perfusion—Steady flow was simulated through the right lobe only (SRL and IRL) in order to correspond with the liver specimens and flow conditions used in the experiments (see Section 2.1). Perfusion was simulated through the

PV and HV trees (Fig. 3), since perfusion to experimental specimens was applied through the cannulated portal vein and exited via the unobstructed inferior vena cava. Flow through the HA tree was set to zero. The resistor that simulates whole body vascular resistance distal to the inferior vena cava was deactivated in order to mimic experimental ex vivo perfusion conditions.

2.3.2 Model Optimization and Validation—Resistance values for the right lobe of native and decellularized liver were optimized for each flow rate (3,6,9,12 mL/min) to predict measured parenchymal fluid pressures (PFP) in the lobe [15]. In the hemodynamic model, PFP was calculated for each flow rate by averaging the model-predicted pressures from the smallest two generations of the PV and HV trees, respectively, to be consistent with the lobule-scale dimensions of the 3.5F (1.2 mm diameter) pressure sensors used to measure experimental PFP. During optimization, model R_s values (Eq. 1) were adjusted by identifying a multiplication coefficient that minimized the difference between the model-predicted PFP and measured PFP, using custom Matlab code. All R_s values in a given model were then multiplied by the same coefficient. Distinct coefficients were found for each flow rate, to account for possible dependence of vascular resistance on flow rate in ex vivo native or decellularized liver. Using this approach, it was possible to tune the native and decellularized hemodynamic models to accurately predict measured PFP for each experimental flow rate. For model validation, the optimized total model resistance for the entire right lobe (SRL and IRL) was compared against the experimental estimate of the total resistance of the right lobe obtained from pressure drop experiments (see Section 2.1), for each perfusion flow rate, since this data was not used during the optimization process (Fig. S.20). As further validation, the total right lobe resistance values from the pressure drop experiments (Section 2.1) were compared against similar experimental data obtained in the right lobe of rat livers, both native and decellularized, in a recent study by Uzarski et al. [18] (Fig. S.1).

2.4 Tissue Scale Model of Lobule Perfusion

2.4.1 Model Development—Two 3D poroviscoelastic (PVE) finite element models, one native and one decellularized, were created using Abaqus software (v6.12, 3DS Dassault Systemes, Waltham, MA) in order to predict fluid flow characteristics within parenchymal space of a simulated hepatic lobule. PVE is an extension of poroelastic or biphasic theory, which model material as a fluid-saturated porous linear elastic solid in which the fluid flows relative to the deforming solid [22]. In a PVE model, the solid matrix material is viscoelastic rather than linear elastic. Thus, rate dependence in a PVE model stems from two sources: flow-dependent mechanisms (fluid escaping the material) and flow-independent mechanisms (relaxation of the solid matrix) [14]. Accordingly, PVE models are better equipped to capture the significant relaxation behavior of liver [14,23,24], which is known to exhibit as much as 80% stress relaxation when subjected to constant strain. Since a PVE model is capable of predicting pore fluid pressure and pore fluid velocity in addition to solid matrix stress and strain, this modeling strategy is attractive for examining the behavior of perfused liver tissue in native and decellularized states. Mathematical details of PVE theory and its application to liver tissue have been reported previously [14,23,25]. Constitutive equations of the poroviscoelastic model are given in Supplemental Information (Section S.5).

A common geometry was used for both models (Fig. 4), based on the classic hexagonal lobule of liver histology [26]. The central vein (CV), terminal portal vein (tPV) and pre-terminal portal vein (pre-tPV) were presented as cut channels along the edge of the lobule (Fig. 4 a,b). Lobule dimensions and vessel radii (Table 1) were obtained from image analysis of native and decellularized liver histology from published literature [16,27–29]. Each quarter-lobule model (Fig. 4a) contains 178,283 elements. Quadratic hexadron (Abaqus element type: C3D20RP) elements were used for the hexagonal parenchymal space, and quadratic tetrahedron (Abaqus element type: C3D10MP) elements were used for the tPV region to accommodate its complex shape. Convergence studies confirmed appropriateness of the mesh density. The simulation time was 150 seconds, with a minimum time increment of 0.15 seconds. Simulations were run on the Miami Redhawk Cluster, a 36-node research computing cluster at Miami University, and on the Oakley Cluster, a 694-node supercomputer at the Ohio Supercomputer Center.

2.4.2 Material Properties—All model material properties are listed in Table 2. Elastic and viscoelastic material properties for native and decellularized liver were taken from previous nano-indentation experiments on perfused native and decellularized liver tissue reported by Evans et al. [14]. Seepage coefficients were introduced to avoid reverse flow from the boundary input regions. The specific weight value corresponds to saline, the perfusate fluid used in the experiments. For a fluid-saturated porous material, the void ratio, e , is defined as the ratio of the fluid and solid volume fractions of the material. The void ratio for native liver was previously estimated from histological image analysis as reported by Raghunathan et al. [23] and Evans et al. [14]. To determine the void ratio of decellularized liver, published histological sections of decellularized liver tissue [5,6,29] were analyzed using ImageJ software. Color-based segmentation of the images was performed to determine the solid and void regions within each section. Hydraulic conductivity, K [m/s], is a measure of the ease with which fluid moves through a porous medium in response to a pressure gradient. A review of the literature revealed large variation in reported hydraulic conductivity values for native liver, ranging from approximately 0.24×10^{-6} to 9.9×10^{-6} [m/s] [30,31], and no previously reported values were found for the hydraulic conductivity of decellularized liver. Therefore, in this work the hydraulic conductivities of native and decellularized liver were calculated from the void ratio of the lobule (based on histological image analysis), fluid properties (density and viscosity), and the mean radius of the sinusoids, using Equation S.1 given in Supplemental Information (Section S.1).

2.4.3 Boundary Conditions—One fourth of the hexagonal liver lobule was modeled in order to reduce required computational time; therefore, symmetric boundary conditions were applied to the X , Y , and Z planes for both native and decellularized liver models (Fig. 4). We assumed uniform expansion in the axial direction and no expansion in the longitudinal direction, consistent with the work of Bonfiglio et al. [32]. CV surface pressure was set to 0 mmHg to serve as a fluid sink. Preterminal PV and terminal PV pressures were obtained from the electrical analog model for each flow rate. Non-zero baseline CV pressures predicted by the electrical analog model were subtracted from pre-tPV and tPV pressures before these values were implemented as pre-tPV and tPV surface pressure boundary

conditions in the lobule finite element models (Table 3). Finally, a linear relation was used to model pressure variation along the length of the pre-tPV in the Z direction (Fig. 5). This boundary conditions is consistent with reported patterns of linearly decreasing hydrostatic pressure in microvasculature [33], and it avoids having multiple boundary conditions assigned to adjacent nodes between tPV and pre-tPV surfaces.

2.4.4 Model Optimization and Validation—Material property values were obtained from literature or calculated from image analysis (Table 2). It was found that no model optimization was needed. Multiple strategies were used for model validation. First, the native and decellularized lobule models were validated across the four experimental perfusion rates (3, 6, 9, 12 mL/min) by comparing model-predicted pore fluid pressure, averaged across the lobule, with measured PFP for each flow rate. As a second, independent method of validation, the native lobule model was run using *physiological* intravascular pressures estimated from [16] and [34] as the pre-tPV, tPV, and CV boundary conditions driving the flow (Table S.3). Model-predicted pore fluid velocities, averaged across the lobule, were then compared against measured sinusoidal plasma velocities reported in animal studies using intravital microscopy and other methods [35–37]. Finally, to validate the hydraulic conductivity equation (Section S.1), the calculated hydraulic conductivity for the native liver lobule model was compared to native liver hydraulic conductivities from literature [30,31]. Validation strategies are summarized in Figure S.21.

3. RESULTS

3.1 Organ Scale Model of Liver Hemodynamics

3.1.1 Baseline Model Results—To establish a baseline of model performance prior to optimization, preliminary simulations were performed. For the baseline model, longitudinal steady flow resistance of the blood vessel (R_v) at each blood vessel generation was obtained from Eq. 1 and the vasculature geometries given in Table S.5 [16]. PFP results predicted by the baseline model are shown in Figure 6a in comparison with experimental measurements of PFP [15], for four flow rates. As expected, the baseline model prior to optimization was a poor predictor of PFP in perfused decellularized liver, with an average percent difference of 270% between model and experimental PFP. The baseline model performed somewhat better at predicting PFP in perfused ex vivo native liver, with an average percent difference of 32% between model and experimental PFP. In general the baseline model under-predicted PFP in native liver, but the predictions fell either within or just below 1 standard deviation of the experimental mean for each flow rate. In addition, the slope of the PFP versus flow rate relationship for the baseline model differed only by 6.2% from that of the native liver data set. It should be noted that the baseline model, prior to optimization, was not designed to account for the significant changes to vascular architecture wrought by the decellularization process, or changes associated with cell death in a saline-perfused ex vivo native liver. Hence, the significant discrepancies between model and experiment were anticipated and served primarily as a frame of reference for illustrating the impact of the optimization process on model performance.

3.1.2 Optimized Model Results—Model-predicted PFP, after optimization, is shown in Figure 6b along with experimental measurements of PFP. On average, the percent difference between model and experimental PFP was 0.1% for the native model and 0.3% for the decellularized model, showing excellent agreement across all flow rates. Thus, the tuned electrical analog models of ex vivo perfused native and decellularized liver were able to predict downstream tissue-scale fluid pressures as a function of flow rate through the portal vein inlet. Optimized total model resistance values are given in Table 4. Boundary condition values for the FE lobule model were obtained from the optimized electrical analog model outputs (Table 3).

3.1.3 Model Validation—Total model resistance values, optimized to experimental PFP data, are illustrated in Figure 7a as a function of flow rate. For the native liver model, total vascular resistance decreased with increasing flow rate. For the decellularized liver model, total vascular resistance was consistently lower than the native case and was independent of flow rate. To validate these optimized resistance values, they were compared against the estimated total vascular resistance values for native and decellularized liver obtained from the pressure drop experiments (Figure 7b), since this data was not used in the model optimization process. The same trends were observed in the experimental results as in the optimized model results. Specifically, the resistance of ex vivo perfused native liver decreased as flow rate increased, while decellularized liver resistance was independent of flow rate and much lower than its native counterpart. Some differences were noted in the magnitudes of total vascular resistance between the experimental and optimized model values, but the trends were quite similar. Similar trends in right lobe resistance were also observed for rat liver experiments conducted by Uzarski et al. (Figure S.1) [18].

3.2 Tissue Scale Model of Lobule Perfusion

3.2.1 Native Lobule Results—Finite element model results for the native liver lobule are shown in Figure 8. This figure illustrates the distributions of pore fluid pressure, pore fluid velocity, and solid matrix displacement across the lobule for one flow rate (9 mL/min). Results for the other three flow rates are given in Supplemental Information (Section S.2), along with void ratio and solid matrix stress and strain results for each flow rate. In addition to 3D distributions (Fig. 8a–c), the pressure, velocity, and displacement results were calculated for three parallel paths (Fig. S.2) running from the pre-terminal portal vein to the central vein. Results for the three paths were then averaged in order to visualize the variation in pressure, velocity, and displacement from the lobule periphery (pre-tPV) to its center (CV) (Fig. 8d–e).

Pore fluid velocity exhibited a U-shaped distribution, with maximum velocities of approximately 1600–1700 $\mu\text{m/s}$ around the inlet and outlet regions on the pre-tPV, tPV, and CV surfaces (Fig. 8a,d). A steep change in fluid velocity was observed from the inlet and outlet regions to the mid-parenchymal region. Approximately 80% of the lobule parenchyma showed fluid velocities in the range of 300–900 $\mu\text{m/s}$. The average pore fluid velocity across the three paths was 609 $\mu\text{m/s}$ for the 9 mL/min flow rate (Table 5).

Pore fluid pressure decreased in a sigmoidal curve from the average inlet pressure of approximately 13 mmHg at the pre-tPV surfaces to the outlet pressure of 7 mmHg at the CV surface (Fig. 8b,e). The rate of change of pore fluid pressure was higher near the inlet and outlet regions compared to the mid-parenchymal region. The average pore fluid pressure across the three paths was 10.2 mmHg for the 9 mL/min flow rate (Table 5).

Symmetrical boundary conditions on the two interior surfaces perpendicular to the X and Y axes (Fig. 4) restricted displacement of these interior surfaces to only the Y and X direction, respectively. Thus displacement is larger at outer edge of the model. Displacement of the solid matrix decreased linearly from approximately 18 μm at the inlet to about 1.2 μm at the outlet (Fig. 8c,f). The average displacement across the three paths was 7.2 μm for the 9 mL/min flow rate (Table 5).

3.2.2 Decellularized Lobule Results—Finite element model results for the decellularized lobule are shown in Figure 9. As above, the figure illustrates the pore fluid velocity, pore fluid pressure, and solid matrix displacement results for the 9 mL/min flow rate (Fig. 9a–c). Additional results are provided in Supplemental Information (Section S.3). The same three parallel output paths (Fig. S.2) used for the native model averages were again used for the decellularized liver model. Results for the three paths were averaged in order to visualize the variation in pressure, velocity, and displacement from the lobule periphery (pre-tPV) to its center (CV) (Fig. 9d–e).

The fluid velocity for the decellularized lobule model showed a U-shaped distribution similar to that of the native model. In general fluid velocities were similar or slightly higher in the decellularized model compared with the native (Table 5). For the 9 mL/min flow rate, maximum fluid velocities were approximately 1600–1700 $\mu\text{m/s}$ near the inlet and outlet surfaces, and 80% of the lobule parenchyma showed fluid velocities in the range of 300–1100 $\mu\text{m/s}$ (Fig. 9 a,d). The average fluid velocity across the three paths was 618 $\mu\text{m/s}$ for the 9 mL/min flow rate (Table 5).

The pore fluid pressure was lower in the decellularized lobule model compared to the native case. The averaged pressure results for the three paths showed a similar sigmoidal distribution, decreasing from about 2.5 mmHg at the periphery to 1.3 mmHg at the CV for the 9 mL/min flow rate (Fig. 9 b, e). The overall average pore fluid pressure across the three paths was 1.9 mmHg for the 9 mL/min flow rate (Table 5).

Solid matrix displacement for the decellularized model showed linear decrease from about 16 μm at the inlet region to about 1.1 μm at the outlet region. The trend and the values were similar to the displacement results from the native FE model although a slight decrease in average displacement was observed. The average displacement across the three paths was 6.3 μm for the 9 mL/min flow rate (Table 5).

3.2.3 Model Validation—FE model pore pressure results at each flow rate, averaged along the paths, were compared to the measured PFP as shown in Figure 10. The results from both native and decellularized lobule models showed good agreement with the measured PFP for each flow rate. The percent difference between averaged native FE model results and

measured PFP were 4.3%, 0.6%, 2.0%, and 9.4% for 3, 6, 9, and 12 mL/min respectively. Since the native FE model over-predicted the slope decrease that was observed in the PFP experimental data from the 9 to 12 mL/min flow rates (Fig. 10), the percent difference at 12 mL/min is about twice that of the other flow rates. For the decellularized lobule, the percent difference between averaged FE model results and measured PFP were 1.8%, 3.3%, 3.1%, and 2.3% for 3, 6, 9, and 12 mL/min flow rates, again showing good agreement between model and experiment.

For the second method of model validation, lobule fluid velocities were calculated for the native model using physiological intravascular pressures (pre-tPV, tPV, CV) as boundary conditions, and the resulting average fluid velocity was compared against fluid velocities from lobule-scale fluid velocity measurements in previous animal studies [34–36]. Model results are shown in Supplemental Information (Section S.4). The average model pore fluid velocity across the three paths was 254 $\mu\text{m/s}$. This value falls within the 100–750 $\mu\text{m/s}$ plasma velocity range reported in the literature for in vivo perfused liver tissue [34–37].

Lastly, the calculated hydraulic conductivity for native liver, $1.85 \times 10^{-6} \text{ m/s}$ (Section S.1), was compared against previously reported literature values. The native liver hydraulic conductivity in the current study fell within the literature range of 0.24×10^{-6} to $9.9 \times 10^{-6} \text{ m/s}$ [30,31].

4. DISCUSSION

Previous investigators have developed computational models of *native* liver perfusion at various length scales [38–40]. For example, Raghunathan et al. [23] and Moran et al. [25] developed continuum-level poroviscoelastic (PVE) models to study liver interstitial fluid pressure under compression. Microscopic fluid flow was studied by Rani et al. [41] and Debbaut et al. [31] with computational fluid dynamics (CFD) models of sinusoids and liver parenchymal space. Ricken et al. [40] and Bonfiglio et al. [32] developed 2D liver lobule models that account for tissue compliance using poroelasticity, and Debbaut et al. [42] reported a rigid porous media model of a 3D liver lobule. However, fluid flow conditions within *decellularized* liver scaffolds have rarely been studied [14]. Difficulties associated with modeling decellularized scaffolds stem mainly from their complex structure and limited availability of experimental hemodynamic data.

We report the development and validation of a multiscale computational model of decellularized liver perfusion. The first tier of the model, an organ scale electrical analog model of hemodynamics, successfully simulated downstream microvascular flow conditions based on flow rate through the cannulated portal vein of decellularized liver. Optimized resistance values agreed with experimental measures of total right lobe resistance in decellularized livers (Figs. 7 and S.1). Using electrical analog model-predicted pressures in specific microvessels as boundary conditions, a tissue scale finite element model was then created of decellularized lobule perfusion. The decellularized lobule FE model accurately predicted lobule pore fluid pressure, for four different experimental flow rates, using a single set of material property parameters. Importantly, no optimization of FE model material properties was needed. The solid matrix material properties were obtained from indentation

experiments conducted on perfused decellularized liver tissue [14] at a length scale appropriate for the present work (indentation depths of 75–80 μm). In addition, decellularized lobule hydraulic conductivity was calculated from fluid properties (density, viscosity), vessel geometry, and void ratio data obtained from microscopy image analysis. Therefore, it is possible to extend the present multiscale computational model to incorporate new void ratio and hydraulic conductivity values resulting from cell proliferation within the scaffold. Thus, the modeling strategy reported here potentially could be used to develop advanced computational models of cell growth and organization within perfused decellularized scaffold systems, for any organ of interest.

Although the present modeling scheme was closely integrated with experimental data, there are some limitations. First, the version of the electrical analog model presented here is based only on vascular resistances and does not account for inertia of the blood, vessel compliance, or vessel wall viscoelasticity [16]. This simplification was made because the experiments simulated in the present work only included steady flow, which is analogous to direct current conditions. Therefore it was considered reasonable to neglect contributions from time-varying components of the circuit such as pulsatile flow. A second limitation is that, in the lobule FE model, the tissue was assumed to be isotropic. Isotropic mechanical properties have been used by other investigators to simulate native liver [43]; however, Debbaut et al. have reported that the permeability of native liver is anisotropic based on sinusoidal architecture [31]. Interestingly, decellularization of the tissue causes obliteration of the sinusoidal structure and leaves uniformly distributed void spaces which were previously occupied by hepatocytes and other cells [5]. Thus, isotropic permeability may be more appropriate for decellularized than for native liver. Finally, the hepatic artery, bile duct, and lymphatic vessel components of the portal triad are absent from the FE model geometry, since flow in our experimental system was applied only through the portal vein. Therefore, fluid behavior changes due to hepatic artery perfusion, bile production, or lymph production cannot be predicted with the present model. However, fluid behavior changes due to changes in void ratio, based on cell proliferation, can be simulated with the current model as noted above.

Since both native and decellularized models were created in the present study, it is possible to compare native versus decellularized parameters to gain quantitative insights into the effects of the decellularization process. For example, the average total resistance for the ex situ perfused native liver specimens was 9.12×10^9 [Ns/m⁵], while the average total resistance for the decellularized specimens was 1.65×10^9 [Ns/m⁵]. Thus, the total resistance of the decellularized specimens decreased by 81.9% compared with the native, when averaged across the four flow rates. This finding is consistent with the much lower pore fluid pressures observed in the decellularized lobule model compared with the native lobule (1.6 vs 8.5 mmHg, averaged across the four flow rates). In a similar vein, the calculated hydraulic conductivity of the decellularized liver lobule was 5.6 times that of the native lobule. This is as expected, since decellularization represents the removal of barriers to flow. Larger hydraulic conductivity suggests that fluid moves more easily through the tissue for a given pressure gradient. Somewhat surprisingly, the average pore fluid velocities in the decellularized lobule were nearly the same or only slightly faster than the native lobule. While one would expect that removing barriers to flow would increase the fluid

velocity, this effect may be counterbalanced by the significant increase in void space in the decellularized tissue, such that the perfusing fluid must fill a larger volume within the tissue and thus the increase in pore fluid velocity is somewhat dampened. Finally, the displacement of the solid matrix resulting from perfusion was quite similar in the native and decellularized lobule models, with mean values of 5.7 and 5.2 μm , respectively, when averaged across the four flow rates. Lobule displacements yielded matrix strain predictions ranging from 1.7% to 6.1% (Sections S.2, S.3). To put these values in context, hepatic stellate cells have exhibited gene expression changes with application of cyclic 10% strain [44]. Thus, the ~5% strain observed in the present model is in the range of biologically meaningful mechanical stretch. Constant ECM displacement, such as that observed in the present model due to the steady flow conditions, may not affect cell behavior; however, under pulsatile flow or time-varying tissue deformations, ECM displacement can result in stretching of the attached cells that could trigger downstream mechano-responsive signaling events. To illustrate lobule deformation, the deformed and undeformed lobule models are given in Figure 11.

5. CONCLUSION

This study reports the development and validation of a multiscale computational model of decellularized liver perfusion. The model consists of two integrated components. The organ scale electrical analog model can predict microvascular pressure and flow conditions based on macrovascular input through the cannulated portal vein. The tissue scale finite element model can predict three-dimensional spatial distributions of pore fluid pressure, pore fluid velocity, and ECM stress and deformation throughout decellularized lobule parenchyma. Coupled with histology and further experiments, this integrated modeling strategy can be used to understand the physical forces in perfused decellularized scaffolds and their effects on cell seeding, growth, and organization of functional bioengineered liver tissue.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Pressure drop experiments to estimate total resistance. (a) Experimental setup. (b) Schematic of inlet and outlet pressures (P_{in} and P_{out}) and inlet and outlet flows (Q_{in} and Q_{out}). (c) Decellularized right lobe. (d) Native right lobe. Cannula is shown entering the portal vein.

Figure 2.

Diagram of branching topology and corresponding resistance of liver vasculature, adapted from [17]. Blood vessels of the n^{th} branch are categorized as the n^{th} generation with corresponding resistance R_{s_n} . The portal vein (PV) and hepatic artery (HA) trees are connected at the sinusoidal generation, represented as the smallest generation in the hepatic vein (HV) tree.

Figure 3.

Schematic of vascular circuit used to simulate flow in ex vivo right lobe specimens perfused through the portal vein. The right lobe is taken to include the superior right lobe (SRL) and inferior right lobe (IRL). No flow is applied through the HA vascular tree, and systemic vascular resistance distal to the HV tree is deactivated due to the ex vivo condition.

Figure 4.

Finite element model geometry for the native and decellularized lobule models. (a) Quarter lobule geometry, with labeled edge width (w), edge length (l), terminal portal vein (tPV), pre-terminal portal vein (pre-tPV), and central vein (CV) surfaces. (b) Hexagonal lobule model after symmetrical boundary conditions are applied (X, Y surfaces). Figure shows element density.

Figure 5. Pressure boundary condition along pre-tPV surface. (a) Z-axis orientation along pre-tPV (shown in red). (b) Pressure variation along pre-tPV in Z direction, shown for decellularized lobule model at the 9 mL/min flow rate.

Figure 6.

PFP results from electrical analog model simulations for four flow rates. (a) Baseline model results (triangles) compared with measured PFP in native (squares) and decellularized (circles) liver specimens. For reference, the mean in situ PFP recorded in rats, for physiological flow conditions, was 2.86 ± 1.04 mmHg [15]. (b) After resistance optimization, two separate native and decellularized electrical analog models can accurately predict PFP for each flow rate.

Figure 7.

Validation of optimized resistance values. (a) Total right lobe resistance obtained from model optimization to PFP data. (b) Total resistance from pressure drop experiments on perfused right lobes of native and decellularized livers.

Figure 8.

Results of native liver lobule model for 9 mL/min flow rate. Contour plots for pore fluid velocity (a), pore fluid pressure (b), and solid matrix displacement (c) across the lobule. Graphs (d,e,f) show model results averaged across three paths from the pre-tPV to CV, as illustrated in Fig. S.2.

Figure 9.

Results of decellularized liver lobule model for 9 mL/min flow rate. Contour plots for pore fluid velocity (a), pore fluid pressure (b), and solid matrix displacement (c) across the lobule. Graphs (d,e,f) show model results averaged across three paths from the pre-tPV to CV, as illustrated in Fig. S.2.

Figure 10.
Lobule FE model results compared to measured PFP values.

Figure 11.
Undeformed lobule model (green) visualized in relation to deformed lobule (wireframe).
Results shown for native model at 9 mL/min flow rate.

Table 1

Liver lobule model dimensions, estimated from [16,27–29].

Geometry (μm)	
CV radius	26.5
tPV radius	16
pre-tPV radius	23
Edge length (l)	350
Edge width (w)	150

Table 2

Material properties used in native and decellularized lobule FE models.

	Model Property	Native	Decell
Elasticity	E (Pa)	4400	914
	ν	0.35	0.35
Viscoelasticity	g_1	0.53	0.481
	τ_1 (s)	2.00E-05	2.00E-05
	g_2	0.376	0.48
	τ_2 (s)	1	0.349
	g_3	0.027	0.017
	τ_3 (s)	7.65	0.5
	g_4	0.01	0.001
	τ_4 (s)	100	100
Seepage Coefficient	k_s (m ³ /Ns)	0.08	0.8
Specific Weight	γ (N/m ³)	9855	
Hydraulic Conductivity	K (m/s)	1.85E-06	1.03E-05
Void Ratio	e	0.2	13

Pressure boundary conditions used in native and decellularized lobule FE models, for four experimental flow rates.

Table 3

	Flow rate (mL/min)	3	6	9	12
Native BC (mmHg)	pre-tPV	3.635	6.265	8.775	9.510
	tPV	1.850	3.190	4.465	4.750
	CV	0			
Decell BC (mmHg)	Baseline P	2.75	4.73	6.62	7.06
	pre-tPV	0.5700	1.150	1.640	2.030
	tPV	0.2850	0.5850	0.8350	1.035
	CV	0			
	Baseline P	0.44	0.87	1.23	1.53

Table 4

Total right lobe resistance, reported for each experimental flow rate.

		Flow rate (mL/min)	3	6	9	12
Measured R (Ns/m ⁵)	Native	R _s	1.481.E+10	1.329.E+10	1.212.E+10	1.208.E+10
		St Dev	1.513.E+10	1.011.E+10	5.807.E+09	3.612.E+09
	Decell	R _s	3.306.E+09	4.026.E+09	4.093.E+09	3.852.E+09
		St Dev	3.888.E+09	2.107.E+09	1.380.E+09	8.065.E+08
Preliminary Model (Ns/m ⁵)		R _s	6.321.E+09			
Optimized Model (Ns/m ⁵)	Native	R _s	1.087.E+10	9.368.E+09	8.743.E+09	7.510.E+09
		Coefficients	1.719	1.482	1.383	1.188
	Decell	R _s	1.707.E+09	1.726.E+09	1.631.E+09	1.517.E+09
		Coefficients	0.270	0.273	0.258	0.240

Average pore fluid velocity (FLVEL), pore fluid pressure (POR), and solid matrix displacement (U) results for native and decellularized lobule models at four experimental flow rates. Averages were calculated across three paths from pre-IPV to CV (Fig. S.2).

Table 5

	Flow rate (mL/min)	3	6	9	12
FE Native	FLVEL (µm/s)	253.7	408.4	609.1	753.4
	POR (mmHg)	4.136	7.399	10.20	10.81
	U (µm)	2.843	5.290	7.163	7.546
FE Decell	FLVEL (µm/s)	211.4	455.4	618.0	767.1
	POR (mmHg)	0.6653	1.328	1.894	2.365
	U (µm)	2.180	4.338	6.343	7.988