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Breakthroughs in Computational Modeling of Cartilage Regeneration in Perfused Bioreactors.

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Abstract — We report about two specific breakthroughs, relevant to the mathematical modelling and numerical simulation of tissue growth in the context of cartilage tissue engineering in vitro. The proposed models are intended to form the building blocks of a bottom-up multiscale analysis of tissue growth, the idea being that a full Microscale analysis of the construct, a 3D PDE problem with internal moving boundaries, is computationally unaffordable. We propose to couple a PDE Microscale model of a single functional tissue sub-unit with the information computed at the Macroscale by 2D-0D models of reduced computational cost. Preliminary results demonstrate the effectiveness of the proposed models in describing the interplay among interstitial perfusion flow, nutrient delivery and consumption and tissue growth in realistic scaffold geometries.

Index Terms — tissue engineering, bioreactor, multiphysics, multiscale.

I. Introduction

A common technique for in vitro cartilage regeneration is to seed a porous matrix with cartilage cells and to culture the construct under medium interstitial perfusion in a bioreactor. An essential step toward the development of functional cartilage is to understand and control the tissue growth phenomenon in such a system. The growth process depends on various space- and time-varying biophysical variables of the environment surrounding the cartilage cells, primarily mass transport and mechanical variables, all involved in the cell biological response [1]. Moreover, it is inherently multiscale, since cell size (10 µm), scaffold pore size (100 µm), and cellular construct size (few mm) pertain to three different spatial scales.

To obtain a quantitative understanding of tissue growth in this complex multiphysics system, advanced mathematical models and relevant scientific computing techniques have been developed (see, e.g., the approaches presented in [2]-[4]).

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In this Letter, we report about two specific breakthroughs (denoted in the following as Model 1 and Model 2, respectively), relevant to the methods for the numerical simulation of tissue growth within this context. The philosophy underlying the proposed approach is based on the evidence that a full Microscale analysis of the whole construct, a 3D PDE problem with internal moving boundaries, is computationally unaffordable. For this reason, we propose a Multiscale approach, consisting of a PDE Microscale analysis of a single functional tissue sub-unit, driven by information computed by reduced 2D-0D models at the Macroscale.

II. Materials And Methods

Model 1: 2D Macroscale - 3D Microscale Analysis

Model 1 is built using the software COMSOL Multiphysics® 3.5a (COMSOL). Two sub-models are set up and coupled (Fig.1): a 2D model of a whole scaffold seeded with a cell monolayer (the biomass) and subjected to interstitial medium perfusion, and a 3D model of a functional sub-unit of such construct. This model is referred to a published experimental set-up [5].

In both the 2D and 3D sub-models, the geometrical domains are defined as follows: the scaffold fibers (cylinders, 100 µm in diameter) are inactive domains, the biomass is a solid diffusion sub-domain, with oxygen volumetric consumption rate following Michaelis-Menten kinetics (see Table I), the medium is a fluid convection-diffusion sub-domain, and the interface between the biomass and the medium is a moving boundary, with displacement velocity prescribed as a function of the local oxygen concentration and of the fluid-induced shear stress, both computed at the fluid-biomass interface.

The model solved by COMSOL consists in a system of nonlinearly coupled PDEs. The nutrient field is calculated using the mass conservation law in advection-diffusion-reaction form, while the fluid velocity vector is calculated using the Stokes equations, the medium being represented as an incompressible Newtonian fluid.

The time evolution of the biomass thickness r is given by the phenomenological relation:

$$\frac{dr}{dt} = f(c)\cdot g(\tau) \qquad (1)$$

where c and τ are the oxygen concentration and the fluid-induced shear stress, respectively, both computed at the fluid-

biomass interface. We have assumed again a Michaelis-Menten model for f, while g is a corrective function, defined as:

$$g(t) := \begin{cases} 0.6 + 4t, & t \in [0,0.1)\text{Pa} \\ 1, & t \in [0.1,0.6)\text{Pa} \\ 1 - 2.5(1 - x), & t \in [0.6,1)\text{Pa} \\ 0, & t \geq 1\text{Pa} \end{cases}$$

The function g accounts for the beneficial versus harmful effects of hydrodynamic shear on cell proliferation, observed experimentally at low versus high shears, respectively [1]. The computational mesh is then regenerated according to the new position of the biomass interface given by (1).

At each time level, corresponding to one culture hour, the 2D model is run on the updated geometry and the fluid pressure at the scaffold location corresponding to the 3D sub-model is extracted and imposed as a boundary condition for the subsequent 3D analysis.

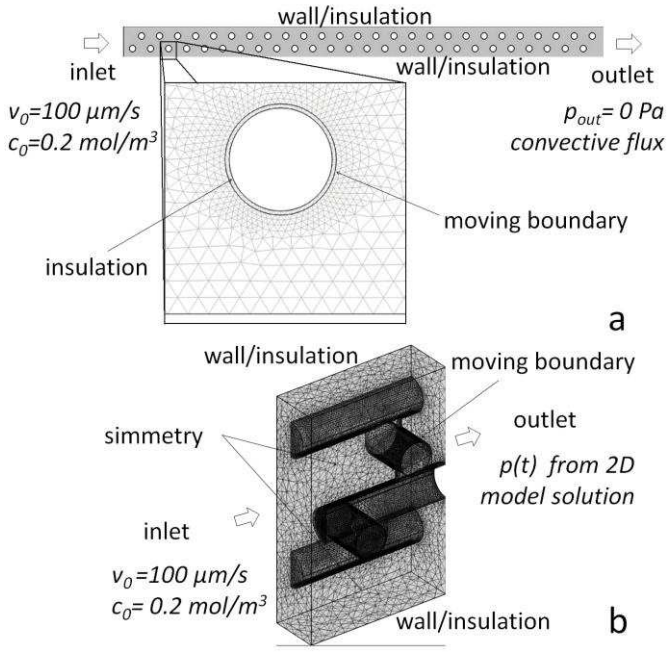

Fig. 1. Set up of Model 1. Geometry of the fiber scaffold and boundary conditions assumed for the (a) 2D Macroscale and (b) 3D Micro-scale simulations.

Model 2: Macroscale Lumped Analysis

Model 2 represents an alternative way to impose boundary conditions to a Microscale 3D model, with a significantly reduced computational cost. To this purpose, following [6], we devise an electrical equivalent network which is a lumped (0D) discrete approximation to the problem of mass transfer, nutrient uptake and biomass growth at the Macroscopic scale of the scaffold. In this description, we assume the scaffold to be represented by a series of layers, each composed of a periodic array of interconnected pores (representing here the functional sub-units). In Fig.2 we show the equivalent electrical network of the scaffold.

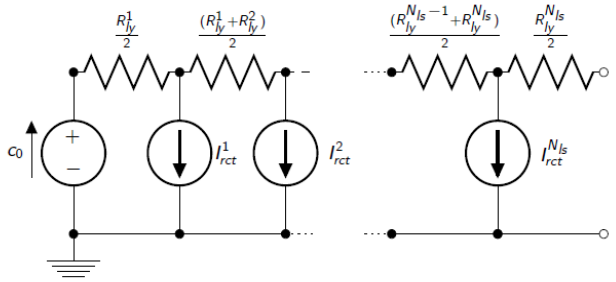

Fig. 2. Model 2. Equivalent electrical network representing the Macroscale 0D model of the scaffold. In the electric analogy, voltage represents concentration and current represents the flow (transfer or conversion) of matter per unit time.

The resistance $R^i_{ly} = R^i_p/n_p$ of each layer $i=1,\ldots,N_{ls}$ is the result of the parallel of the equivalent resistances R^i_p of the n_p pores belonging to the i-th layer. The nonlinear current sources I^i_{rct} represent the Michaelis-Menten kinetics of equivalent mass consumption due to cell metabolism of all the pores of the i-th layer. The ideal voltage source at the left of the circuit represents the inlet Dirichlet boundary condition for the concentration, while the open circuit at the right represents the no-flux Neumann boundary condition at the outlet. Notice that, while it is assumed that in each layer the pore resistances and current sources are all equal, they are, in general, a function of the scaffold layer, since at different depths, the phase composition of the pores can vary, due to different nutrient supply and thus different cellular metabolism and biomass production. Referring to the geometry proposed in [7], consisting of a cubic-shaped hollow box of side 2b, we compute the equivalent resistance $R^i_p = (2bD_{eff})^{-1}$ of the single pore, D_{eff} being the effective nutrient diffusivity in the multiphase pore system. The results obtained for this geometry provide a remarkably accurate representation of more realistic (and more complex) porous constructs. The model proposed in [7] has also been suitably modified to account for biomass growth, which is supposed to occur in a uniform manner along the direction normal to the internal solid walls. Denoting by a the half-side of the hollow part of the cube, such that t:=a/b, and by h_b the thickness of the biomass layer, such that s:=h_b/b, we have the following result

$$\frac{D_{eff}}{D} = \left(\frac{1-t}{(t-s)^2 + D_b/D\, s(2t-s)} + \frac{t-s}{(1-(s+1-t)^2) + D_b/D\, s(s+2(1-t))} + \frac{s}{(t-s) + D_b/D\,(1-(1-t)^2-(t-s))} \right)^{-1} \quad (2)$$

where D is the diffusivity of the clear medium (see Tab.1).

III. RESULTS

Model 1

Representative results from Model 1 are gathered in Fig. 1. The average fluid pressure extracted from the 2D sub-model (Fig.1a) increases linearly with culture time, due to the corresponding biomass growth which increases the hydraulic resistance to flow.

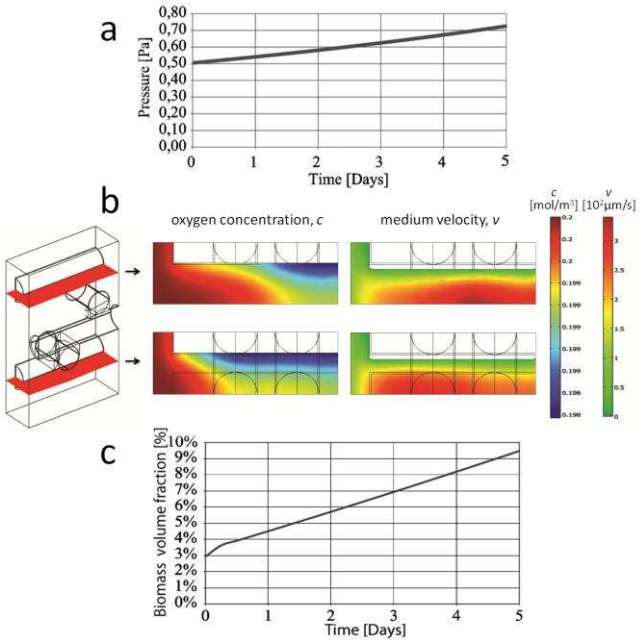

Fig. 3. Results of simulations from Model 1. (a) 2D model - average fluid pressure predicted at the scaffold location assumed for the 3D sub-model. (b) 3D model - oxygen concentration and medium velocity mapped on horizontal sections at 5 days and (c) biomass volume fraction.

The progressive depletion of oxygen from the medium along the flow direction, is mapped on two sections of the 3D sub-model in Fig.2b. Since the total oxygen drop within the 3D domain is only 1% and the velocity gradients are constant, the predicted biomass thickness is fairly homogeneous in this domain, according to Eq.(1). The 3D sub-model predicts an increase of the biomass volume fraction (Fig.1c), from initial 3% to 9% at five culture days, with a subsequent increase in the median shear stresses predicted at the biomass surface of values up to 5 mPa, due to the increasing velocity gradients.

Model 2

Eq.(2) predicts how the diffusion process is altered by the multiphase system represented by the pore perfused by the medium and hosting the biomass under growth. If we consider different biomass thicknesses (taking for example the data reported in [9] for a similar configuration), corresponding to a time evolution of a few days in the bioreactor system, we find

TABLE I
NUMERICAL VALUES OF THE MODEL PARAMETERS USED IN THE SIMULATIONS

Symbol	Parameter [units]	Value
μ	medium dynamic viscosity at 37 °C [Pa·s]	$8.1\cdot10^{-4}$
ρ	culture medium density at 37 °C [kg/m^3]	$1\cdot10^{3}$
D	O_2 diffusivity in water at 37°C [m^2/s]	$2.1\cdot10^{-9}$
D_b	O_2 diffusivity in the biomass	$0.5\cdot10^{-9}$
r_0	initial biomass thickness [µm]	4
V_m	max. O_2 cell consumption rate [mol/m^3/s]	$3.84\cdot10^{-3}$
K_m	Michaelis-Menten constant [mol/m^3]	0.15
c_0	medium inlet O_2 concentration [mol/m^3]	0.2
v_0	medium inlet velocity [µm/s]	100
N_{ls}	number of layers in the lumped 0D model	10
n_p	numer of pores in a layer	400

that when the biomass invades the empty space the effective diffusivity is reduced by a factor of almost 50% with respect to the uncellularized scaffold (see Fig.4a). Notice also the influence of the porosity Φ of the scaffold, which significantly modifies the value of the effective diffusivity in the uncellularized condition. Fig.4b shows the oxygen concentration profile computed from the equivalent electrical network as a function of the scaffold depth for different prescribed thicknesses of the biomass layer.

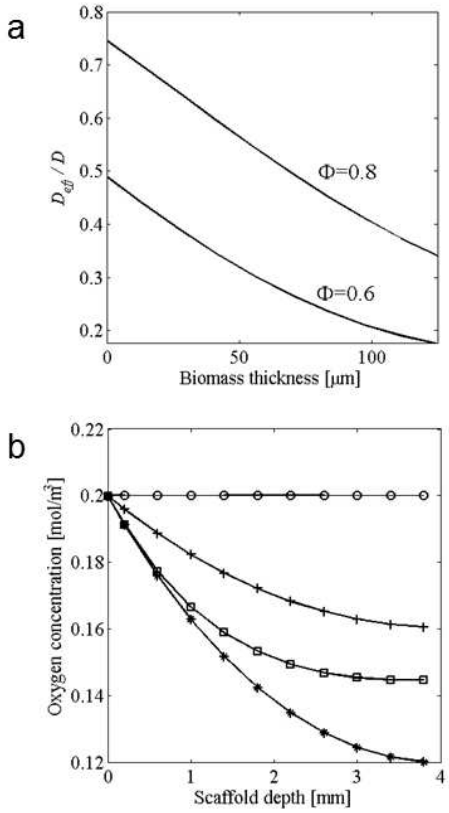

Fig. 4. Results from Model 2. (a) effective diffusivity for the multiphase pore, normalized over medium diffusivity as a function of the biomass thickness (pore radius 250µm, values as in [9]), parametrized for two different porosities of the scaffold. (b). Oxygen concentration computed from the equivalent electrical network as a function of the scaffold depth. Legend: 'o' no biomass; 'x' constant biomass thickness equal to 20µm; '□'

biomass thickness with a linear variation from 100 to 0 μm along the scaffold depth; '*'' constant biomass thickness equal to 100μm.

IV. Discussion

Tissue Engineering (TE) represents a promising example of how scientific and technological development may be able, in a close future, to transform the medical practice and to significantly improve the life conditions of millions of patients. At present, however, the extraordinarily complexity of the artificial reconstruction of biologically functional tissues makes the widespread application of TE a yet unreached goal.

A cellular construct cultured in a bioreactor can be schematically described as a multi-layered structure consisting of a porous/fibrous matrix (scaffold) immersed into a perfusion bath of physiological fluid which delivers nutrients to the growing cells seeded into the scaffold until maturation into functional tissue. Ideally, cells should be provided with optimal conditions of nutrient delivery, fluid-mechanical loadings and interaction with the artificial substrate in order to obtain a biologically functional tissue. However, only functional tissues of several tenth of microns of thickness have been successfully engineered in the laboratory, whereas any clinical application would require a thickness of at least a few millimeters. To gain a quantitative understanding of cultured cell growth phenomena, one must take into account the fact that bioreactor systems exhibit an intrinsic multi-physics/multi-scale structure and phenomenological behavior, and are characterized by the presence of material heterogeneities and of several interplaying processes that occur within a wide range of temporal and spatial scales. A sound mathematical investigation based on a proper inclusion of the governing phenomenological principles occurring at all the different scales is thus in order.

In this Letter, we have presented two predictive models that form the building blocks of a bottom-up multiscale analysis of mass transport and tissue growth, from the length scale of an individual tissue sub-unit up to the length of a relevant tissue or biomaterial construct. At the present stage of our research activity, the Microscale Model 1 computes the time dependent nutrient delivery/consumption in a 3D geometry updated by a prescribed phenomenological growth function, while the Macroscale lumped Model 2 computes, in a highly computationally efficient manner, an updated nutrient concentration profile along the scaffold, under the input of local information from finer models (i.e., geometry, biomass thickness).

Results from Model 1 provide a quantitative description of significant aspects of the non-homogeneous tissue growth process observed in vitro, such as the decrease in the biomass growth rate during the course of the culture, either along the flow direction, due to progressive depletion of oxygen from the flow, and in areas of higher biomass volume fraction, due to the inhibition effect of high non physiological fluid-induced shears. Results from Model 2 indicate that a significant concentration drop may occur in the scaffold when almost half of the radius of the pore is occupied by the biomass.

V. Conclusion

In this Letter, we have proposed and numerically validated two computational models for the simulation of engineered tissue growth in interstitially perfused scaffolds. The philosophy that has guided our investigations is based on the development of a multiscale approach that allows, in a modular implementation, to describe the various complex interplaying biophysical phenomena driving device performance.

Models 1 and 2 represent a first attempt to integrate in a coordinate fashion the analysis at the scale of a single functional sub-unit (Microscale) with the analysis at a macroscopic scale based on a lumped parameter representation of the system.

Future research directions will be devoted to: 1) improve the interaction between experimental information and data obtained through modern imaging (spatial and temporal) techniques with the mathematical modeling framework; 2) improve the synergy between simulation and experimental phases with the purpose of identifying appropriate observables to drive model parameter calibration and, in turn, to provide more accurate model output predictions; 3) include electro-mechano-transduction mechanisms in the Microscale cellular analysis; 4) investigate mass transport of other nutrients (for example, glucose) as well as the role of growth factors and catabolite waste removal in the overall mass transport and consumption in the cellular construct.

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