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3-D computational modeling of media flow through scaffolds in a perfusion bioreactor

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Abstract

Media perfusion bioreactor systems have been developed to improve mass transport throughout three-dimensional (3-D) tissue-engineered constructs cultured in vitro. In addition to enhancing the exchange of nutrients and wastes, these systems simultaneously deliver flow-mediated shear stresses to cells seeded within the constructs. Local shear stresses are a function of media flow rate and dynamic viscosity, bioreactor configuration, and porous scaffold microarchitecture. We have used the Lattice–Boltzmann method to simulate the flow conditions within perfused cell-seeded cylindrical scaffolds. Microcomputed tomography imaging was used to define the scaffold microarchitecture for the simulations, which produce a 3-D fluid velocity field throughout the scaffold porosity. Shear stresses were estimated at various media flow rates by multiplying the symmetric part of the gradient of the velocity field by the dynamic viscosity of the cell culture media. The shear stress algorithm was validated by modeling flow between infinite parallel plates and comparing the calculated shear stress distribution to the analytical solution. Relating the simulation results to perfusion experiments, an average surface shear stress of 5×10^{-5} Pa was found to correspond to increased cell proliferation, while higher shear stresses were associated with upregulation of bone marker genes. This modeling approach can be used to compare results obtained for different perfusion bioreactor systems or different scaffold microarchitectures and may allow specific shear stresses to be determined that optimize the amount, type, or distribution of in vitro tissue growth.

Keywords: Fluid shear stress; Bioreactor; Scaffold; Micro CT; Imaging; Computational fluid dynamics

1. Introduction

Static culture of cell-seeded 3-D scaffolds typically produces thin tissue growth localized to the construct periphery (Ishaug et al., 1997). The observed heterogeneity in matrix synthesis is believed to be a result of inadequate distribution of nutrients and removal of waste products within the constructs (Freed et al., 1993; Pazzano et al., 2000). Improving in vitro mass transport is therefore a critical challenge in producing thick cellular constructs. Many groups have developed bioreactors that perfuse cell-seeded constructs and have demonstrated beneficial effects of perfusion on cell function and tissue growth (Glowacki et al., 1998; Goldstein et al., 2001; Bancroft et al., 2002; Cartmell et al., 2003). While flow rate is the independent variable typically reported in these studies, the same flow rate through two scaffolds with different pore sizes, porosity or pore anisotropy can impart vastly different shear stresses on the cells within the constructs.

In addition to enhancing chemotransport, parallel plate flow systems apply flow-mediated shear stresses, to which bone cells are highly responsive. Shear stresses in the range of 0.5–1.5 Pa (5-15 dynes/cm^2) affect osteoblast proliferation as well as production of alkaline phosphatase, nitric oxide (NO) and prostaglandin (PGE_2), indicating that shear stress is an important regulator of cell function (Reich and Frangos, 1991; Hillsley and Frangos, 1997; Smalt et al., 1997; Klein-Nulend et al., 1998; McAllister et al., 2000; Jiang et al., 2002). These short-term 2-D flow experiments suggest that flow-induced shear stresses may also modulate the

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Nomenclature

U_e	average flow speed in experimental setup (mm/s)
U_{LB}	average flow speed in the LB model (lattice units/timestep)
τ	shear stress tensor
v	experimental dynamic viscosity
U	3-D velocity vector
lu	lattice unit, the length of one side of an element in the LB model
$U_{x(avg)}$	bulk flow rate
W	width of the channel
P	pressure
x	length in x direction along parallel plates
μ	dynamic viscosity
Y	percent error underestimation
x	lu/channel

function of bone cells seeded on 3-D scaffolds and subjected to perfusion for longer culture periods.

However, calculating flow-mediated shear stresses within a complex 3-D porous structure is not trivial. Botchwey et al. (2003) used theoretical and experimental methods to estimate shear inside 3-D microcarrier scaffolds during high aspect ratio rotation. Assuming that flow through the scaffolds with an idealized pore structure of varying tortuosity obeyed Darcy's law, they estimated shear stresses within their scaffolds. While this approach provides an order of magnitude estimate of the average shear stresses, the distribution of shear stresses within complex 3-D architectures cannot be determined. Combining 2-D histology images with finite element computational fluid dynamics techniques, Raimondi et al. estimated local shear stresses on $400 \times 300\,\mu m$ sections of perfused mesh scaffolds seeded with chondrocytes (Raimondi et al., 2002). Although this method does estimate local shear stresses, it only includes a small 2-D slice of a much larger 3-D system.

The purpose of this study was to use the Lattice–Boltzmann (LB) method with equilibrium distribution functions described by Martys and Chen (Martys and Chen, 1996; Stockman et al., 1997; Stockman, 1999), to simulate cell culture media flowing through 3-D structures with complex microarchitectures that have been reconstructed using microcomputed tomography. Multiplying the symmetric part of the gradient of the resulting 3-D velocity field by the dynamic viscosity gives a distribution of the local internal shear stresses throughout the structure. This technique was validated by simulating flow through parallel plates and comparing the estimated shear stresses to the analytical solution shear stress values. Finally, shear stresses were estimated within 3-D constructs from a previous perfusion experiment in which cell function was shown to vary with flow rate.

2. Methods

2.1. Microcomputed tomography

Microcomputed tomography (μCT) imaging systems with associated stereology programs are widely used to non-destructively assess the three-dimensional morphology of radio-dense materials (Feldkamp et al., 1989). A μCT system (μCT-40, Scanco Medical, Switzerland), consisting of a microfocus X-ray tube, a 2-D detector, and a fan beam reconstruction algorithm, was used to create a three-dimensional image of human trabecular bone with a voxel resolution of 34 μm. This image was used to define the physical boundary conditions for computational fluid dynamic simulation of 3-D fluid flow through a perfused scaffold.

2.2. 3-D computational fluid flow modeling

A computational fluid dynamic modeling code based on the LB method was developed at Sandia National Laboratories (Stockman, 1999) and adapted for this study at Henry Ford Hospital (Detroit, Michigan). The LB method breaks physical space into a large number of nodes, each comprised of a set of mass probability distributions. In each timestep, the distributions translate from node to node along any one of 18 fixed velocity vectors (Fig. 1), and then undergo a collision step governed by Boltzmann theory that conserves physical properties such as momentum. We used the vector model described by Martys and Chen (1996). The end result is that the LB method approximates the Navier–Stokes equations to the second order and the model simulates a Newtonian fluid. Advantages of this method include the ability to model very complex geometries with few gridding constraints, as well as to run complicated models on modest computer hardware (e.g. 10 million elements on a PC with 1.0 Gbyte of RAM). This LB code was previously validated for flow in complex geometries (Stockman et al., 1997; Stockman, 1999).

The code used for this study employs the "reinterpreted bounce-back condition" at solid–fluid boundaries (Cournubert et al., 1991). In this method, a no-slip condition is implicitly imposed on solid–fluid interfaces, via inversion of mass distribution on solid nodes during the collision step (Chen et al., 1996). This condition places the zero velocity flow position approximately $\frac{1}{2}$ lattice units into the fluid, from the nodes in the solid wall, as shown in Fig. 2. Black circles denote the "nodes" of the automaton. A solid at node (x,y) is