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SIMPLE 3D VASCULARIZATION MODELS FOR PERFUSION BIOREACTORS

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Abstract: Growing cells in a perfusion bioreactor for tissue engineering is challenging. Cell viability and distribution is determined by complex interactions between a medium flowing through a porous scaffold and cell growth. Using a mathematical model that includes oxygen transport by diffusion/convection and cell growth mechanism, the effect of simple vascularization within the scaffold is investigated. As an initial approximation, straight channels through the length of the scaffold are considered, with performance assessed using a systematic set of criteria. Results indicate the approach can inform the choice of suitable channel size and spacing, and should enable enhanced scaffold design.

Keywords: Perfusion bioreactors, biomedical systems, mathematical models, dynamic models, performance analysis.

1. INTRODUCTION

Growing *in vitro* graft for tissue engineering presents many challenges. Achieving a uniform oxygen distribution as well as a high cell density within a three-dimensional (3D) scaffold is one of the major issues (Lewis *et al.,* 2005; Obradovic *et al.,* 2000; Radisic *et al.,* 2006). In recent years, perfusion bioreactors have been successfully used to overcome problems related to oxygen delivery (Bancroft *et al.,* 2003; Glowacki *et al.,* 1998; Goldstein *et al.,* 2001) and these are the subject of this study. Problems remain in growing tissues of clinical relevant thickness and further advances are required.

1.1 The vascularization approach.

In vivo tissues receive the necessary substrate for proliferation from the circulatory system through capillaries. Mimicking the natural tissue vascularization can lead to the formation of more homogeneous tissues and prevents implant failure caused by hypoxia (Markowicz *et al.,* 2005). Unfortunately, the production of an intricate network of capillaries within a polymeric scaffold is difficult to achieve. It has been demonstrated that low oxygen partial pressure outside the cells (Semenza 2001) can induce vasculogenesis (new blood vessel formation) and angiogenesis (formation of new branches of pre–existing vessels). Some experimental work was done on vascularization *in vitro* (Kirkpatrick *et al.,* 2003; Levenberg *et al.,* 2005), but hardly any paper considers this issue from a modelling point of view, as an aid to provide an understanding of the fundamental mechanisms regulating oxygen flow to a scaffold. Pettet *et al.* (1996) correlate density of vessels to oxygenated areas for a wound–healing process, but results seems difficult to adapt to a scaffold–bioreactor system. Ma *et al.* (2005) modelled the effects of structure on the nutrient distribution for *in vivo* bone marrow, however, for an RWPV bioreactor as opposed to the total perfusion type considered here. For perfusion bioreactors, Radisic *et al.* (2004) model oxygen distribution in a scaffold with a parallel channel array as a mimic to real tissue vascularization, but assume constant cell density and the model, therefore, is in steady–state. A major limitation is that velocities in the porous matrix are neglected so that only diffusion and

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reaction but not perfusion are accounted for in the tissue space.

A systematic modelling study of the effects of channel structure in a scaffold in perfusion bioreactors, aimed at understanding the evolution of cell density and distribution, appears to be lacking.

2. METHODS AND OBJECTIVES

The long term goal is to develop a fundamental understanding of vascularization on oxygen transport and cell growth within a perfusion bioreactor. A mathematical model already developed (Coletti *et al.,* 2006) is used which describes a 3D cell culture in cylindrical perfusion bioreactors, including the complex interactions between fluid dynamics of a nutrient medium flowing by convection and diffusion through a scaffold, modelled as a porous medium, and cells growth kinetics. Here, the model is adapted to include a simple vascularization, in the form of straight channels through the scaffold.

First the effect of a single, straight channel at the centre of the scaffold is analysed in 2D, exploiting the symmetry of this geometry with respect to the angular coordinate. A more complex, 3D geometry with several channels is then considered, with the assumption of angular symmetry relaxed. Results are compared to a reference base case with full perfusion (no channels) under the same operating conditions (medium flowrate and composition, cell type, etc.). The parameters used are for immortalised murine C_2C_{12} cell culture on a collagen scaffold.

The aims are to keep the oxygen concentration in the scaffold above a viability level below which the culture enters critical conditions (hypoxia), and to achieve a high and homogeneous final cell distribution as fast as possible. To properly evaluate alternative configurations and operations, some systematic criteria are needed and these are described first.

2.1 Performance assessment criteria.

Changing operating conditions, reactor or scaffold geometry, or cell line parameters affect cells growth in a very complex way. Oxygen and cell concentration are a function of local conditions and evolve in time, and in a 3D scaffold are therefore functions of (z,r, ϑ ,t). Aggregate criteria are however useful to compare overall conditions at any one time, and their evolution in time. A set of 7 systematic criteria by which to evaluate the performance of alternative operations proposed by Coletti and Macchietto (2006) is briefly summarized here, with performance defined in terms of oxygen concentration and uniformity of its distribution within the scaffold. Some of the criteria may be applied to other relevant quantities of interest, such as cell density or oxygen uptake rate.

Criterion A1. $\overline{c}_{O_2}$, at time *t*, the oxygen average concentration in the scaffold:

$$\overline{c}_{O_2} = \frac{\int_{V_s} c_{O_2}(r,z,\vartheta,t)\, dV_s}{V_s} \tag{1}$$

where V_s is the scaffold total volume, r, z and ϑ are the systems coordinate and c_{O2} is the local oxygen concentration in the scaffold.

Criterion A2. $\overline{c}^{\%}_{O_2}$, at time *t*, $\overline{c}_{O_2}$ as a percent of the limit oxygen concentration for viability, c_{lim}:

$$\overline{c}^{\%}_{O_2} = \frac{\overline{c}_{O_2}}{c_{\lim}} \cdot 100 \tag{2}$$

Criterion B. $\tilde{t}_B$: the time when $\overline{c}_{O_2}$ first falls below c_{lim}:

$$\tilde{t}_B = t\big|_{\overline{c}_{O_2} < c_{\lim}} \tag{3}$$

Criterion C. The location ($\tilde{r}$, $\tilde{z}$, $\overline{\vartheta}$) and time $\tilde{t}_C$ where the oxygen concentration first falls below the viability level, $c_{lim:}$

$$\begin{cases} \tilde{r}_C, \tilde{z}_C, \overline{\vartheta}_C = (r,z,\vartheta)\big|_{\overline{c}_{O_2} < c_{\lim}} \\ \tilde{t}_C = t\big|_{\overline{c}_{O_2} < c_{\lim}} \end{cases} \tag{4}$$

Criterion D. $\tilde{V}_D$, at time *t*, the fraction of scaffold volume (in percent) with $c_{O2} < c_{lim:}$

$$\tilde{V}_D = \frac{\int_{V_s} V\big|_{\overline{c}_{O_2} < c_{\lim}}\, dV_s}{V_s} \cdot 100 \tag{5}$$

Criterion E. $\tilde{t}_E^{\,p\%}$, the time when a given percentage volume *p*% of the scaffold has $c_{O2} < c_{lim}$:

$$\tilde{t}_E^{\,p\%} = t\big|_{\tilde{V}_D < p} \tag{6}$$

Criterion F1. A_{O_2} , at time *t*, the amount of oxygen available for cells metabolism in the scaffold:

$$A_{O_2} = \int_0^R \int_0^{2\pi} N_{O_2}\big|_{z^{out}} - N_{O_2}\big|_{z^{in}}\; d\vartheta dr \tag{7}$$

where N_{O_2} is the oxygen total flux into (Z^{in}) and out (Z^{out}) of the scaffold.

Criterion F2. $A^{\%}_{O_2}$, at time *t*, the amount of oxygen used by the cells as a % of the total oxygen inlet:

$$A^{\%}_{O_2} = \frac{A_{O_2}}{\int_0^R \int_0^{2\pi} N_{O_2}\big|_{z^{in}}\; d\vartheta dr} \tag{8}$$

Criterion G. At time *t*, oxygen distribution, as a histogram where each bar is the fraction of scaffold volume that has oxygen concentration between two