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Towards a quantitative understanding of oxygen tension and cell density evolution in fibrin hydrogels

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ABSTRACT

The in vitro culture of hydrogel-based constructs above a critical size is accompanied by problems of unequal cell distribution when diffusion is the primary mode of oxygen transfer. In this study, an experimentally-informed mathematical model was developed to relate cell proliferation and death inside fibrin hydrogels to the local oxygen tension in a quantitative manner. The predictive capacity of the resulting model was tested by comparing its outcomes to the density, distribution and viability of human periosteum derived cells (hPDCs) that were cultured inside fibrin hydrogels in vitro. The model was able to reproduce important experimental findings, such as the formation of a multilayered cell sheet at the hydrogel periphery and the occurrence of a cell density gradient throughout the hydrogel. In addition, the model demonstrated that cell culture in fibrin hydrogels can lead to complete anoxia in the centre of the hydrogel for realistic values of oxygen diffusion and consumption. A sensitivity analysis also identified these two parameters, together with the proliferation parameters of the encapsulated cells, as the governing parameters for the occurrence of anoxia. In conclusion, this study indicates that mathematical models can help to better understand oxygen transport limitations and its influence on cell behaviour during the in vitro culture of cell-seeded hydrogels.

1. Introduction

Biodegradable hydrogels become more and more prevalent in tissue engineering as a biomimetic matrix that can organise three-dimensional (3D) cell proliferation and differentiation [1–3]. However, controlling the culture environment inside hydrogels of a clinically relevant size remains a major challenge [4]. Tissue substitutes formed in vitro lack vasculature; hence, they suffer greatly from mass transport limitations due to slow diffusion of nutrients and high density of metabolically active cells [5]. In particular, the adequate supply of oxygen has been suggested as one of the most challenging design parameters in a tissue engineering process because of the high cellular oxygen consumption and the low solubility and diffusivity of oxygen molecules in culture medium and hydrogel matrix [6]. As a result, hypoxia is common within the central regions of tissue engineered constructs [7]. For example, oxygen gradients have been measured in cartilaginous constructs using microelectrodes [8,9]. Oxygen gradients were also visualised in constructs for cardiac tissue engineering by using a hypoxia-sensitive pimonidazole staining [10]. The size of the hypoxic region was found to increase with time, while it was decreased significantly when metabolism was inhibited. This observation indicates that oxygen consuming cells in the peripheral regions hinder oxygen penetration, even in situations in which sufficient oxygen is delivered to the construct surface.

In a skeletal tissue engineering approach, the presence of oxygen gradients within constructs causes inhomogeneous cell distribution and new matrix formation. Local reduction of cell viability has been attributed to oxygen gradients [11], since mesenchymal stem cells (MSCs) were shown to die when exposed to anoxia for 5 days [12]. Additionally, local increases in cell density were reported in constructs that only relied on diffusion for their oxygen supply, due to enhanced proliferation near the surface of the scaffold [8,13,14]. In addition to viability and proliferation, numerous other cellular processes were shown to be regulated by oxygen [15,16]. In general, oxygen levels around 5% were reported to upregulate proliferation of progenitor cells [17–20]. However, conflicting results have been observed when studying osteogenic differentiation at reduced oxygen levels [15,16,21,22].

Spatial and temporal control of the oxygen tension inside cell-seeded constructs would allow the tissue engineers to use oxygen to

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their advantage and guide cellular function. To achieve this, a good knowledge and understanding of the different processes that contribute to the oxygen microenvironment inside a tissue substitute is needed. Mathematical models that describe the interaction between oxygen tension and cell density during 3D culture have been used to help researchers understand the mechanisms and dynamics of experimental observations [23]. Model predictions of the oxygen mass balance within tissue engineered constructs have shown to correlate well to experimentally observed oxygen profiles for fixed cell distributions or at steady state conditions [8,10,11,24,25]. By incorporating cell growth as well, Lewis et al. were able to explain experimentally monitored oxygen and cell density gradients within cartilaginous constructs [13]. However, the model only considered the first 14 days of the in vitro culture due to the relatively simple description of the relation between cell proliferation and oxygen concentration within a construct. Gross et al. modelled both cell growth and death, in addition to the oxygen transfer, within a spherical pancreatic substitute [26]. This approach allowed them to describe the dynamic changes in cell distribution that were observed experimentally by histological examination of the encapsulated cell systems.

In this study, a combined experimental and computational approach was followed to test the hypothesis that the occurrence of cell density gradients inside fibrin hydrogels can be related to limitations in oxygen mass transfer. To this end, a mathematical model was developed that describes the spatiotemporal evolution of oxygen tension and density of human periosteum derived cells (hPDCs) in fibrin hydrogels for bone tissue engineering applications. Apart from static culture experiments of fibrin-hPDC constructs, dedicated in vitro experiments were performed to quantify model parameters related to hPDC proliferation and death, their modulation by oxygen tension, and the oxygen diffusivity inside fibrin. Model predictions on cell density, distribution and viability within fibrin-hPDC constructs were correlated to experimental results. Finally, a sensitivity analysis was carried out to examine which model parameters were most influential to the model outcomes.

2. Mathematical model

2.1. Geometry

During the in vitro experiments, cell-seeded fibrin hydrogels were placed in 12-well plates and submerged in 2 mL of medium (Fig. 1A). The medium surface was exposed to an ambient oxygen tension of 21% in the incubator. Due to the axisymmetry of this setup, the cell density and the oxygen tension were modelled for one half of a vertical cross-section (Fig. 1B). Three regions were defined in the model: hydrogel, surface, and medium. The hydrogel region corresponded to the cell-seeded fibrin carrier, while the surface region comprised the cells that resided on the top and side surfaces of the carrier. The medium region was a cell-free zone that simulated the presence of culture medium around the carrier. The total modelled geometry had a height (Z) of 5.7 mm and radius (R) of 11 mm. The fibrin carrier, with a height and radius of 4 mm, was positioned in the centre of the well corresponding to the experimental conditions (see 'Materials and methods'). The surface region had a thickness $t_{surface}$, which was measured experimentally.

2.2. Model equations

In the presented mathematical model, the spatiotemporal evolution of oxygen tension (c_{O_2}) and cell density (c_{cell}) are mathematically described by two partial differential equations. Oxygen is assumed to move due to diffusion with a diffusion coefficient D and to be consumed by the cells at a rate Q. The cells are assumed to be immobile in the carrier, but to increase and decrease in number at a proliferation rate P and death rate d, respectively.

$$\frac{\partial c_{O_2}(z,r,t)}{\partial t} = D(z,r,t)\cdot\nabla^2 c_{O_2}(z,r,t) - Q(z,r,t)\cdot c_{cell}(z,r,t) \quad (1)$$

$$\frac{\partial c_{cell}(z,r,t)}{\partial t} = P(z,r,t)\cdot c_{cell}(z,r,t) - d(z,r,t)\cdot c_{cell}(z,r,t) \quad (2)$$

These coupled partial differential equations were solved numerically using a previously developed finite volume code [27,28]. The numerical algorithm took the axisymmetric geometry into account and made use of the code ROWMAP [29] for the time integration. The grid size was 0.2 mm in axial and radial direction in the hydrogel and medium region. In the surface region, 5 elements were used to span the thickness of this region. Doubling the amount of elements in this region did not alter the model predictions, demonstrating that the numerical results were mesh-independent.

2.3. Oxygen diffusion

Oxygen molecules had to diffuse through the medium and the fibrin matrix to reach the cells in the centre of the carrier. This diffusive mass transfer of oxygen through medium and fibrin is determined by the diffusivity of oxygen in water ($D_{medium} = 3 \times 10^{-9}$ m^2/s [30,31]) and in fibrin (D_{fibrin}), respectively. However, at locations with high volume fractions of cells, diffusing molecules will be physically hindered by the cells, in addition to experiencing an increased consumption [10], due to a low diffusivity of oxygen through cells ($D_{cell} \approx 3 \times 10^{-10}$ m^2/s [32]). The effective diffusion coefficient of oxygen was calculated from the diffusion coefficients in cells and in medium for the surface region and from the diffusion coefficient in cells and in fibrin for the hydrogel region:

$$D(z,r,t) = D_i \frac{2/D_{cell} + 1/D_i - 2\cdot\phi_{cell}(z,r,t)\cdot(1/D_{cell} - 1/D_i)}{2/D_{cell} + 1/D_i + \phi_{cell}(z,r,t)\cdot(1/D_{cell} - 1/D_i)} \quad (3)$$

where D_i represents D_{medium} or D_{fibrin} in the surface or hydrogel region, respectively. This equation is derived from a Maxwell-like model that regards cells as randomly

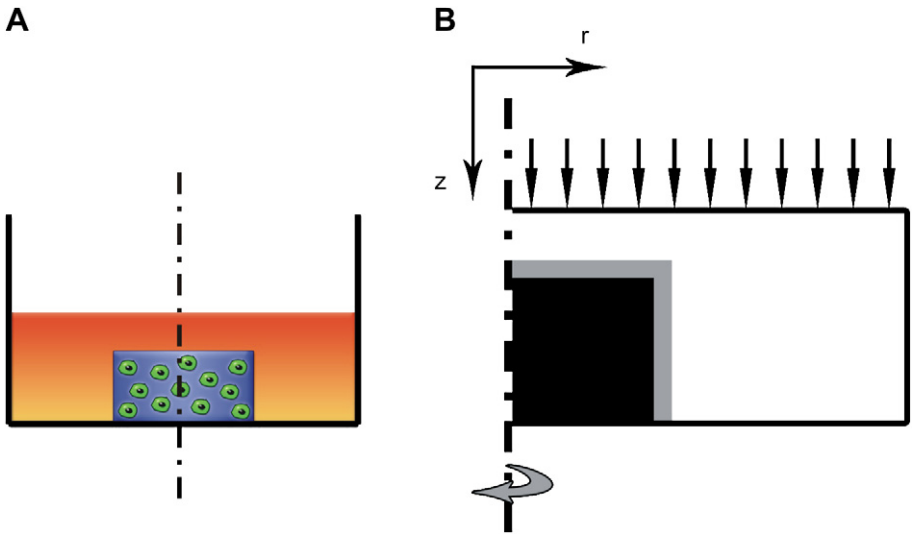

Fig. 1. (A) Schematic representation of the three-dimensional culture of cells in fibrin hydrogels. (B) Geometry of the axisymmetric model (half of vertical cross-section) which consists of three distinct regions: cells encapsulated in the hydrogel (black), cells on the hydrogel surface (grey, not shown proportionally) and culture medium (white).