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Mathematical modelling of tissue-engineered angiogenesis

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ABSTRACT

We present a mathematical model for the vascularisation of a porous scaffold following implantation *in vivo*. The model is given as a set of coupled non-linear ordinary differential equations (ODEs) which describe the evolution in time of the amounts of the different tissue constituents inside the scaffold. Bifurcation analyses reveal how the extent of scaffold vascularisation changes as a function of the parameter values. For example, it is shown how the loss of seeded cells arising from slow infiltration of vascular tissue can be overcome using a prevascularisation strategy consisting of seeding the scaffold with vascular cells. Using certain assumptions it is shown how the system can be simplified to one which is partially tractable and for which some analysis is given. Limited comparison is also given of the model solutions with experimental data from the chick chorioallantoic membrane (CAM) assay.

1. Introduction

1.1. Background

The field of tissue engineering, the primary aim of which is to replace diseased and damaged organs with tissues grown *in vitro*, is set to play a central role in meeting the health care needs of society in the future [1,2]. However, a major factor impeding progress of the field is an incomplete knowledge and understanding of the many different processes that contribute to the production of a functioning organ or piece of tissue [3,4].

A basic procedure used in tissue engineering to produce an *ex vivo* tissue implant is to seed cells of the desired tissue type into a porous biodegradable scaffold produced for example by the method of salt leaching [5], supercritical fluids [6] or electrospinning [7]. The implant is then incubated inside a bioreactor so as to encourage the tissue to become established prior to implantation [8]. After being surgically implanted into the patient, the scaffold degrades and the implanted cells become integrated with the host tissue. A particular approach that is receiving much attention is to seed scaffolds with stem cells [9,10], and the use of embryonic stem cells (ESCs) will potentially have an enormous impact on the field of regenerative medicine [11,12]. By providing an abundant source of cells with unlimited proliferation and differentiation potential, the use of ESCs will circumvent the problem of supplying sufficient quantities of autologously derived cells from a patient. However, the specific biochemical clues that need to be applied to direct the ESCs into the desired tissue lineage are not yet fully known.

As the experimental side of tissue engineering develops, there is a strong need to develop mathematical frameworks for studying the underlying tissue-growth processes [13,14]. That sufficient knowledge of these processes is often lacking poses a significant challenge for the mathematical modeller; however, building and analysing mathematical models can be useful as a means of understanding the interplay between the mechanisms that are known. Ideally the modelling should be carried out to complement experimental work so as to allow the model to be validated and tested, and on the basis of the model predictions, to suggest protocols to improve experimental outcomes. Meaningful comparison between theory and experiment requires appropriate techniques for quantifying tissue composition such as confocal microscopy and micro-computed tomography [15].

To date tissue engineering has been successful in producing simple avascular tissues, such as skin and cartilage, which are thin enough for oxygen and other nutrients to diffuse through passively. However, diffusion limits the thickness of these tissues to

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be at most a few millimetres [16,17], although larger specimens can be sustained *ex vivo* by perfusing nutrient through them in a bioreactor [18]. To sustain a piece of tissue with larger dimensions inside a patient, it is necessary that blood vessels be made to penetrate the implant from the surrounding tissue [19].

In normal tissue the vasculature ensures that the cells receive an adequate supply of nutrients and also provides for the removal of wastes. Blood is pumped from the heart through arteries which branch repetitively, eventually down to the level of the capillaries, a fine network of tubes such that a cell typically is never more than a few cell diameters from a capillary. Blood cells passing through the capillary network exchange oxygen and carbon dioxide through the capillary walls. Angiogenesis refers to the process by which new capillaries form from existing vessels in a tissue. Vasculogenesis refers to the manner in which capillaries form from the migration and differentiation of endothelial progenitor cells (EPCs) in the absence of a pre-existing vasculature. Both processes are required for the growth and maintenance of healthy tissues in an organism, including during embryonic development and in the adult stage where they are essential for wound healing and as a physiological response to exercise.

Given that the use of synthetic materials constrains the minimum size of the diameter of vascular prostheses to be about 5 mm [20], angiogenesis is essential for the tissue engineering of microvascular networks [21–24]. However, the ways in which cells respond to the myriad of biochemical and biophysical clues in order to form new blood vessels is highly complex and not completely understood [25,26]. Given the additional complications associated with cell growth inside porous biomaterials, it is clear that harnessing angiogenesis for tissue engineering poses special challenges [27,28].

The *in vivo* testing of the angiogenic response of a porous scaffolds commonly involves direct implantation into a mammalian laboratory animal followed by incubation for periods of up to several months, see [29–31] for examples. However, the extent of vascularisation can only be assessed after excision of the scaffold, which requires sacrificing the animal. An advantage of the dorsal skinfold chamber is that it allows implants, for example in mice [32] or rabbits [33], to be viewed in real time. The use of mammalian based assays carries several draw-backs, being both costly and labour intensive, and raises ethical concerns [34]. In these respects the use of the chorioallantoic membrane (CAM) assay, where the scaffold is implanted into an extra-embryonic membrane of a chicken, is regarded as preferable.

1.2. The chick chorioallantoic membrane assay

The chorioallantoic membrane of a chicken embryo is a membrane of tissue approximately 200 μm thick that surrounds the developing chick (see Fig. 1), the function of which is to support respiratory capillaries and to provide ion transport and gas exchange [35]. In addition to providing a means of testing the biocompatibility of materials [36–39], the CAM assay is used for experiments concerning drug toxicity, tumour growth, wound healing [40] and angiogenesis [41]. The testing procedure typically consists of making an opening in the shell, placing the drug or implant onto the CAM, and then covering the window with a transparent film to allow ease of observation as the experiment proceeds. The extent of vascularisation is usually assessed by measuring the change in vessel density in the membrane around the implant. As well as being a straightforward procedure with lower costs and labour requirements than other assays, a particular advantage is that the biology of the CAM is much better defined than for laboratory animal based assays. Disadvantages of the CAM assay include the relatively short maximum implantation time and the limited vascularising potential of the CAM, which

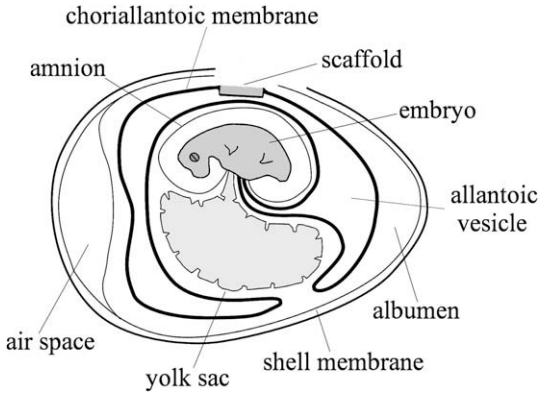

Fig. 1. Diagram showing some features of a fertilised chicken egg, with a scaffold implanted onto the chorioallantoic membrane.

only allows a small amount of ingrowth to be achieved [42]. Further, the differences between avian and mammalian biology brings into question the suitability of drawing conclusions for mammalian models based on results from the CAM assay. Nevertheless, like the corneal assay [43] the CAM assay is useful because it can establish whether the scaffold is liable to cause an adverse reaction if used as a therapeutic implant [21].

This paper describes the formulation and theoretical analysis of a mathematical model of tissue-engineered angiogenesis, complemented by experimental work using the CAM assay. The overall aim of the experiments is to determine the experimental factors that will encourage vascularisation of scaffolds thereby promoting the survival of seeded cells in particular murine embryonic stem cells (mESCs). Scaffolds were produced using size sieved salt (300–400 μm) as a pore producing material with polylactic acid (PLA) polymer cast over this and then removed by leeching with water. Porous scaffolds were hydrated, warmed and placed onto the CAM with or without adsorption of vascular endothelial growth factor (VEGF). Scaffolds were implanted at day 4 of gestation, by which time the CAM has developed to a suitable size, via an opening cut into the egg shell. Fig. 2 shows typical images obtained from experiments with unseeded 5 mm^3 salt leached PLA scaffolds. Fig. 2(a) shows a view through the egg shell window of a scaffold *in situ* that has been incubated on the CAM for two days while Fig. 2(b) shows a scaffold that has been excised after four days and stained with osmium tetroxide (OsO_4: an X-ray contrast agent) prior to imaging using micro-computed tomography (μCT) techniques. This staining technique is useful for determining the extent of soft tissue encroachment into the porous biomaterials [44,45] and such data are applied to the model developed in this paper. Fig. 2(c) shows an optical microscopy image of a histological section of a scaffold, where tissue infiltration off the CAM into the scaffold is apparent. Details of the experimental procedures are given in Appendix C.

1.3. Vascularisation of porous biomaterials

The manner in which tissue invades a scaffold that has been implanted into an animal is complicated and involves several different types of cells. Various authors have described the process of angiogenesis for mammalian based assays [46], as well as the CAM assay [47,48]. Vascularisation of porous biomaterials has aspects in common with that of wound healing, but the type of scaffold can have a significant effect on the ultimate extent of vascularisation [38,49].

Angiogenesis involves a complicated sequence of biochemical and biophysical signalling events between the infiltrating cells,