Continuum Modelling of In Vitro Tissue Engineering: A Review

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Abstract By providing replacements for damaged tissues and organs, in vitro tissue engineering has the potential to become a viable alternative to donor-provided organ transplant, which is increasingly hampered by a shortage of available tissue. The complexity of the myriad biophysical and biochemical processes that together regulate tissue growth renders almost impossible understanding by experimental investigation alone. Mathematical modelling applied to tissue engineering represents a powerful tool with which to investigate how the different underlying processes interact to produce functional tissues for implantation. The aim of this review is to demonstrate how a combination of mathematical modelling, analysis and in silico computation, undertaken in collaboration with experimental studies, may lead to significant advances in our understanding of the fundamental processes that regulate biological tissue growth and the optimal design of in vitro methods for generating replacement tissues that are fully functional. With this in mind, we review the state-of-the-art in theoretical research in the field of in vitro tissue engineering, concentrating on continuum modelling of cell culture in bioreactor systems and with particular emphasis on the generation of new tissues from cells seeded on porous scaffolds. We highlight the advantages and limitations of different mathematical modelling approaches that can be used to study aspects of cell

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population growth. We also discuss future mathematical and computational challenges and interesting open questions.

1 Introduction

The interdisciplinary field of tissue engineering is emerging as a valuable tool in the quest for viable clinical solutions to health problems associated with tissue damage, degeneration and failure. Currently, the most successful surgical approaches involve the implantation of tissue grafts, or entire replacement organs, taken from suitable donors. Due in part to greater longevity in society (Palferman 2003), there is a chronic shortage of donor tissue. For example, in the UK during 2009/2010 552 patients died while awaiting transplants, and at 31 March 2010, there were approximately 8000 NHS patients registered for organ transplant (Johnson 2010). Furthermore, engineered tissues with the correct in vivo properties have applications in toxicology screening and drug testing. While therapeutic tissue engineering has the potential to address the issue of donor tissue shortage, this field remains in its infancy, since tissue growth is exceedingly complex, being regulated by an enormous variety of processes, from intracellular transduction pathways to tissue-level mechanics. Understanding these mechanisms is crucial to the development of reliable methods for engineering viable replacement tissue; mathematical analyses can provide fundamental insight into these mechanisms. In addition, collaboration between experimental and theoretical researchers enables in silico testing of experimental protocols (thereby reducing experimental cost) and stimulates the generation of model-driven experimentally-testable hypotheses. In this way, mathematical modelling can provide a key scientific tool with which to improve tissue engineering approaches.

In this chapter, we review the contribution of mathematical modelling to the understanding of tissue growth processes. We focus on continuum approaches which consider the influence of cells' biochemical and biophysical environment on tissue growth. Before surveying such theoretical analyses (Sects. 3.1–3.3), it is instructive to consider the key biological considerations in more detail.

1.1 Tissue Engineering

Broadly speaking, there are two distinct approaches to tissue engineering: in vivo and in vitro tissue engineering. The latter involves growing replacement tissue in the laboratory for implantation into patients. A common in vitro method involves seeding porous scaffolds with cells of the desired tissue type. After a period of incubation, during which the cells proliferate and colonise the scaffold, the resulting tissue construct is implanted into a patient. In contrast, in vivo tissue

engineering involves implanting (e.g.) degradable scaffolds or gels loaded with cells directly into the body. In each case, degradation of these artificial supporting structures and their replacement by extracellular materials—such as collagen and proteoglycans (Freed et al. 1994; Hutmacher 2000)—leads to eventual tissue repair. In vivo tissue engineering uses the human body as a natural bioreactor, the perceived advantage being that the human body offers the correct physical and biochemical cues to enable creation of functional, viable tissue. However, the mechanisms by which these cues are employed by the cells are not well understood; a thorough review of in vivo tissue engineering considerations is given by Zdrahala and Zdrahala (1999). In what follows, we concentrate on in vitro tissue engineering, where tissue growth occurs under closely monitored and controlled environmental and operating conditions.

1.1.1 Cell Populations

The tissue engineering concepts outlined above are conceptually straightforward; however, in practice many barriers remain to be overcome. Fundamental problems include stimulating sufficient cellular proliferation to colonise the scaffold and preventing dedifferentiation of the seeded cell population. An approach mitigating the former problem involves using tissue precursor cells or multipotent stem cells, which have high proliferative capacity and can be induced to differentiate to a number of different cell types (Risbud and Sittinger 2002). The literature regarding the use of stem cells in tissue engineering and, for instance, the methods by which they can be induced to differentiate along different cell lines is extensive; a good introduction is given by Salgado et al. (2004) and references therein. We choose not to review this literature here, preferring to focus on continuum modelling of biochemical (and biophysical) aspects of tissue growth; implicit in the mathematical models that we analyse are the assumptions that, on the timescale of interest, the cell population has sufficient proliferative capacity to colonise the scaffold.

The mechanical forces that cells experience affect their differentiation, proliferation, orientation, gene activity and a host of other activities; indeed, as we indicate below, culturing cells in an in vivo-like mechanical environment can maintain differentiated function of the seeded cells. The stimuli are integrated into the cellular response via a process known as mechanotransduction. The mechanical environment of the tissue comprises both internally generated and externally applied forces. Internally generated forces may include active forces generated by cells during movement and adhesion, or residual stresses brought about by tissue growth and remodelling; the presence of such stresses has been observed in a variety of soft tissues (examples include arterial and venous tissue, myocardium and the trachea) and, in many cases, is crucial to their correct function. For example, residual stresses act to minimise the peak stress across the depth of the arterial wall (Chuong and Fung 1986) and are involved in wall remodelling (Fung 1991). Externally applied loads that act in vivo include macroscale forces due to movement and muscle contraction, and shear stress induced by fluid flow. Such

forces are known to be important in the correct functioning of various tissues; placing patients with broken limbs under traction to prevent incorrect bone repair or misshapen bones is a simple example of this phenomenon that has been practised in hospitals for many years. Furthermore, flow-mediated shear stress has been shown to effect the culture of a variety of mechanosensitive cell types such as bone, cartilage, muscle, liver and blood vessels. For example, many studies have shown how stimulation via fluid shear stress enhances extracellular matrix formation (Bakker et al. 2004a; Klein-Nulend et al. 1995b; You et al. 2000).

Many studies have investigated the influence of the mechanical environment on cells' phenotype. For example, the response of osteocytes to mechanical loading has been investigated by Klein-Nulend et al. (1995b) and Bakker et al. (2004a). It is generally accepted that these terminally-differentiated human cells are the most mechanosensitive in bone and that they direct the formation and resorption of bone tissue at the microscopic level (Noble and Reeve 2000). Osteocytes have several thin processes which extend into the porous structure of bone and respond to interstitial fluid flows which exist in bone under loading. In this way, bone remodelling can be directed by physiological loading, despite the small strains allowed by the stiff calcified matrix.

Due in part to its avascular nature, cartilaginous tissue is notorious for its poor capacity to self repair and much experimental work has concentrated on developing suitable implants. Experimental studies (reviewed in Urban 1994) indicate that, under physiological conditions, moderate levels of mechanical stress regulate cartilage cell (chondrocyte) metabolism and ensure maintenance of extra-cellular matrix (ECM) integrity. Further, these processes are profoundly influenced by mechanical compression and hydrostatic pressure, such stimulation leading to accelerated chondrocyte growth and ECM synthesis, depending upon its regime of application (e.g. loading magnitude or, in the case of cyclic loading, frequency).

Another important area of tissue engineering is the culture of sheets of keratinocytes, which are used as replacement epithelium in a host of clinical settings (notably wound closure and/or skin grafts for severe burns). Mechanical strain is known to influence the proliferation rate of keratinocytes and activate them to express keratin, the constituent of intermediate filaments expressed specifically in keratinocytes (Yano et al. 2004).

1.1.2 Artificial Scaffolds

As indicated above, in vitro tissue engineering often involves seeding a porous scaffold with cells, to create a 'tissue construct'. The properties of the scaffold are therefore of central importance to the success of this approach.

As the ultimate aim is the in vivo implantation of the scaffold, the first requirement is that it is compatible with the host tissue, and does not elicit an immune response (Salgado et al. 2004). Furthermore, the scaffold acts as a surrogate for the significant amount of acellular material that is present in living tissue and defines its mechanical properties (for instance the collagen and elastin fibres present in ECM,

or the calcium hydroxyapatite which lends bone tissue its rigidity). The scaffold material must, therefore, be chosen so that its mechanical integrity is maintained under physiological conditions, a factor of especial importance when the construct is load-bearing (as is the case for bone or cartilage implants). Additionally, since tissue repair is effected by the replacement of artificial scaffolds by extracellular materials, ensuring that the rates of nascent tissue growth and scaffold degradation (e.g. due to hydrolysis) are appropriately matched is crucial in maintaining the mechanical integrity of the construct. Lastly, porous scaffolds with a highly porous, interconnected structure are required to encourage cell penetration, vascularisation of the construct from surrounding tissue (in vivo) and efficient mass transfer of nutrients and waste products. There is, therefore, a trade-off between mechanical loading and mass transfer considerations in scaffold design.

Topographical and biochemical characteristics of the supporting scaffold can be used to enhance cell-scaffold adherence (crucial in most cells' growth), and to direct cell movement or differentiation (Weiss 1945; Salgado et al. 2004); for example, scaffolds may be engineered to deliver growth factors or DNA (Sipe 2002), or to contain specific cellular recognition molecules (Freed and Vunjak-Novakovic 1998). The suitability of a wide range of materials for tissue engineering has been investigated; examples include hydroxyapatite, poly(α-hydroxyesters), and natural polymers such as collagen and chitin. Poly(α-hydroxyesters) comprise a range of materials, including widely used polymers such as poly(lactide-co-glycolide)(PLGA), poly(glycolic acid) and poly(L-lactic acid). A comprehensive discussion of scaffolds for bone and cartilage tissue engineering applications is given by Hutmacher (2000); more general treatments are given by Atala et al. (1997) and Hollister (2005).

1.1.3 Bioreactor Systems

In a tissue engineering context, a bioreactor may be viewed as a cell culture system, in which biochemical and/or biophysical processes are closely monitored and controlled. For the reasons described above, many in vitro culture systems aim to mimic the in vivo environment; bespoke bioreactors are therefore required to provide the appropriate biochemical and biophysical conditions for individual tissue engineering applications. Specific examples of bioreactor types, which we investigate in more detail in later sections, are described below; reviews of bioreactor designs for specific applications are given by Martin et al. (2004), Cartmell and El Haj (2005), Eibl and Eibl (2009).

For the purposes of this review, it is convenient to classify cell culture techniques into two distinct groups, termed static and dynamic culture, respectively. Static culture refers to the simplest scenario in which mass transfer of (e.g.) nutrients or waste products through the culture is effected by diffusion; dynamic culture encompasses a range of bioreactor systems, designed to improve mass transfer to the cell population and/or to provide mechanical stimulation. Typically, enhanced mass transfer is effected via advection of nutrients and waste products by an imposed flow of culture medium (two strategies for achieving this are discussed

below); mechanical stimulation of cells may be restricted to the influence of such a flow, or by additional cell stimulation strategies. We pause to remark that the operating parameters (e.g. flow rate) of the majority of dynamic culture systems do not change during the cell culture period, or are manually adjusted. Butler et al. (2009) highlight the need for the development of bioreactor systems which regulate automatically the biochemical and biophysical environment to accommodate the evolving requirements of the developing tissue.

The simplest example of a static culture bioreactor is a petri dish. Here, cells are grown in a monolayer to confluence. A weakness of this approach is rapid cell de-differentiation and loss of phenotype due to the absence of a three-dimensional architecture (Lin and Chang 2008). Cell populations cultured as three-dimensional multicellular spheroids (via culture in a non-adhesive environment) aim to address this by maximising cell-cell contacts, and have been found to positively affect tissue functionality (Riccalton-Banks et al. 2003). However, since the transport of nutrients and waste products is by diffusion only, scale-up to produce tissue of a size appropriate for implant results in the formation of constructs with a viable, proliferating periphery but a necrotic core (Cartmell and El Haj 2005). Bioreactors which employ dynamic culture conditions aim to mitigate the effects of diffusion-limited transport. Two such systems are described below.

Rotating wall bioreactors comprise a cylindrical vessel of circular cross-section rotating about its longitudinal axis with constant angular speed (see Fig. 1a). While specific designs vary, key parameters with which to vary the culture environment include: (i) rotation speed, (ii) bioreactor aspect ratio and (iii) bioreactor orientation. See Hammond and Hammond (2001) for more details of rotating-wall bioreactors. The system can be used as a suspension culture method, in which an initial cell suspension spontaneously self-assembles to form 3D spheroids (Lappa 2003); alternatively, a porous scaffold can be added, either pre-seeded, or to which the suspended cells adhere. A wide range of cell types have been cultured in this bioreactor system. Representative examples include human breast carcinoma cells cultured in suspension to produce in vivo-like nodules suitable for in vitro drug screening [experimental investigation discussed in Sawyer et al. (2007) and Waters et al. (2006)], and osteoblasts seeded on porous scaffolds (Yu et al. 2004).

Commonly, the longitudinal axis of the vessel is oriented horizontally and the rotation speed is chosen such that the upward hydrodynamic force balances the downward gravitational force and the spheroid or scaffolds contained within exist in a state of perpetual free-fall. This is thought to provide an optimal environment for tissue engineering applications, since the relative motion between the culture medium and tissue construct ensures that the culture medium remains mixed, aiding mass transport, while the shear stresses experienced by the cells can be controlled (Martin et al. 2004; Waters et al. 2006).

An alternative approach is to directly perfuse a cell-seeded scaffold with culture medium via a pump. By confining the scaffold such that culture medium is driven through its interconnected pores (perfusion bioreactor), rather than around the periphery (perfusion chamber), mass transfer limitations throughout the tissue

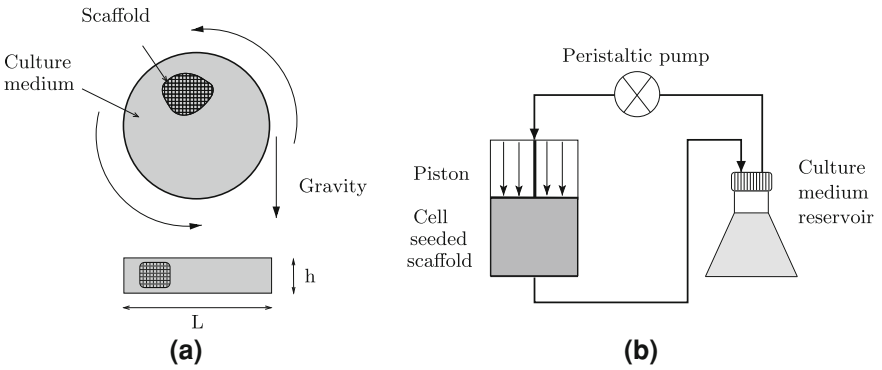

Fig. 1 **a** A rotating bioreactor system comprising a cylindrical vessel of diameter L and depth h rotating about its longitudinal axis, which contains a porous cell-seeded scaffold immersed in culture medium. *Upper figure* is a face-on view of the bioreactor. *Lower figure* is a side view showing the small gaps between the scaffold and the bioreactor walls. **b** The perfusion/compression bioreactor system of El-Haj et al. (1990), comprising a cell-seeded scaffold contained within a cylindrical vessel and constrained by a perforated piston, through which culture medium is perfused via a peristaltic pump. The perforations in the face of the piston allows simultaneous perfusion and compression of the scaffold

construct are reduced. In the rotating wall bioreactor, the rotation speed determines the mechanical environment of the cells (and the mass transfer characteristics of the system); here, the fluid shear stress experienced by the cells may be controlled by varying the perfusion rate. Indeed this type of bioreactor is frequently employed in the generation of bone tissue constructs, due to the sensitivity of such cells to stimulation by fluid shear stress (McCoy and O'Brien 2010). A modified perfusion bioreactor system is employed by El-Haj et al. (1990), in which cells seeded in a porous scaffold are subjected to perfusion with media and direct compression using a piston. This system is illustrated in Fig. 1b.

The above bioreactors provide convenient model systems with which to investigate aspects of tissue growth modelling relevant to tissue engineering applications. Outstanding problems that need to be addressed in order to optimise the culture environment in these bioreactors include the following

1. How can diffusion-limited mass-transfer limitations be overcome?
2. How do the culture medium, cells and scaffold and other extracellular materials interact to produce a viable tissue construct?
3. What influence does the mechanical stimulation and biochemical environment provided by the bioreactor have on tissue growth?

The models that we review in the remainder of this chapter illustrate how mathematical models can be used to address these questions and, in so doing, improve the efficiency of cell culture systems.

2 Mathematical Modelling Approaches

As outlined above, it is clear that a wide variety of factors influences the formation of tissue, and considerable effort has been invested in elucidating the mechanisms by which cells experience and respond to these stimuli. The bioreactor system employed is specific to the tissue engineering application under consideration, and generates its own unique biochemical and biomechanical environment, tailored to the growth of a particular tissue. Such variety necessitates a range of versatile mathematical modelling approaches, reflecting the environment of the cells and the experimental questions being posed. Such theoretical models can be used to predict the flow and nutrient transport characteristics within a specific bioreactor system, and in particular to determine local information about nutrient and shear stress fields that is not straightforward (or even possible) to obtain experimentally. The resulting models may be validated against measurable experimental data, such as perfusion flow rate, outlet nutrient concentration, and then exploited to reveal details of the mechanical and nutrient fields within the bioreactor system. Once validated, the model can then be used to predict the outcome of a particular experimental scenario (limiting the need for numerous and expensive bioreactor experiments, potentially saving time and resources) and to optimise the bioreactor operating conditions.

We focus our review on the use of multiphase modelling to describe biological tissues, and present a brief overview in Sect. 2.1 below. We conclude this section with a short description of alternative modelling approaches, before describing in detail models specific for bioreactor systems in Sect. 3.

2.1 Multiphase Modelling

Biological tissue is a composite material, comprising a wide variety of interacting constituents, including, for example, a large number of different cell types, their associated ECM and other deposited materials, and interstitial fluid. Multiphase, or mixture theory, models provide a natural continuum framework within which to investigate such interactions. These models are based upon the idea that tissues may be represented by a mixture of continua, which are able to occupy the same region of space; interactions between the different 'phases' are specified via mass and force balance equations, together with appropriate constitutive relations, the choice of which allows a wide variety of physical systems to be modelled. This methodology also reflects the idea that, as composite materials, tissue properties are reflected by the relative volume fractions (and properties) of their constituents (Trelstad and Silver 1981); changes in tissue composition occur via processes such as mitosis, apoptosis, necrosis, (de-)differentiation and ECM production.

Since the study of (Treusdell and Noll 1960), an enormous number of studies have been dedicated to formulating a rigorous framework of conservation laws for

mixtures of interacting continua (Bowen 1976; Marle 1982; Passman and Nunziato 1984; Whitaker 2000; Kolev 2002; Ateshian 2007). Such approaches have been used widely to model biological tissue mechanics (Mow et al. 1980; Lai et al. 1991) and, more recently, tissue growth and remodelling (Please and McElwain 1998; Please et al. 1999; Landman and Please 2001; Preziosi and Tosin 2009; Ambrosi et al. 2010). Since the aim of this review is to highlight modelling approaches and challenges, we do not provide a historical perspective of multiphase modelling approaches, nor do we present a comprehensive derivation of relevant multiphase equations; it is markworthy, however, that although by definition such a multiphase continuum approach does not account for the precise microscopic detail of (for example) cell–cell interactions, the averaging process involved in deriving models of this type ensures that terms present in the model equations arise from appropriate microscopic considerations (see, e.g. Drew 1983). In Sect. 3, we provide some example studies which highlight modelling issues in tissue engineering and mixture theory approaches with which to investigate them. We remark that in many cases, modelling tumour growth was the original focus of these studies; the formulations are, nevertheless, relevant to tissue engineering applications.

As indicated above, the basis of these multiphase models is a set of conservation equations, governing mass and momentum transfer between the constituent phases. For a system comprising n incompressible phases, if inertial effects and body forces are negligible, then the equations governing the ith phase (with density ρ_i, volume fraction θ_i, velocity $\boldsymbol{u}_i$ and stress tensor σ_i) may be expressed:

$$\rho_i \left\{ \frac{\partial \theta_i}{\partial t} + \nabla \cdot (\theta_i \boldsymbol{u}_i) \right\} = S_i \,, \tag{1}$$

$$\nabla \cdot (\theta_i \boldsymbol{\sigma_i}) + \sum_{j \neq i} \mathbf{F}_{ij} = 0 \,, \tag{2}$$

where t denotes time, ∇ is the spatial gradient operator, S_i is the net rate of mass transfer into the ith phase and $\mathbf{F}_{ij}$ denotes the force acting on phase i as a result of interactions with phase j. We note that in some cases, flow in bioreactors is modelled by the full Navier–Stokes equation, in which case, inertial terms are retained in Eq. (2).

Within the multiphase modelling context, assumptions can be made that simplify the resulting systems of equations. For example, one approach is to assume that the cells occupy no volume, and therefore have no effect on the fluid flow. When determining the mechanical load that the flow exerts on the cells, it is assumed that the shear stress exerted on the substrate will be that experienced by the adherent cells; nutrient transport is incorporated by considering cells to be sinks or sources of metabolites or waste products. In some cases, such flow and transport problems require equations for the flow of nutrient-rich culture medium surrounding a porous scaffold to be coupled to those describing the flow within the scaffold. These equations are linked by specifying boundary conditions at the

interface between the single-phase flow domain (the surrounding fluid) and the multiphase flow domain (the porous scaffold in which both scaffold and fluid volume fractions must be considered). For example, Navier–Stokes equations may be used to describe the surrounding fluid via

$$\nabla \cdot \boldsymbol{u} = 0, \frac{\partial \boldsymbol{u}}{\partial t} + \boldsymbol{u} \cdot \nabla \boldsymbol{u} = -\frac{1}{\rho}\nabla p + v\nabla^2 \boldsymbol{u} \tag{3}$$

where $\boldsymbol{u}$ is fluid velocity, p is fluid pressure, ρ is the fluid density and v is the kinematic viscosity. The flow within the porous scaffold may be described by Darcy's law

$$\nabla \cdot \boldsymbol{u} = 0, \boldsymbol{u} = \frac{k}{\mu}\nabla p, \tag{4}$$

where k is the permeability of the porous medium and μ is the dynamic viscosity. Alternatively, the Brinkman equations may be used:

$$\nabla \cdot \boldsymbol{u} = 0, \boldsymbol{u} = \frac{k}{\mu}\nabla p - k\nabla^2 \boldsymbol{u}. \tag{5}$$

In Eqs. (4) and (5) the Darcy flux $\boldsymbol{u}$ is the fluid velocity weighted by the scaffold porosity.

Both the Darcy and Brinkman equations are examples of multiphase models that may be obtained from Eqs. (1) and (2) via appropriate choices of constitutive laws. Models of this type are reviewed in Sect. 3.2. A key aspect of the macroscale Darcy and Brinkman models is that the microscale properties are captured via a parameter at the macroscale, for example, the scaffold permeability, without the need for detailed knowledge of the pore geometry, information that is expensive to obtain, and moreover, changes from one scaffold to another. Furthermore, if details of the pore geometry *are* known, they can be incorporated into an expressions for the permeability, for example via homogenisation techniques (Shipley et al. 2009).

An alternative to the above macroscale approach when considering flow and nutrient transport problems is to consider a *microscale* one in which the fluid flow within the interconnected pores is solved using Navier–Stokes equations. Such a method, especially when complemented with imaging techniques such as μ-CT that provide detailed information about the geometry and the microstructure of the porous scaffold, is a powerful tool for the full characterisation of the 3D flow fields and stresses within dynamic culture systems. However, this can be computationally intensive, and requires a simulation to be run for every scaffold architecture. Examples of such studies are reviewed in Sect. 3.2.

As an alternative to assuming that the cells occupy no volume, they may be assumed to occupy volume, but that their interaction with the flow can be neglected (so that the role of any culture medium in the system is simply to provide a supply of nutrients to the cells). Such models, which are particularly appropriate for static culture systems where the transport of nutrients and

metabolites is by diffusion, are reviewed in Sect. 3.1. Finally, in some applications, detailed information regarding culture medium flow and cell population dynamics is required; here it is necessary to consider explicitly the cells as a separate phase (with their own volume fraction, local velocity, and so on), and to consider their interaction with the surrounding fluid flow. Examples of models using this approach are given in Sect. 3.3.

2.1.1 Mathematical Reduction

When adopting a multiphase approach (and the various modelling simplifications) the resulting continuum models comprise coupled partial differential equations (PDEs). However, their solution may be computationally intensive and may not reveal details of the mechanisms underlying observed tissue growth phenomena. An alternative approach is to exploit the typically disparate length- and time-scales inherent in these systems: for example, the bioreactor may be long and thin, or the timescale for cell growth may be long compared to that for diffusion of solutes. In the former case, the spatial dependence of the problem may be reduced, in the latter, quasi-steady assumptions may be made. Alternatively, the magnitude of problem specific parameters (such as the Reynolds number or Peclet number) may be exploited to simplify the equations. For example, the Reynolds number characterises the ratio of inertia to viscous effects, and the Peclet number the ratio of advection to diffusion timescales, and by considering these parameters to be either large or small, the governing equations may be simplified to retain only the key aspects of the underlying physics of the system under consideration. In all cases, sophisticated asymptotic methods can be used rationally to simplify the governing equations, leading to reduced models that are tractable, yet remain physically well-grounded. The reduced models can then be attacked using analytical techniques and simpler numerical methods. By allowing maximum analytical progress to be made (enabling, for instance, the influence of various governing parameters to be relatively easily investigated), the analysis of reduced models can provide significantly more physical insight into the underlying mechanisms than would be obtained by computational simulations of the full system alone. Such approaches are reviewed in Sect. 3, and contrasted with more computational studies.

2.2 Alternative Modelling Approaches

When modelling cell population growth, many authors have employed a discrete, rather than a continuum, approach and considered individual cells explicitly. Such models provide a natural framework within which to accommodate, for example, cell signalling interactions, movement and proliferation, thereby providing comprehensive and detailed information about the dynamics of the cell population. Representative studies include Ouchi et al. (2003), in which a cellular Potts

(Graner-Glazier-Hogeweg) model is employed and formal rules for cellular interactions are postulated; such a modelling approach has been widely used, capturing cell sorting phenomena as well as a range of morphogenetic processes in, for example, the development of the slime mould *Dictyostelium discoideum*. To accommodate the physics of cell–cell or cell–environment interactions, Meineke et al. (2001) incorporate linear over-damped springs connecting cell centres within the modelling framework. This approach was included in a multiscale computational model developed to investigate the role of Wnt signalling in regulating tissue renewal in the intestinal crypt by Van Leeuwen et al. (2009). The preceding models employ a deterministic approach to model cell behaviour; however, at the single-cell scale, biological processes are inherently stochastic. Biological systems are profoundly affected by such stochastic noise; for example, genetic selection may be influenced by the stochastic nature of mRNA transcription (see Wilkinson 2009 and references therein). The importance of such stochasticity to a variety of tissue growth processes has been widely investigated. Common approaches include the use of master and Langevin equations (Othmer et al. 1988; Hadeler et al. 2004).

The analysis of discrete models typically necessitates a computational approach, since realistic simulations demand large numbers of cells, for which analytic results may be difficult or impossible to obtain. In an attempt to circumvent this problem, multiscale (homogenisation) techniques have been employed to derive continuum models directly from underlying discrete systems, enabling some of these discrete effects to be incorporated into tissue-scale models in a mathematically precise way. Representative examples include Turner et al. (2004); Murray et al. (2009) and Fozard et al. (2010), who employ such techniques to represent collective movement of adherent cells within a continuum framework, while (O'Dea and King 2011a, b) analyse pattern formation due to cell signalling processes. Shipley et al. (2009) determine the macroscale flow and transport properties of a specific type of tissue engineering scaffold, in order to specify criteria for effective tissue growth. In all cases, the resulting continuum models are formulated as small systems of PDEs which may be amenable to analysis and/or numerical investigation. For these reasons, much research has concentrated on continuum representations of tissue growth, and it is models of this type for tissue engineering bioreactors that we now consider in detail below.

3 Mathematical Modelling of Bioreactor Systems

The biochemical and biomechanical cues which lead to optimum growth are specific to the tissue under consideration; correspondingly, the mathematical modelling approach required is determined by both the bioreactor system and the particular biological processes under investigation. In the following we review theoretical studies of such systems, concentrating on the following broad themes: (i) models of static culture systems which focus on biochemical effects such as

nutrient-limited growth, (ii) dynamic culture models in which the cells are viewed as point sources and sinks of metabolites and nutrients respectively, and the cells occupy no volume; and (iii) dynamic culture models in which the volume fraction of cells is finite and biochemical and biomechanical interactions with the environment are incorporated.

3.1 Modelling Static Tissue Culture

In this section we focus on mathematical models that have been developed to describe tissue growth in static culture, when the delivery of vital nutrients and removal of waste products are diffusion-limited. The motivation for many of these models, particularly those of Galban and Locke (1997, 1999a, b), stems from experiments performed by Freed and coworkers in which highly porous polymer scaffolds of varying thickness were seeded with chondrocytes and immersed in fluid containing (diffusible) nutrients such as oxygen and glucose (Freed et al. 1993, 1994). The average cell density achieved within the scaffolds was found to decrease as the thickness of the scaffold increased, leading the authors to conclude that limitations in nutrient transport, caused by an increase in cell mass, could be responsible for inhibiting cell growth. As we explain below, the continuum models of tissue growth in static culture range from phenomenological ones (Galban and Locke 1997) to multiphase models that distinguish between different components of the developing tissue (Galban and Locke, 1999a, b). Some models are cast as moving boundary problems, the free boundary typically delineating regions of the scaffold that have been colonized by cells and tissue matrix from regions that are devoid of cells and tissue matrix (Galban and Locke 1997; Wilson et al. 2007). A key and unifying feature of these models is the assumption that nutrient availability is growth-rate limiting.

3.1.1 Quasi-Steady Nutrient Distribution

In an attempt to determine whether Freed et al.'s mechanistic explanation was consistent with their experimental observations, Galban and Locke developed a series of mathematical models for the growth of chondrocytes seeded in a porous polymer matrix (Galban and Locke 1997, 1999a, b). In their initial work, two moving boundary problems were developed to study the influence of nutrient transport and consumption on cell growth (Galban and Locke 1997). They focused on a single, growth-rate-limiting nutrient (e.g. oxygen or glucose) whose consumption rate was assumed to be highest in regions containing cells. By exploiting the difference in time-scales for nutrient diffusion within a scaffold ($\sim$minutes) and cell growth ($\sim$hours), they were able to make a quasi-steady approximation, neglecting time-derivatives in the reaction–diffusion equation that defines the nutrient distribution within the porous matrix. The position of the moving

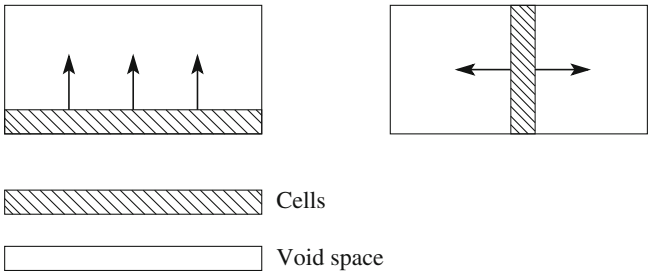

Fig. 2 In Galban and Locke (1997), moving boundary problems were developed to study the influence of nutrient transport and consumption on cell growth. The moving boundaries separate regions devoid of cells from those containing cells. Two seeding protocols were considered: in one case, cells were seeded on the scaffold periphery (where nutrient levels are highest), and in the second case the cells were seeded on a plane passing through the centre of the scaffold. The direction of cell growth is indicated by the arrows in the figure

boundary separating regions containing cells from those devoid of cells was determined by assuming that cell growth is localised at the moving boundary where it occurs at a rate which is proportional to the local nutrient concentration. This phenomenological model was used to compare two different seeding protocols: in one case, cells were seeded on the periphery of the scaffold, where nutrient levels are highest, and in the second case the cells were seeded in a plane passing through the centre of the scaffold. The direction of cell growth in each case is indicated in Fig. 2.

Numerical simulations revealed that the second case, with cells initially migrating outwards from the centre of the scaffold and in the direction of increasing nutrient levels, gave results which were in better agreement with Freed et al.'s experimental data than those obtained for the case in which the cell migrate towards the centre of the scaffold and into regions with lower nutrient levels. Good agreement was, however, only achieved for scaffolds of intermediate thickness (i.e. neither excessively thick nor overly thin). This deficiency motivated Galban and Locke to consider more detailed, multiphase models, in which the scaffold was viewed as a two-phase mixture of (effectively) cells and extracellular fluid, and cell growth was no longer localised on a moving boundary (Galban and Locke 1999a, b). In Galban and Locke (1999a), a one-dimensional geometry was employed, and the nutrient concentration assumed to vary parallel to the axis of the cylindrical scaffold only. The cell and fluid volume fractions were taken to be spatially uniform and to evolve over time, the cell volume fraction increasing at a rate which depends on the nutrient distribution within the scaffold. As in Galban and Locke (1997), the nutrient distribution was governed by a quasi-steady, reaction–diffusion equation. However the diffusion coefficient in Galban and Locke (1999a) was no longer taken to be a constant: it was assumed to decrease as the cell volume fraction increased. In this way, cell growth was found to be self-limiting: as cells proliferate, their volume fraction increases but this hinders nutrient transport through the scaffold and, hence, slows the rate of cell growth until, eventually, nutrient levels are too low to support

further growth. This behaviour was evident in their numerical results which showed the cell volume fraction increasing towards a fixed value at long times, this value depending on the system parameters and, for example, decreasing as the scaffold thickness was increased. In this respect, the one-dimensional model yielded results which were in good agreement with the experimental observations reported in Freed et al. (1993, 1994). However, the predicted changes were not large enough to match those observed in the experimental data, possibly because the cell volume fraction was assumed to be uniform throughout the scaffold. Therefore, in Galban and Locke (1999b), the two-phase model was generalised by extending it to two spatial dimensions and allowing the cell and fluid volume fractions, and, hence, the effective nutrient diffusion coefficient, to vary with position and time. For typical simulations, spatial variation in the cell volume fraction and the effective diffusion coefficient became more pronounced over time, with the cell density being maximal on the periphery of the scaffold, where nutrient levels were highest, and declining towards the centre, where nutrient levels were lowest (the diffusion coefficient exhibited the opposite behaviour, with transport inhibited where the cell volume fraction was high). The agreement between the simulations obtained from this model and Freed et al.'s experimental data was much better than that obtained for the earlier, simpler models: the model was able to capture the dynamic trends that had been observed in the spatial distribution of the cell volume fraction and nutrient concentration when the scaffold thickness was varied.

The predictions generated from Galban and Locke's work, particularly the results in Galban and Locke (1999b), highlight some of the problems associated with culturing cells in scaffolds under static conditions. For example, as the thickness of the desired tissue construct (and, hence, the scaffold) increases, limitations associated with transporting nutrient to cells at the centre of the scaffold can lead to the formation of nutrient-deprived regions which are characterised by cell quiescence and even necrotic cell death. As we explain in Sects. 3.2 and 3.3, perfusion bioreactors, in which nutrient-rich culture medium is driven through the scaffold, offer scope for increasing nutrient supply to the central regions of the scaffolds and, thereby, improving the integrity of the engineered tissue.

3.1.2 Reaction and Diffusion of Nutrients

Galban and Locke's work has inspired the development of a number of similar mathematical models. For example, Lewis et al. (2005) develop a simple, one-dimensional model to investigate interactions between the evolving nutrient profiles and cell distributions within cartilaginous tissue constructs. The nutrient profile is modelled with a reaction–diffusion equation in which the diffusion coefficient is assumed to be constant and the local rate of nutrient consumption taken to be proportional to both the nutrient concentration and the cell density. The cells are assumed to be immobile and to proliferate at a rate proportional to that at which they consume nutrients. By comparing their simulations with experimental data for the spatio-temporal evolution of the oxygen and cell distributions, they

found that their model represents a good description of cartilaginous tissue growth for the first two weeks after seeding. At later times, mechanisms not included in the model, such as contact inhibition of cell proliferation and reduced nutrient transport, are likely to become significant and may explain the poor agreement with the data after two weeks in culture.

Dunn et al. (2006) extended Lewis et al.'s model to account for contact inhibition of growth, using a logistic growth law to describe cell growth, the growth rate being proportional to the nutrient concentration, and by considering a two-dimensional cylindrical geometry. Their model was found to give good agreement with data from experiments in which pre-osteoblasts were initially seeded uniformly throughout a porous scaffold, these results being qualitatively similar to those reported by Freed et al. for chondrocytes. Dunn et al.'s model was, however, unable to reproduce experiments in which the scaffolds initially consisted of a series of thinner scaffolds that alternated between cell-seeded and unseeded: when the initial conditions were altered to mimic the layered structure, the experimentally observed peak in cell density at the interface between seeded and unseeded regions was not evident in the numerical simulations. Two possible explanations for this discrepancy were proposed. First, convective flow, which is not included in the mathematical model, may enhance nutrient transport at the boundaries between the seeded and unseeded regions. Alternatively, cell migration may not be negligible: cells may migrate away from regions of low oxygen and accumulate at the interface between the seeded and unseeded regions, where oxygen levels are higher.

More recently, Jeong et al. (2011) have focussed on developing an efficient numerical method for solving an extension of Lewis et al.'s model for the growth of cartilaginous tissue constructs in two-dimensional cylindrical geometry, in which the cells are allowed to move by random motion. Jeong and coworkers propose an operator-splitting algorithm to solve the resulting pair of coupled reaction–diffusion equations. Their approach involves alternating finite-difference approximations and analytical solutions in order to update the cell and nutrient profiles on successive time-steps. We remark that the model that Jeong et al. solve is similar in form to an earlier model by Obradovic et al. (2000) that was used to investigate how the local oxygen concentration within a cartilaginous construct influences the rate at which cells seeded within the scaffold produce glycosaminoglycan, an important component of cartilage.

The work of Chung et al. (2006) places the phenomenological reaction–diffusion models developed in Obradovic et al. (2000) and Jeong et al. (2011) on a stronger theoretical foundation. Following Galban and Locke (1999a, b), Chung and coworkers use a volume-averaging approach to derive an extension to Galban and Locke's two-phase model that accounts for a small degree of random cell movement. Their model simulations suggest that cell motility leads to more uniform cell distributions within the scaffold and higher overall rates of cell growth than when cell movement is neglected. Chung et al. also use their model to demonstrate that uniform cell seeding is likely to be a better strategy for tissue engineering (in static culture) than non-uniform seeding, arguing that concentrated

cell seeding results in competition for nutrients which, when combined with limitations in nutrient transport, will reduce the overall cell growth rate.

3.1.3 The Cells' Mechanical Environment

While nutrient availability undoubtedly plays a crucial role in regulating cell growth within tissue constructs, mechanical effects are also important. In Wilson et al. (2007), Wilson and coworkers develop a simple deterministic model of tissue growth in which the cells are initially seeded around the periphery of a porous scaffold. They assume that nutrient is freely available, and use Darcy's law to model cell movement towards the centre of the scaffold. As in Galban and Locke (1997), a moving boundary problem is introduced to delineate regions of the scaffold that have been colonised by cells from regions which are devoid of cells. The Baiocchi transformation is used to transform the model to a linear complementarity problem for which one-dimensional analytical solutions and two-dimensional numerical ones are presented. Attention focusses on the behaviour of the moving boundary as the cells reach the centre of the scaffold and the colony approaches confluence: asymptotic techniques are used to derive approximate expressions for the time to confluence and to show that, near closure, the moving boundary evolves to an ellipse and that the ratio of its semi-major and semi-minor axes is identical to that of the two-dimensional, rectangular scaffold. The pressure within the scaffold is found to increase considerably shortly before it is filled, highlighting the potential problem for tissue engineers of a "slit" persisting within the tissue construct and compromising its mechanical integrity (see Fig. 3).

3.2 Modelling Dynamic Tissue Culture: Cell Volume Neglected

As discussed in Sect. 1.1.3, since static culture systems rely on diffusive transport of solutes, it is not possible to engineer constructs of a size suitable for implantation. Dynamic culture systems exploit the flow of culture medium to enhance nutrient and waste product transport by advection. Furthermore, such systems can provide mechanical load to mechanosensitive tissues, e.g. via the application of fluid shear stress to the cells. We review here models in which the cells occupy no volume, and have no affect on the fluid flow. We start by describing computational approaches and then consider approaches in which simplifying assumptions are used to derive reduced models that may be solved using analytical or simpler numerical techniques.

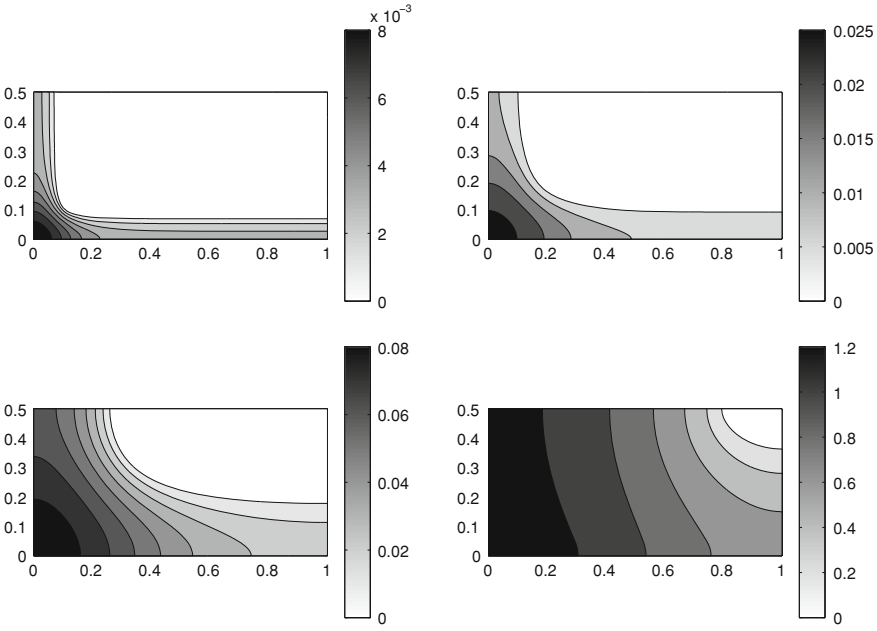

Fig. 3 Simulation results from the model developed in Wilson et al. (2007) showing how cells seeded around the periphery of a two-dimensional, rectangular porous scaffold eventually colonise the scaffold. The pressure profiles for the quadrant are plotted at dimensionless times $t = 0.5$, (*top left panel*), 1.0, 1.5 and 1.66 (*bottom right panel*), and show how the maximum pressure within the scaffold increases as the cells colonise more of the scaffold. The origin $(x, y) = (0, 0)$ is in the far corner so that the profiles may be easily viewed

3.2.1 Computational Approaches

When using computational fluid dynamics (CFD) approaches, the geometry of the system is retained (i.e. no simplifying assumptions are made e.g. due to the bioreactor being long and thin), and the reactor geometries can be generated using CAD packages. Such an approach was taken in Lawrence et al. (2008) who considered the effect of bioreactor geometry on the fluid flow through porous constructs. Rectangular and circular bioreactors were considered, with and without porous scaffolds, and with different inlet and outlet patterns. The governing equations (in this case, Navier–Stokes for the single-phase fluid, and Brinkman equations for flow through the porous scaffold) were solved using computational fluid dynamics software (COMSOL Multiphysics and ANSYS CFX). The authors identified non-ideal fluid distribution profiles by looking for "channeling" (where the fluid finds a short cut and leaves the reactor without dispersing through it), and "dead zones" which reduce the effective volume of the reactor and prolong the residence time of some fluid elements. Such non-uniform flow patterns can lead to poor nutrient distribution, and non-uniform shear stress distribution. The authors focus on fluid flow only, and highlight nutrient transfer as an open problem. Many

authors have considered similar approaches, using either Brinkman (Lawrence et al. 2008; Devarapalli et al. 2009) or Darcy constitutive laws Cioffi et al. (2008) to model flow through the porous scaffold.

In addition to the above macroscale approach, there are two alternative ways to incorporate scaffold topology into the models. In the first, imaging techniques are used to provide detailed information about the pore scale geometry, which in turn provides a realistic computational domain within which to simulate the governing model equations. As an example, Cioffi et al. (2006) reconstructed the scaffold micro-geometry from μ-CT images acquired from a sample of the actual scaffold. The steady-state Navier–Stokes equations through the domain were solved using the commercial CFD software ANSYS FLUENT, which enabled quantification of the flow field and fluid shear stresses acting on the internal walls of the matrix in a tissue engineered construct subject to direct perfusion of culture medium within a bioreactor. A second approach is to idealise the micro-scale geometry. Boschetti et al. (2006) used a simplification of the geometry of a polymeric scaffold obtained by particulate leaching. A micro-domain of the scaffold was idealised as 27 sub-units arranged in a honey-comb pattern. Each sub-unit was obtained by subtracting a solid sphere from a concentric solid cube. The aim was to predict how the shear stress experienced by the cells depends on physical quantities such as scaffold porosity, pore size, and the imposed flow rate. By solving the Navier–Stokes equations in this complex domain, subject to appropriate boundary conditions (such as no-slip), it was possible to determine the exact nature of the flow properties. In Cioffi et al. (2006), the results of the simulations using the exact detailed scaffold architecture were compared with that from a simplified micro-scale model (Raimondi et al. 2004). A key finding is that, within the parameter regime considered (low Reynolds number flow and interconnected pores), the micro-geometry of the scaffold did not affect significantly the median shear stress acting on the inner scaffold walls. Hence, for the scaffolds considered in Cioffi et al. (2006), it is not necessary to build a detailed model for each new scaffold geometry, when a simple estimation of the median, mode and mean shear stress is required. The use of 3D geometrical models simplifies the scaffold geometry and reduces significantly the cost of the computation. Cioffi et al. (2008) considered a combined macro-scale/microscale computational approach to quantify oxygen transport and flow-mediated shear stress experienced by cells cultured in three-dimensional scaffolds in perfusion bioreactor systems. The macro-scale model consisted of Darcy equations for flow in the porous scaffold and an advection–reaction–diffusion equation for nutrient transport. The micro-scale approach was based on μ-CT reconstruction of the scaffold geometry. While both modelling approaches predicted similar average oxygen levels at different depths, the μ-CT model captured the micro-scale variations associated with the scaffold architecture. Thus, the choice of modelling approach for the scaffold geometry should be motivated by the required model outputs—if estimations of average shear stress and oxygen concentration are of interest, a simplified modelling approach can be taken, whereas if precise details of the spatial distribution of shear stress and oxygen

concentration are required, then a more detailed modelling approach, where the scaffold geometry is reconstructed using μ-CT images is more appropriate.

3.2.2 Asymptotic Model Simplification

As outlined in Sect. 2.1.1, asymptotic methods can be used to simplify the governing equations, leading to reduced models that provide insights into the key underlying mechanisms involved in tissue growth within bioreactors. This approach is exemplified in Cummings and Waters (2006) (for complementary computational approaches see e.g. Lappa 2003) where a model for a rotating bioreactor is developed, in which a cylindrical vessel of circular cross-section rotates about its longitudinal axis. The bioreactor is filled with nutrient-rich culture medium and contains a growing tissue construct, which is modelled as a cylindrical solid object of circular cross-section. The axial length of the bioreactor is small relative to its radius. This fact, together with the observation that the reduced Reynolds number for the system is small, leads to a simplified model, in which the dependent variables are averaged across the axial direction, reducing the problem to two space dimensions. The rotation of the system introduces Coriolis and centrifugal force terms in the governing equations. The resulting fluid-dynamical problem is similar to that for flow around a moving circular obstacle (the tissue construct) within a rotating Hele-Shaw cell (see, e.g. Schwartz 1989; Waters and Cummings 2005). Additionally, the Peclet number is typically large in these biological applications, which simplifies the nutrient transport problem to one in which the nutrient concentration is constant along the two-dimensional fluid flow streamlines, with diffusion taking place perpendicular to the streamlines.

The authors exploit the fact that fluid flow, nutrient transport, and construct growth occur on very different time-scales, and decompose the problem into four distinct stages. Firstly, the fluid flow around the tissue construct is determined, assuming its location within the bioreactor is known. Then the position of the construct is determined by considering the balance of forces acting on the construct. Two classes of periodic motions are found, distinguished by whether or not the centre of the construct orbits the bioreactor centre (a special subcase of the latter being where the tissue construct remains stationary in the laboratory frame of reference). The fluid flow and construct trajectory model were validated in Cummings et al. (2009) by comparing the theoretical results with an experimental study of the trajectory of a large solid cylindrical disc suspended within a fluid-filled rotating vessel. All three flow regimes described above were observed experimentally, and good agreement between experiment and theory was found.

Having successfully solved the fluid flow problem, the nutrient distribution around the construct was then found, assuming a fresh supply of nutrient is maintained at the bioreactor's circular boundary and that at the tissue construct boundary nutrient is taken up at a rate proportional to how much is available in the surrounding culture medium. Using the nutrient concentration solution, and

assuming the cells grow in response to the nutrient field, the growth rate of the construct was determined.

The theoretical study led to the important biological insight that if the construct position is stationary when viewed from the laboratory frame, then a nutrient depletion zone will form in the neighbourhood of the construct. Thus it is more desirable to operate the bioreactor in a regime in which the construct undergoes a time-dependent trajectory when viewed from the laboratory frame, mixing the nutrient concentration as it goes, and eliminating the development of nutrient depletion zones.

Still motivated by tissue growth in rotating bioreactors, Waters et al. (2006) considered a two-region, single-phase model to determine the morphology of a tissue construct formed from a single-cell suspension in culture media, within a rotating bioreactor. Experimental results indicated that at rotation rates below a critical value, the cells 'self-assemble' to form smooth nodules which are approximately elliptical in cross-section. However, at higher rotation rates, an amorphous construct forms with a highly irregular boundary. Histological studies show that the construct consists of a fluid interior which is a mix of apoptotic cells and culture media, surrounded by a membrane of proliferating cells and collagen. Motivated by these observations, Waters et al. (2006) consider a more dense viscous fluid drop surrounded by an extensible membrane of constant membrane tension in a less dense immiscible viscous fluid within a rotating bioreactor system. Both thin-disc and slender-pipe bioreactors (for which the bioreactor aspect ratio is small or large respectively) were considered so that, similarly to Cummings and Waters (2006), the fluid-dynamical equations may be simplified leading to a series of spatially 2D problems. The authors consider the interfacial stability of the initially circular fluid-fluid interface to small-amplitude, oscillatory perturbations; the instability arises as a result of the competition between the destabilising effects of centrifugal forces, and the stabilising effects of surface tension. The theoretical results indicate that culture within thin-disc bioreactors is more likely to result in irregular shape constructs than culture within slender-pipe bioreactors, and that in the thin-disc regime the wave number of the most unstable mode increased as the rotation rate increases, in line with experimental observations. Of course, the instability mechanism examined in Waters et al. (2006) is not the only one by which irregular constructs might be expected to arise, and the modelling highlighted here does not include any biochemical features, e.g. growth in response to nutrient fields, or cell proliferation, in the model.

In Cummings and Waters (2006) bioactive processes that modify the porous scaffold are neglected—rather the construct is modelled as an impermeable solid object. However, a number of studies have explicitly accounted for the properties of the porous scaffold and their effect on the fluid flow and nutrient transport. In Whittaker et al. (2009) a simple mathematical model is developed for forced flow of culture medium through a porous scaffold. Flow is forced through the scaffold via inlet and outlet pipes, and also through identical porous-walled fibres inserted through the scaffold: fluid is pumped into one end of each fibre and the other end is sealed so that the fluid is forced to travel through the porous fibre walls into the

scaffold. There are therefore two flow domains—the fibres and the space occupied by the scaffold around them—and the flows are coupled via continuity conditions at the interfaces. The scaffold is modelled as a uniform isotropic porous medium, with a point source and point sink modelling the inlet and outlet pipes respectively, and line sources representing flow from the fibres. A separate fluid dynamical problem is solved within the fibres to compute the strength of the source terms in the line sources representing flux of fluid from the fibres into the scaffold. Again, geometric features are exploited to simplify the governing equations: the slender geometry of the fibres allows lubrication theory to be used to simplify the full Navier–Stokes equations. A Poisson problem is obtained for flow in the scaffold, which can be solved numerically, and is a much easier to solve than direct numerical simulation of the full Navier–Stokes equations within the complex scaffold geometry. Having solved for the fluid flow, the authors were then able to determine the distributions of shear stress, nutrients and waste products, and asses the implications for cell proliferation.

3.2.3 Bioreactor Design

In a series of papers (Shipley et al. 2010, 2011; Shipley and Waters 2011), Shipley and co-workers consider flow and nutrient transport problems in hollow fibre membrane bioreactors (HFMB). HFMBs are designed to enhance nutrient delivery to, and metabolite removal from, cells by using fluid flow to provide advective transport in addition to diffusion. A single hollow fibre consists of a central lumen, surrounded by a porous fibre wall or membrane, which separates the lumen from the extra-capillary space (ECS). Cells are seeded in a single layer on the fibre walls, or throughout a matrix surrounding the fibre. Fluid is driven through the fibre lumen under the action of an applied pressure gradient. The porous fibre wall allows the passage of nutrients, metabolites and growth factors both to and from the cells, and acts as a membrane to protect the cells from the direct effect of fluid shear due to flow through the lumen, thus enabling relatively high flow rates to be used without cell damage. A bundle of such fibres is then housed within a bioreactor: in addition to flow through the fibre lumen, the bioreactor has entry and exit ports for ECS flow. By developing a series of theoretical models, Shipley et al. were able to specify a set of operating conditions which the end user can use to prescribe the bioreactor geometry (e.g. fibre length and ECS depth) and operating parameters (e.g. pressures, flow rates, nutrient inlet concentrations, cell-seeding density) to obtain the optimum cell culture environment for the tissue under consideration.

In Shipley et al. (2010) theoretical predictions for the fluid retentate (lumen outlet flowrate) and permeate (ECS outlet flowrate) are derived, and compared against experimental data to determine the membrane permeability and slip. Fluid transport in the lumen and ECS is described using Navier–Stokes equations, and flow through the porous fibre walls using Darcy equations. The model is simplified by exploiting the slender geometry of bioreactor system, so that lubrication theory

may be used to describe flow in the lumen and ECS. Analysis of the width of the boundary layer region where slip effects are important, together with the sensitivity of the retentate and permeate equations to the slip parameter, showed that slip was not significant for these membranes. The model was then further reduced by assuming no-slip conditions at the membrane surface, and comparison of the theoretical predictions for the retentate and permeate with the experimental data enabled the membrane permeability to be determined. The validated and parameterized model was then used to determine the operating conditions that enable the lumen inlet flowrate and pressure at the lumen outlet to be controlled to provide a specific permeate to lumen inlet flowrate ratio. In Shipley et al. (2011) the complementary nutrient transport problem was studied: although the modelling was for a generic nutrient, the parameters were specialised for oxygen. Here fluid flow in the lumen was modelled by Poiseuille's law, while membrane and ECS flow were neglected. Nutrient concentration was governed by an advection–diffusion equation in the lumen, a diffusion equation in the membrane, and an advection–reaction–diffusion equation in the ECS, with Michaelis–Menten kinetics for the reaction term. These equations were coupled via specification of continuity of concentration and flux at the lumen-membrane and membrane-ECS boundaries. Analytical progress was made in the limit in which the ECS oxygen concentration is much larger than the half-maximal concentration of oxygen, so that the Michaelis–Menten reaction term can be approximated by a constant. These solutions were complemented by computations of the full system of equations, using COMSOL. The study enabled operating conditions to be defined to enable the user to determine the medium flow rate, lumen length and ECS depth that ensures a minimum oxygen concentration throughout the HFMB. In Shipley and Waters (2011), this work was extended to consider flow throughout the HFMB and the transport of lactate, in addition to oxygen. The study indicated that oxygen (as opposed to lactate) is the limiting solute with respect to bioreactor design, and that opening the ECS port to promote radial flow through the bioreactor provides significant benefit, enabling a greater volume of cells to be cultured within the desired nutrient environment.

All the models described above consider timescales appropriate to transport processes rather than cell proliferation, differentiation etc, and neglect neo-tissue formation, so that, for example, the effect of cell proliferation on the scaffold permeability and hence the resulting fluid flow is not considered. In Sect. 3.3 we consider models which account for the evolution of cell volume fraction, and the full coupling between the cell dynamics and their environment.

3.3 Modelling Dynamic Tissue Culture: Multiphase Phase Flow Models

As discussed in Sect. 3.2, a simple study of perfusion within porous tissue constructs was presented by Whittaker et al. (2009); here, the scaffold geometry is fixed, and the effects of cell proliferation on scaffold porosity, and hence fluid flow and nutrient transport fields, are neglected. Multiphase models which consider these features include Coletti et al. (2006) and Causin and Sacco (2011). In these studies, the dynamics of a cell seeded porous scaffold incubated in a perfusion bioreactor were considered. The scaffold was modelled as a rigid porous material, saturated with a viscous fluid. To account for cell proliferation, the porosity of the rigid scaffold is defined to be the sum of a time-invariant component (the porosity of the decellularised porous scaffold) and a time-dependent component (due to the proliferation of the cells); that is, the cell phase is assumed to be immobile, and with identical material properties to the scaffold phase. By appealing to the separation of timescales between flow and cell proliferation, the momentum balance equation for a viscous culture medium is simplified by assuming that fluid flow may be modelled by the Brinkman equations. Occlusion of scaffold pores due to cell proliferation and its influence on the flow field is accommodated via a cell density-dependent Darcy permeability of Carman-Kozeny type. The influence of this flow field on the distribution of a passive nutrient is modelled by reaction–diffusion–advection equations; nutrient consumption by the cells is modelled by Michaelis–Menton kinetics. Cell proliferation in response to this nutrient field is described by a Contois equation.

A more comprehensive study, investigating such ideas is presented by Chung et al. (2007). Here, cell movement is accommodated via linear diffusion, the fluid flow and nutrient fields being modelled in a similar manner to that described above. The cell density and nutrient profiles were calculated by numerical simulation, to demonstrate that perfusion of cell cultures can lead to enhanced proliferation and a more spatially-uniform cell distribution. Shakeel et al. (2011) extend this approach by employing nonlinear diffusion to represent cell movement, and by accommodating the influence of the mechanical environment on cell proliferation. Such a change in cell phenotype may be accommodated by suitable specification of the mass transfer rates S_i; Shakeel et al. (2011) capture the dependence of cell proliferation and nutrient consumption on fluid shear stress by adapting functional forms proposed by O'Dea and coworkers (reviewed below) to describe enhanced proliferation and nutrient consumption when cells are exposed to physiologically-relevant levels of fluid shear stress. By employing the commercial finite element software COMSOL Multiphysics, the influence on eventual tissue construct composition of different scaffold porosity distributions, and cell seeding protocols was investigated. The authors conclude that an effective means to ensure nutrient delivery to large tissue constructs in such bioreactors is through the use of high-porosity channels which span the construct.

3.3.1 Cell-Environment Interactions: Momentum Transfer

The above authors dramatically simplify the momentum transfer between phases by employing the Darcy or Brinkman equations to relate the fluid flow to the fluid pressure. A comprehensive multiphase formulation relevant to tissue growth processes within a perfusion bioreactor system, which addresses in detail the interactions between phases was presented by Lemon et al. (2006). The modelling framework accommodates an arbitrary number of fluid phases (representing, for instance, cells and culture medium), contained within a porous medium (such as an artificial scaffold and/or ECM). The model differs from other fluid-based models (such as Breward et al. (2002), Franks and King (2003) and references therein) by the explicit consideration of a tissue's solid constituents (here, interpreted as a porous scaffold and/or ECM), and the general nature of the interphase interaction terms, which extends the range of tissue engineering applications that can be addressed. In general, the formulation allows for deformation of the porous medium; however, in Lemon et al. (2006), attention is restricted to a rigid porous scaffold and as such, the stimulation provided by mechanical bioreactors (e.g. the bioreactor system of El-Haj et al. 1990) may not be accommodated. The adoption of fluid-like constitutive assumptions for the phases contained within the porous scaffold, which is appropriate on the timescale of tissue growth, simplifies significantly the modelling of tissue growth processes since mass transfer between phases (representing tissue growth) does not generate stress, as is the case when elastic constitutive modelling assumptions are made.

Referring to Eq. (2) in Sect. 2.1, the interphase force terms $\mathbf{F}_{ij}$ are assumed to comprise contributions from viscous drag, accommodated via terms proportional to the difference in phase velocity, and active forces (such as those which arise from interphase interactions), these being modelled via additional pressure contributions (Lemon et al. 2006):

$$\mathbf{F}_{ij} = p_{ij}\left(\theta_j \nabla\theta_i - \theta_i \nabla\theta_j\right) + k\theta_i\theta_j(\boldsymbol{u}_j - \boldsymbol{u}_i); p_{ij} = p + \psi_{ij}; j \neq i \tag{6}$$

$$p_i = p + \phi_i + \sum_{j\neq i} \theta_j \psi_{ij} \tag{7}$$

where p_{ij} denote the 'interphase pressures' which are assumed to comprise a contact-independent pressure, p, common to all phases in the mixture, and an additional term ψ_{ij} which accounts for the effect of tractions between phases (e.g. cell–ECM interactions). The pressure p_i of each phase accommodates an additional internally-generated pressure ϕ_i (due to cell–cell interactions, for example).

The multiphase approach embodied by Eqs. (1), (2), (6) and (7), together with suitable constitutive assumptions about σ_i and S_i, have been employed by many authors to investigate a range of tissue engineering problems. In each case, the relevance to the biological system under consideration is ensured by restriction to an appropriate numbers of phases, and by specification of σ_i, S_i, and the extra pressures ϕ_i and ψ_{ij}.

For example, Lemon and coworkers specialise the modelling framework to consider two fluid phases (cells and culture medium being modelled by a viscous fluid and an inviscid fluid, respectively) contained within a rigid porous scaffold, employing the resulting model to investigate proliferation, aggregation, dispersal and travelling wave behaviour of a population motile cells within an artificial scaffold. In Lemon et al. (2006), linear stability analysis was employed to determine how aggregative or dispersive behaviour depends upon the model parameters; in Lemon and King (2007b), by considering travelling wave solutions, taking distinguished limits of certain model parameters (such as the viscous drag between the cell and scaffold phases) and obtaining numerical solutions in one spatial dimension it was shown that this model displays a wide range of travelling wave behaviour. Notably, both backward and forward-travelling waves may exist, an unusual feature of models for tissue growth. By introducing a generic passive solute whose spatio-temporal dynamics are governed by an advection–reaction–diffusion equation, the influence of nutrient limitations on tissue growth was considered in Lemon and King (2007a). In addition, different initial cell seeding strategies were studied, and spatial heterogeneity of the scaffold was accommodated in a limited sense by prescribing a scaffold density distribution which is constant except near the periphery where it tapers to zero. Numerical simulation (in one spatial dimension), as well as simplifying asymptotic limits, indicate preferential tissue growth near the scaffold's periphery due to nutrient depletion by the cells; reduction of cell-scaffold drag ameliorates this feature.

These models reveal that scaffold properties (drag and porosity) are key determining factors affecting the expansion of the cell population to colonise the scaffold, and provides a methodology with which to determine model parameter regimes in which the cell population is able to colonise uniformly the scaffold.

In the above studies by Lemon and coworkers, attention was restricted to a single cell phase, which was assumed to proliferate at a constant rate (or at a rate proportional to the local nutrient concentration). O'Dea et al. (2008, 2010) employed the general multiphase formulation (1), (2), (6) and (7) to investigate the ability of different mechanical stimuli to influence tissue construct growth. By suitable specification of the mass transfer rates S_i (and employing viscous constitutive assumptions for both the cell and culture medium phases), progression between three different cell phenotypes was considered: (i) proliferative, (ii) ECM-depositing and (iii) apoptotic. Guided by experimental studies (Roelofsen et al. 1995; Klein-Nulend et al. 1995a; You et al. 2000; Bakker et al. 2004b; Han et al. 2004; Yourek et al. 2004), simple functional forms were proposed to model phenotypic changes in response to cell density (reflecting contact inhibition), and culture medium pressure and shear stress (reflecting the accepted influences of these stimuli on bone tissue growth). This approach was also exploited in the study of Shakeel et al. (2011), described above. Numerical simulations presented in O'Dea et al. (2010) indicated that the mechanical stimuli to which the cells respond can alter significantly the composition and morphology of the resulting tissue construct. This suggests that, in real applications, the histology of engineered tissue constructs

may be employed to infer the dominant regulatory mechanisms at play in a given cell line.

3.3.2 Tissue Engineering Scaffolds

While the studies of Lemon, O'Dea and coworkers accommodate explicitly the momentum transfer between phases (unlike that of Shakeel et al. 2011) the models are significantly simplified by assuming that the tissue construct's solid components (scaffold, ECM) are rigid, spatially-homogeneous and constant in time, despite experimental evidence to the contrary (Lemon and King (2007a) consider spatial variation in scaffold density only near the scaffold periphery). By including additional mass conservation equations of the form (1) to govern the scaffold and ECM density distributions and employing experimental data to initialise the scaffold distribution, O'Dea et al. (2012) have shown that, due to cell-scaffold adhesion, spatial variations in scaffold density may lead to significant heterogeneity in cell and ECM distributions, with implications for their mechanical suitability for implant. In addition, it was highlighted that the model simulation results can be employed to demonstrate how rates of scaffold degradation and ECM deposition may be matched in order to maintain mechanical integrity of tissue constructs.

Despite the general nature of the multiphase formulation (1), (2), (6) and (7), all of the foregoing investigations employ significant modelling assumptions, or exploit suitable asymptotic limits, to simplify the governing equations, leading to reduced models that are analytically tractable or more straightforward to solve numerically. Lemon and co-workers study linear stability, travelling waves and numerical solution in one spatial dimension; Coletti et al. (2006) and Causin and Sacco (2011) consider an immobile cell phase; Chung et al. (2007) and Shakeel et al. (2011) simplify the momentum transfer between phases; and O'Dea and co-workers consider the simplifying limit of asymptotically-small tissue construct aspect ratio to obtain a one-dimensional model and calculate travelling wave and numerical solutions. To determine the applicability of such geometric simplifications, Osborne et al. (2010) use finite element methods to construct two dimensional solutions for bioreactors of varying aspect ratios. Comparison with results obtained by O'Dea et al. (2010) in the long-wavelength limit, demonstrated that the simplified model captures the majority of the qualitative behaviour; however, to capture accurately mechanotransduction-affected tissue growth, spatial effects in (at least) two dimensions are required.

The aforementioned studies employ the general multiphase model (1), (2), (6) and (7) in various incarnations to investigate in detail cell–cell and cell–substrate interactions. However, in all cases, the scaffold phase is modelled as a rigid porous medium. Deformation of the tissue construct forms a key feature of many mechanical bioreactor systems (in the specific example outlined in Sect. 1.1, this is provided by macroscale compression of the scaffold by a piston). This can be accommodated within the multiphase framework by appropriate choice of the

constitutive law for the solid component(s) of the tissue. Furthermore, if the tissue stresses induced by volumetric growth are of interest, as is certainly the case for the engineering of soft tissues, fluid-based constitutive choices for the cell phase are inappropriate, since such stresses will dissipate (Araujo and McElwain 2005b).

Among the first multiphase formulation to accommodate such ideas, Roose et al. (2003) presented a poroelastic model for the growth of an avascular tumour spheroid in which the tissue comprises a porous solid phase, saturated by a fluid whose flow is governed by Darcy's law. The stress tensor for poroelastic materials (Biot, 1941) was modified by the addition of a term accommodating volumetric tissue growth. Byrne and Preziosi (2003) provide a more thorough derivation of equations appropriate to model tissue as a deformable porous media (comprising a solid and a liquid phase), as well as considering explicitly environmental influences on tissue growth.

A comprehensive derivation for a general *n*-phase mixture is provided by Araujo and McElwain (2005a, b), who state conservation equations for mass, linear and angular momentum, and energy; detailed application to a spherically-symmetric tissue represented as a biphasic mixture of linear-elastic solid (cell) phase, saturated with an inviscid fluid was given. Such a constitutive assumption leads to singular behaviour in stress evolution (Jones et al. 2000), typically regularised by the addition of viscosity to the elastic constitutive law (so that the tissue is modelled as a poroviscoelastic material); Araujo and McElwain show that anisotropic growth in the direction of least compressive stress is an alternative method for regularising growth-induced stress. Preziosi and Tosin (2009) present a similar multiphase formulation accounting for two different cell types, ECM and tissue fluid.

Many other authors have given extensive consideration to the derivation of multiphase models of tissue growth which can accommodate deformation and flow in a physiologically-accurate way (see e.g. Loret and Simões (2005); Ricken and Bluhm (2010) and references therein); however, the complexity of such models means that most studies are restricted to biphasic mixtures, or employ simplifying constitutive laws and/or geometries. An alternative approach is to exploit a CFD approach (see Sect. 3.2) to determine 3D characterisation of the flow and nutrient transport within cell culture systems. Examples include Kwon et al. (2008) and Consolo et al. (2011), in which the optimisation of conditions for embryonic stem cells encapsulated within hydrogel beads was considered, when cultured in a rotating bioreactor (see Sect. 1.1.3). Advection and diffusion of oxygen were coupled to comprehensive multiphase fluid dynamics calculations to investigate how the rotation speed ensures proper oxygen supply, while maintaining a low-stress condition. Theoretical predictions of optimal rotation speed were obtained, ensuring oxygen delivery to the cells while avoiding excessively dense bead packing and collision with the bioreactor walls. These were employed in in vitro experiments showing that the bead motion adheres to the in silico analysis and that such a dynamic culture strategy shows distinct benefits over static culture in terms of cell number and viability.

4 Discussion

In vitro tissue engineering is an emerging field of enormous importance, with the potential to alleviate the current shortage of tissues and organs for transplant, which are required for successful clinical intervention in problems associated with tissue damage, degeneration or failure. In addition, engineered tissues have applications in toxicology testing and drug screening.

Tissue engineering is an intrinsically interdisciplinary field; the complexity of the biological and biochemical processes involved obfuscates investigation by experimental biologists alone. Theoretical contributions from applied mathematics can provide important insight into tissue growth and clarify how the different processes interact. In this review, we have provided an exposition of the contribution of continuum mathematical modelling to the generation of new tissues from cells seeded on porous scaffolds. The mathematical models generate insight into nutrient-limited growth in static culture, and the dual role of fluid flow in enhancing nutrient transfer to the growing tissue construct, and in providing a source of mechanical stimulus to the cells e.g. via fluid shear stress.

It is clear that significant advances in tissue engineering have been made in recent years and that mathematical modelling is starting to play a central role in the design of bioreactors and the interpretation of the resulting experimental data (see, e.g. Shipley et al. (2011), Shipley and Waters (2011), Shipley et al. (2009), Cinar et al. (2003), Julien and Whitford (2007) and references therein). In spite of this progress, many open problems remain to be addressed. We outline below some of the theoretical, computational and modelling challenges that lie ahead.

- Appropriateness of continuum limit

As stated above, the mathematical models that we have reviewed typically treat the developing tissue and its constituents as continua and, as a result, do not distinguish between individual cells. When studying the initial growth phase of cells seeded within a tissue construct, it is natural to question the validity of adopting a continuum approach, particularly if the initial cell seeding density is low. In such cases, it may be more appropriate to use discrete, cell-based models of the type developed by Chung et al. (2010) and Cheng et al. (2009). In Cheng et al. (2009), the model has three components: a reaction–diffusion equation for the nutrient concentration, a cellular automata model describing cell migration, proliferation, and collision, and rules relating cell division rates and migration speeds to nutrient concentration. The hybrid discrete-continuous model was solved to study how transport limitations affect the tissue regeneration rates under conditions encountered in typical bioreactors. In Chung et al. (2010) a similar hybrid cellular automata approach is used to investigate the effect of nutrient-limitation on cell construct development for cartilage tissue engineering. The model was used to identify seeding strategies that result in enhanced cell number and a uniform cell distribution for the tissue engineered construct.

As a further example, The EU funded FP6 project Complex Automata Simulation Techniques (COAST) aims to develop computational methods to solve multi-scale models where a number of single-scale models interact on different length and timescales and pass information between themselves. The approach involves coupling of single-scale cellular automata or agent based models. The success of the approach has been demonstrated in the modelling of in-stent restenosis within blood vessels.

Discrete, cell-based descriptions, such as cellular automata models, are well suited to problems requiring the incorporation of cell signalling processes or subcellular phenomena, including the cell cycle and signal transduction pathways: see, for example, Van Leeuwen et al. (2009) and Alarcon et al. (2010). However, it is not at present clear how to incorporate the influence of mechanical forces into such a formulation; in this case, treating the cells as deformable objects is more appropriate. For example, by following Drasdo and coworkers (Drasdo and Hohme 2005; Byrne and Drasdo 2009) and viewing the cells as homogeneous, isotropic, elastic objects (or ellipsoids in 3D), it should be possible to compute the stresses and strains experienced by individual cells and relate them to the subcellular response that such mechanical stimuli elicit (Mullender et al. 2004).

- Hybrid models

In practice, as cells proliferate, the computational effort needed to track individual cells quickly becomes prohibitive. In such situations, when cell numbers are large, it may be more appropriate to resort once again to a continuum approach. While there are now a number of alternative approaches for modelling at the cell- and continuum scales, it is less clear how to transition between the two approaches or how to relate parameters appearing in the continuum models to those that appear in cell-level ones. A possible resolution to the latter problem is outlined in Byrne and Drasdo (2009): simulations of an individual-based model of avascular tumour growth, parameterised by measurable biophysical quantities, are compared with simulations from a continuum mechanical model and, in this way, parameters in the continuum model related to measurable quantities. The problem of deciding when it is appropriate to switch from a continuum to a discrete description (or vice versa) is considered in Kim et al. (2007). The authors propose a hybrid model for the growth of an avascular tumour embedded within a deformable gel. A cell-based approach is used in the outer annulus of the tumour, where nutrient levels are high and the cells are proliferating, while continuum descriptions are used for the gel surrounding the tumour and the central core of the tumour, where nutrient levels are low and the cells undergo necrosis. Careful consideration is given to the appropriate coupling of these representations at the boundary between the continuum and discrete domains.

- Computational challenges

The systems of governing equations arising from multiphase descriptions of biological tissues are complex: to solve these equations in the complex 2D and 3D geometries typically encountered within bioreactor systems requires the development

of sophisticated numerical schemes. To this end, Osborne and Whiteley (2010) consider a numerical technique specifically for the solution of the multiphase flow equations. They demonstrate that these equations can be written as a mixed system of PDEs, consisting of first-order hyperbolic equations for the volume fraction of each phase, generalised Stokes equations for the velocity of each phase, and elliptic PDEs for the concentration of nutrients and messengers. This complex system of coupled nonlinear PDEs is solved via the development of finite element techniques.

As the mathematical and computational models being used to simulate tissue growth become more detailed and complex, and as they are extended by different researchers, it becomes increasingly important that the underlying software is robust, reliable and fully tested. Several groups are developing software to tackle such problems. For example, the software Cancer Heart and Soft Tissue Environment (CHASTE) has been developed and is continually being advanced to solve multiscale and multiphase problems in areas of physiology that encompass cardiac, cancer and tissue engineering applications (Pitt-Francis et al. 2000).

- Modelling challenges

Within the context of multiphase modelling, a key challenge is the specification of appropriate constitutive laws for the material properties of the constituent phases, and their interactions via interphase mass and momentum transfer. Current modelling approaches, such as O'Dea et al. (2010), have proposed simple candidate constitutive laws and have shown how the characteristic tissue morphologies that arise depend on the mechanical stimuli: by comparing model predictions with experimental data, it should be possible to determine the dominant regulatory stimuli for a given cell line. However, in order to account accurately for the material properties of the tissue construct in question (highlighted as a key consideration in in vitro tissue engineering in Sect. 1.1.2), simplified constitutive assumptions such as those employed by O'Dea et al. (2010) and Osborne et al. (2010) are inappropriate. Detailed modelling of growth-induced stresses and tissue construct deformation is required. Such approaches necessitate a dramatic increase in model complexity (see e.g. Loret and Simões 2005; Ricken and Bluhm 2010) and are therefore heavily reliant on numerical simulation, or model simplification in order to make analytic progress.

Once appropriate models have been developed for the bioreactor culture system, the models must be parameterised for the particular tissue type under consideration e.g. bone, cartilage or cardiac tissue. Such model parameterisation requires ongoing and close collaboration between modellers and experimentalists, in order that the parameters of interest can be identified and successfully measured. A significant barrier to the development of biologically realistic and powerful models is the generation of suitable experimental data against which they can be validated. Since the engineering of a tissue construct is a dynamic process, and it is often technically difficult (or infeasible) to track a single experiment, the collection of reliable quantitative data is an extremely difficult problem. An important output of theoretical models is detailed information regarding the spatio-temporal distributions of (e.g.) stress, cell density, or nutrient levels within evolving tissue

constructs: since this data is difficult, or impossible, to obtain experimentally, it is an extremely challenging problem to validate the developed theoretical models against experimental data. However, by validating key features of the model for which suitable data may be procured, we may employ such models to provide detailed spatio-temporal information with confidence. Such an approach was demonstrated in Cummings et al. (2009) (see Sect. 3.2): here the authors validated the trajectory of a tissue construct within a rotating bioreactor by comparing theoretical and experimental results, and, having successfully done this, were then able to use the model to provide detailed spatial information about the nutrient field surrounding the tissue construct.

- In vitro to in vivo—integration and vascularisation

The models reviewed thus far have focussed on the in vitro generation of tissue constructs rather than their integration with normal tissue when they are implanted into patients. Recently, Lutianov and coworkers have formulated a mathematical model, comprising a system of nonlinear reaction–diffusion equations, to describe the in vivo regeneration of cartilage by isolated chondrocytes and/or mesenchymal stem cells that are seeded into a defect in the knee (Lutianov et al. 2011). Model simulations suggest that it takes around eighteen months for chondrocytes to regenerate a typical defect, of length 10–20 mm and thickness 2–3 mm. The authors also use their model to demonstrate that mesenchymal stem cells are no better at regenerating cartilage than chondrocytes. Landman and Cai (2007) have also used mathematical modelling to study the integration of tissue constructs into host tissues. A key aspect of integration is the development of an appropriate vascular supply to the engineered tissue (Novosel et al. 2011). We do not thoroughly review theoretical models for the vascularisation of engineered tissues here, but refer the interested reader to Landman and Cai (2007) and Lemon et al. (2009). In contrast to Lutianov et al. who consider avascular tissues, Landman and Cai develop a model to investigate the feasibility of stimulating the formation of new blood vessels within the tissue construct to prevent the development of nutrient-starved regions developing within the tissue construct. Lemon et al. (2009) described the evolution of different tissue constituents within an artificial scaffold, including vasculature, via a set of coupled non-linear ordinary differential equations. Bifurcation analysis was used to determine the extent of scaffold vascularisation as a function of the parameter values. The development of models which accommodate the formation and establishment of a vascular supply is a challenging open problem in the field: sophisticated models already exist describing angiogenesis in, e.g. wound healing and cancer, and it will be instructive to draw upon these models when considering vascularisation of tissue engineered constructs (Owen et al. 2009; Anderson et al. 2012).

In conclusion, despite, or perhaps because of, the many challenges outlined above, it is clear that tissue engineering is an exciting multidisciplinary field which is raising many demanding biological questions that can serve as the basis for fascinating, and equally demanding, mathematical problems for many years to come. Continued dialogue between mathematical modellers and tissue engineers,

biologists, clinicians and a variety of other experimental scientists will be crucial to the resolution of these problems and should lead to wider recognition that mathematical modelling can be used as a powerful tool to advance understanding of tissue engineering.

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