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The influence of hydrostatic pressure on tissue engineered bone development

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HIGHLIGHTS

- Novel model of the bone mineralisation response to an applied hydrostatic pressure.
- Three candidate constitutive forms of the pressure dependent response developed.
- A qualitative comparison to experimental observations is made.
- Qualitative agreement with experimental data for one response developed.
- Illustration of the importance of the cell "memory" and cell "recovery time".

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ABSTRACT

The hydrostatic pressure stimulation of an appropriately cell-seeded porous scaffold within a bioreactor is a promising method for engineering bone tissue external to the body. We propose a mathematical model, and employ a suite of candidate constitutive laws, to qualitatively describe the effect of applied hydrostatic pressure on the quantity of minerals deposited in such an experimental setup. By comparing data from numerical simulations with experimental observations under a number of stimulation protocols, we suggest that the response of bone cells to an applied pressure requires consideration of two components; (i) a component describing the cell memory of the applied stimulation, and (ii) a recovery component, capturing the time cells require to recover from high rates of mineralisation.

1. Introduction

In vitro tissue engineering is a method for creating functional tissue and organ samples external to the body, with the aim of replacing damaged or diseased tissues and organs (Rose and Oreffo, 2002; Martin, 2004). Our particular focus is on bone tissue engineering. By using autologous cells (donor and recipient being the same person), often seeded onto or into a scaffold which acts as a template for the developing tissue, tissue engineered products have many advantages for the replacement or treatment of damaged or diseased bone over traditional approaches, such as either bone grafting or non-living prostheses. The quantity of autologous bone that can be harvested for a bone graft is limited and the surgical procedures involved have a high risk of complications, while there can be problems with rejection and infection during allogeneic (donor and recipient being different people) bone grafting (Dimitriou et al., 2011; Schroeder and Mosheiff, 2011). Non-living prostheses, for example metallic or ceramic implants, are not able to easily biologically integrate into the surrounding tissue. Moreover, they have different mechanical properties to that of bone that can lead to weakening at the bone–implant interface, and they can require surgical revision after several years of use (Schroeder and Mosheiff, 2011). The engineering of functional bone tissue implants is an alternative strategy to replace bone, and is free from some of these risks and

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disadvantages. However, to date, only simple avascular tissues have been successfully engineered to a standard appropriate for their use *in vivo* (Orlando et al., 2011; Wong et al., 2010). Research into methods for increasing the quality and quantity of tissue engineered products is essential. This requires a more detailed understanding of the processes involved during tissue development.

The development of the growing tissue construct, a term used to describe the combination of scaffold, cells, extracellular matrix and fluid, often occurs within a bioreactor. The use of a bioreactor enables precise control of the biophysical and biochemical environment experienced by the construct during growth (Rauh et al., 2011; El Haj and Cartmell, 2010; Yeatts and Fisher, 2011). Cells respond to both biomechanical and biochemical cues and thus need to be subjected to the correct mechanical and biochemical environment to function appropriately (El Haj et al., 2005). This is particularly important in the development of mechanosensitive tissues, such as bone (Mullender et al., 2004).

Bone tissue consists of three main components: mineralised bone matrix, cells and interstitial fluid. The mineralised bone matrix consists of an organic matrix along with solid inorganic mineral, mostly in the form of hydroxyapatite (Buck and Dumanian, 2012). The process of mineralisation of the matrix involves the conversion of soluble inorganic ions, dissolved in the bone fluid, into solid apatite crystals deposited on the collagen to form a composite which gives bone its ability to withstand loading forces (Clarke, 2008).

Within the body, bone tissue growth and regulation is coordinated by three main cell types: osteoblasts, osteoclasts and osteocytes. Osteoblasts secrete large amounts of specialised extracellular matrix known as osteoid, composed largely of type I collagen. As the cells and the matrix both mature, the secreted proteome changes to include molecules with adaptations for mineralising and structurally modifying the matrix, including alkaline phosphatase, osteocalcin, osteopontin and osteonectin (Gorski, 2011). The presence of these proteins in the extracellular matrix promotes the crystallisation of calcium and phosphate in the interstitial fluid into a basic form of hydroxyapatite aligned with the collagen fibrils, resulting in it becoming increasingly ossified, a process often termed primary mineralisation (Buehler, 2007; Boivin, 2007). Secondary mineralisation occurs over a longer period of time, between several months to years, in order to strengthen the bone with more resilient matrix and is associated with changes in both the crystalline composition of the bone and the composition of proteins in the extracellular matrix (Henstock et al., 2015; Fuchs et al., 2008; Bala et al., 2010). Osteoclasts degrade existing mineralised bone matrix, while osteocytes are a highly specialised cell type and are the main mechanosensors within bone tissue, converting mechanical signals into the biochemical cues to which osteoblasts and osteoclasts then respond, and hence regulate the local microstructure of the skeleton (Mullender et al., 2004). Osteoblasts arise from mesenchymal stem cells through differentiation, and can further mature into osteocytes, whereas osteoclasts arise from a separate cell lineage, and are differentiated from haematopoietic stem cells (Buck and Dumanian, 2012). Mechanical stimulation is essential for the maintenance and health of bone tissue, and the coordination of the functions of these different cell types (Chen et al., 2010; Liu et al., 2010; El Haj et al., 2005), although the precise cellular response to mechanical loading is still unknown and is an active area of research.

The mathematical model developed in this paper is based on an experimental setup consisting of a mixture of active osteoblasts and osteocytes. Although osteoblasts use certain digestive enzymes to migrate through their environment and remodel their surrounding extracellular matrix, they are generally considered to be tissue-forming, rather than degrading cells (Paiva and Granjeiro, 2014). Therefore, we determine that the main phenomena that we are investigating is that of primary mineralisation, and subsequent changes in the secondary phase of mineralisation are expected to have a very limited input due to the short duration and lack of osteoclasts in the experiment.

Mathematical modelling, in conjunction with biological experiments, has an important role to play in elucidating biological mechanisms occurring during the growth and development of tissues, and providing information that cannot be experimentally measured. Once a mathematical model is validated, it may be used to optimise the experimental strategy, with the aim of improving the quality of tissue engineered products. Several theoretical models have been developed to describe the growth of engineered tissues, as reviewed in O'Dea et al. (2012). However, to the authors' knowledge, no mathematical models have been developed to describe the response of bone-producing cells to hydrostatic pressure stimulation. We note that a series of related papers adopted a multiphase modelling approach to investigate the effect of the pressures generated due to fluid motion and tissue growth within a perfusion bioreactor on the tissue composition, upon which the first of our models is loosely based (O'Dea et al., 2008, 2010; Osborne et al., 2010), although a comparison to experimental data was not made. It should be noted that a number of hypotheses exist for predicting the formation of different tissue types (for example, bone, cartilage and connective tissue) under different mechanical stimulation protocols and magnitudes *in vivo*. Recent reviews of mathematical models based on these hypotheses may be found in Isaksson (2012) and Boccaccio et al. (2011). It is an open question whether the hypotheses proposed are valid for *in vitro* tissue engineering studies (Khayyeri et al., 2009). However, we note that a number of these studies have hypothesised that bone mineralisation is affected by memory of the loading protocol. For example Levenston et al. (1994) included a fading memory component in their model of bone adaptation in response to mechanical loading *in vitro*, although no direct comparison to experimental data was made.

We develop a suite of mathematical models to elucidate the role of applied hydrostatic pressure on the quantity of minerals deposited in the engineering of a bone construct. It is well documented that hydrostatic pressure stimulation promotes stem cell differentiation down the chondrogenic lineage to form cartilaginous tissues (Elder and Athanasiou, 2009). In contrast, the effects of hydrostatic pressure on bone cells, and the resultant effect on the bone tissue composition, has received less attention and is not well understood (Chen et al., 2010; Liu et al., 2010; Hess et al., 2010). It is known that hydrostatic pressure is experienced by cells residing in the marrow space (Chen et al., 2010), and the physiological pressure within the lacunar–canalicular system has been estimated computationally to reach 274 kPa during a typical walking loading strategy, with higher pressures being obtained during impact loading (Zhang et al., 1998a,b). When artificially engineering a bone in a bioreactor, cyclical or dynamic pressure is typically applied because it is physiologically more realistic than a constant applied pressure, and has been shown to produce constructs with more of an osteogenic phenotype (a denser and more mineralised construct) (Basso and Heersche, 2002; Roelofsen et al., 1995). A variety of experimental studies on the effects of hydrostatic pressure on bone tissue development have been performed, using a range of cell types. Results indicate that dynamic hydrostatic pressure has a positive influence on bone development; it has been shown that hydrostatic pressure stimulation causes increased intracellular concentration of calcium ions (Liu et al., 2010), increased matrix mineralisation (Roelofsen et al., 1995) increased collagen and calcium content (Nagatomi et al., 2003), decreased levels of osteocyte cell apoptosis (Liu et al., 2010),