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Modelling the role of surface stress on the kinetics of tissue growth in confined geometries

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ABSTRACT

In a previous paper we presented a theoretical framework to describe tissue growth in confined geometries based on the work of Ambrosi and Guillou [Ambrosi D, Guillou A. Growth and dissipation in biological tissues. Cont Mech Thermodyn 2007;19:245–51]. A thermodynamically consistent eigenstrain rate for growth was derived using the concept of configurational forces and used to investigate growth in holes of cylindrical geometries. Tissue growing from concave surfaces can be described by a model based on this theory. However, an apparently asymmetric behaviour between growth from convex and concave surfaces has been observed experimentally, but is not predicted by this model. This contradiction is likely to be due to the presence of contractile tensile stresses produced by cells near the tissue surface. In this contribution we extend the model in order to couple tissue growth to the presence of a surface stress. This refined growth model is solved for two geometries, within a cylindrical hole and on the outer surface of a cylinder, thus demonstrating how surface stress may indeed inhibit growth on convex substrates.

1. Introduction

Evidence is accumulating that, in addition to biochemical factors, the physical environment in contact with cells and tissues modifies and controls cells' behaviour. Individual cells can sense the stiffness of a substrate, for example, with stem cells being able to differentiate along different pathways depending on the elastic properties of the substrate [1–4]. The surface roughness has also been demonstrated to influence cell adhesion and proliferation of several different cell types [5]. Similarly, the geometry of the sites available for cell adhesion, determined by features such as the density of ligands [6,7], as well as the size [8] and the shape of adhesive areas [9,10], plays a role in cell spreading, apoptosis and differentiation. Finally, cells behave differently depending on whether they are completely surrounded by a matrix in three dimensions or are just sitting on a flat surface in two dimensions [11]. Such geometric effects are also seen at the multicellular or tissue level [12], where an imposed shape gives rise to boundary constraints on the contractile behaviour of tissue, in turn controlling proliferation and further growth [13]. The collective behaviour of cells, such as MC3T3-E1 pre-osteoblasts cultured within three-dimensional holes, is influenced by the shape of the holes' cross-sections, and may be described by a simple model of curvature controlled growth [14]. This model is consistent with the idea that cells, and the extracellular matrix they organize, act as tensile elements within the tissue and predicts the differing growth rates observed experimentally in osteons and hemi-osteons [15]. Furthermore, it has also been shown that after the initial stages of cell spreading, the rate of growth was found to be independent of the substrate material [16], highlighting the crucial role played by geometry. In order to understand such behaviour, it is important to develop suitable theoretical models for tissue growth. One such approach was presented in a previous paper by the authors [17], where tissue growth was described by an eigenstrain (as resulting from cell divisions and synthesis of extracellular matrix by the cells) which depends on the local stress in the tissue. While very encouraging results were obtained with this model, it could not predict experimentally observed asymmetry between tissue growing on convex and concave surfaces [15,18,19]. It is the goal of this paper to remedy this by introducing the additional action of surface stress, as observed to occur in tissue cultures.

2. Motivation

2.1. Experimental motivation

From an experimental standpoint surprisingly few quantitative studies have investigated the role of geometry on tissue formation, although much work has been done on how geometric features control the behaviour of single cells (see e.g. Refs. [6–10]). Observations of bone tissue growth in vivo show that there is a signifi-

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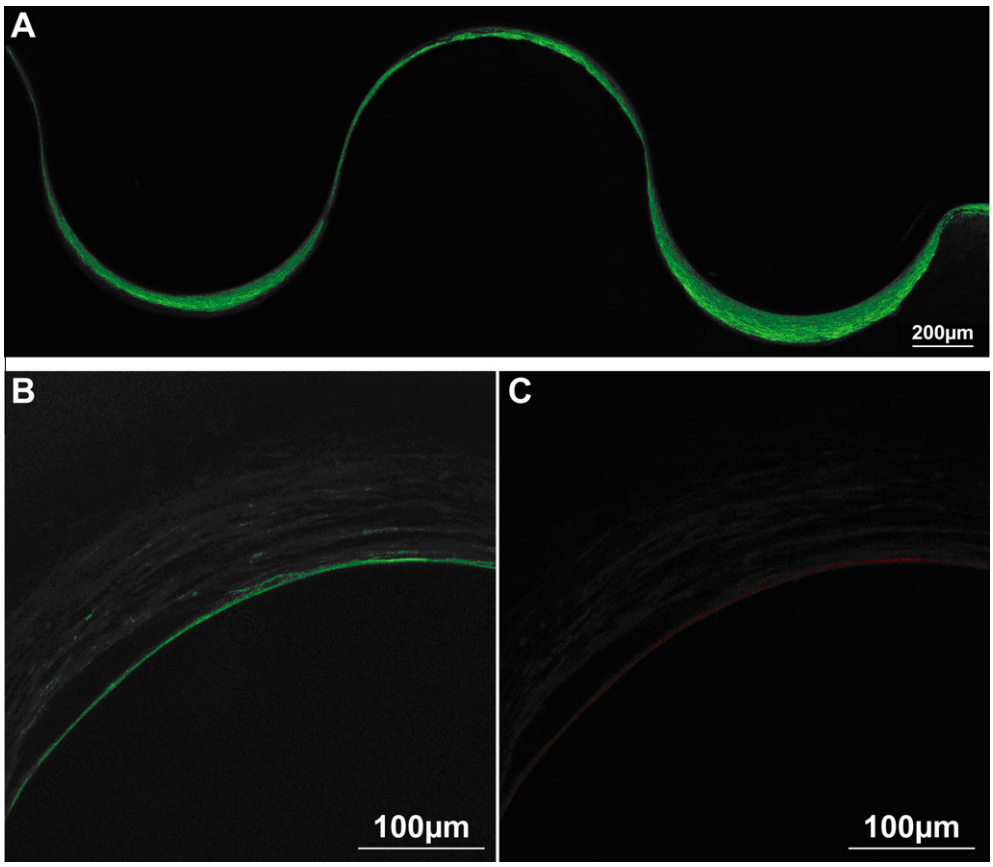

Fig. 1. Experimental evidence for the asymmetry between growth on convex and concave surfaces. (A) A stack of confocal images of tissue stained for actin (green) on a wave-like substrate with the scaffold below and medium above. (B, C) a stack of three slices deep in the tissue showing the high concentration of actin (B, green) and myosin (C, red) at the tissue–medium interface. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

cantly higher amount of bone tissue formed in concavities as opposed to convex (or planar) surfaces (see e.g. Refs. [18,19] and reference contained therein). The geometry of artificial bone defects in turn influences the lamellar arrangement of new bone [20], and also appears to control lamellar bone formation during bone fracture healing [21]. This has of course practical consequences, for instance in periodontal treatment, where it is also observed that the presence of "bony walls", which create local concavities on which new tissue can grow, assists the regeneration of new bone [22]. Finally, evaluations of computed tomography measurements of trabecular bone show that trabecular bone has close to zero mean surface curvature. This implies that, also in vivo, there is indeed a strong control of (and response to) tissue geometry by the bone cells themselves [23].

Quantification of the tissue response to substrate geometry was studied in vitro by observing growth in simplified scaffolds containing only a few straight-sided pores with controlled cross-sections [14,15]. In the initial study by Rumpler et al. [14] pores containing only concave surfaces were produced in hydroxyapatite using rapid prototyping and tested in culture with MC3T3-E1 murine pre-osteoblast cells. It was shown for these shapes that tissue growth occurred only on concave surfaces, with growth occurring on flat surfaces only when the local tissue surface curvature changed due to tissue ingrowth from the surroundings. Bidan et al. [15] extended this work by looking at the response of the same cells to semi-circular channels produced on the surface of the scaffolds. Growth was observed to be "pinned" on the corners of the channels, with only a thin layer of tissue being produced, indicating that tissue growth in vitro is sensitive to the local sign of curvature.

To further investigate this curvature sensitivity, a "wave-like" surface was produced in hydroxyapatite using rapid prototyping with repeating concave channels and convex ridges. The scaffold synthesis and cell culture experiments were performed identically to the experiments described in Refs. [14,15]; for more details see Appendix B.

A typical tissue configuration is depicted in Fig. 1A, which shows a confocal image stack of the tissue formed on the wave-like surface. The lower dark region in the image is the scaffold (opaque) and the upper dark region is the media (no signal); actin stress fibres (present in the tissue) appear at the interface in green.[1] More tissue is formed on the concave surfaces compared to the convex one, again supporting the idea that there is a dependency of "bone-like" tissue growth on the sign of curvature. A potential reason for this sign dependence of growth could come from the high contractility of the cells themselves that develops primarily on the tissue–medium interface as indicated by the colocalization of actin and myosin at the interface (Fig. 1B). A tensile surface stress has a different effect on underlying cells depending on whether the surface is convex or concave. This provides an experimental motivation behind the main goal of this paper, to examine tissue growth from a theoretical perspective in simplified concave and convex scaffolds and to test the role of surface stress on growth.

2.2. Theoretical motivation

In a preceding paper [17] a thermodynamically consistent rate $\dot{\mathbf{g}}$ of a growth tensor $\mathbf{g}$ being an eigenstrain tensor to represent tissue growth (later denominated as eigenstrain rate in a small strain setting) was derived, based on work by Ambrosi and co-workers

[1] For interpretation of colour in Fig. 1, the reader is referred to the web version of this article.