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Buckling of a growing tissue and the emergence of two-dimensional patterns ☆

M.R. Nelson [a,*], J.R. King [a], O.E. Jensen [b]

[a] School of Mathematical Sciences, University of Nottingham, University Park, Nottingham NG7 2RD, UK
[b] School of Mathematics, University of Manchester, Oxford Road, Manchester M13 9PL, UK

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ABSTRACT

The process of biological growth and the associated generation of residual stress has previously been considered as a driving mechanism for tissue buckling and pattern selection in numerous areas of biology. Here, we develop a two-dimensional thin plate theory to simulate the growth of cultured intestinal epithelial cells on a deformable substrate, with the goal of elucidating how a tissue engineer might best recreate the regular array of invaginations (crypts of Lieberkühn) found in the wall of the mammalian intestine. We extend the standard von Kármán equations to incorporate inhomogeneity in the plate's mechanical properties and surface stresses applied to the substrate by cell proliferation. We determine numerically the configurations of a homogeneous plate under uniform cell growth, and show how tethering to an underlying elastic foundation can be used to promote higher-order buckled configurations. We then examine the independent effects of localised softening of the substrate and spatial patterning of cellular growth, demonstrating that (within a two-dimensional framework, and contrary to the predictions of one-dimensional models) growth patterning constitutes a more viable mechanism for control of crypt distribution than does material inhomogeneity.

1. Introduction

This article addresses the mechanism by which biological growth may generate a build-up of residual stresses within a tissue, the relief of which drives deformations. This patterning mechanism has been previously linked to a vast array of biological systems, including plant leaves, stems and petals [1–3], skin wrinkling [4], and cortical development [5]. Here, our primary interest lies in the formation of the regular array of test-tube-shaped invaginations found in the interior wall of the human large intestine. These *crypts of Lieberkühn* (see *e.g.* Figure 3 in [6]) are known to form approximately seven days after birth in mice; prior to this the intestinal wall is smooth [7,8]. In contrast, the chick embryo exhibits the formation of ridges that develop a zig-zag pattern before turning into undulations that subsequently form villi [9]. The mechanisms that underlie crypt formation are not understood definitively; candidate mechanisms include mechanical buckling instabilities [9], viscous fingering instabilities [10], and Turing instabilities [11]. Here, we consider a contributing biomechanical process through which the cells in the developing intestine's epithelial lining proliferate and expand, resulting in the whole layer becoming compressed, and ultimately generating out-of-plane deformations.

The crypts of Lieberkühn are responsible for the maintenance of the healthy epithelium: the base of each crypt houses a population of stem cells which continuously produce new epithelial cells. These epithelial cells migrate up the crypt axis as they differentiate and, on arrival at the crypt opening, ultimately undergo programmed cell death and are released into the intestinal lumen [12]. This constant production, differentiation, migration and loss of epithelial cells results in a full regeneration of the layer every 5–6 days [13]. Malformed or dysfunctional crypts are commonly linked to the onset of intestinal cancer [14–16]. A thorough understanding of the mechanisms underlying crypt formation might therefore be useful in developing new intestinal cancer therapies, for example. This study is motivated by the eventual target of using stem cell and tissue engineering techniques for the successful manufacture of intestinal epithelia *in vitro*, a key question being whether tissue engineers must provide a framework for the crypt structure explicitly, or whether they can instead manipulate the underlying biological processes such that, given the correct environment, a cell population would assemble into crypts spontaneously. Recent experimental studies have identified the latter option as a strong possibility. Examples include the study of Viney

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* Corresponding author. Tel.: +44 (0)115 951 4923; fax: +44 (0)115 951 3837.
E-mail addresses: martin.nelson@nottingham.ac.uk (M.R. Nelson), john.king@nottingham.ac.uk (J.R. King), oliver.jensen@manchester.ac.uk (O.E. Jensen).

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et al. [17], in which collagen was used to support the co-culture of epithelial cells and fibroblasts for the culture and study of intestinal nematodes, and the study of Sato et al. [18], in which Matrigel was used to demonstrate the ability of isolated crypt stem cells to generate an individual crypt. Spence et al. [19] demonstrated that, via a series of growth factor manipulations that mimic embryonic intestinal development, a population of human pluripotent stem cells can be directed to differentiate into functional epithelial tissue.

Previous models of the colorectal crypt have often used a cell-based description of the epithelium to study the division, differentiation and migration of stem cell progeny [16,20–22]. Lattice-free models [23–25] have facilitated realistic descriptions of cell division, migration and deformation, representing cells by points at their centres or vertices connected by elastic springs that capture cell–cell interactions. Stochastic [26] and deterministic [27] models for the out-of-plane deformations induced by in-plane stresses have elucidated the effects of *e.g.* hyper-proliferation, crypt budding and fission in the onset of colorectal cancer. Recent models have also investigated the role of tissue curvature in driving cell differentiation [28] and regulating tissue growth rate [29].

Via an experiment in which intestinal epithelial cells were cultured upon a deformable substrate, Nelson et al. [30] validated the hypothesis that cellular proliferation and expansion against fixed boundaries can result in sufficient in-plane compression to generate out-of-plane deformations of intestinal epithelia *in vitro*. Two parallel biomechanical models, each a one-dimensional (1D) representation of a 2D system, were used to demonstrate that buckling thresholds and post-buckled configurations were largely unchanged under variation of cell-substrate adhesion properties or under spatial patterning of the cells' growth rate. The study showed that mode selection can, instead, be controlled by either patterning the material properties of the substrate, or by tethering the substrate to an underlying foundation. However, these predictions have yet to be fully assessed using more realistic 2D models.

Mathematical models of growing biological organisms have been an active area of research since the 1940s [31,32]. While many early papers deployed geometric arguments to track displacements of material points alone (*e.g.* [33,34]), tissue growth models are now commonly embedded into the theory of nonlinear elasticity. Skalak and Rodriguez [35–37] provided a formalism for this process, suggesting a decomposition of the associated deformation gradient tensor as $F = AG$, where the tensor G captures growth effects (mapping an initially unstressed and unloaded body into an enlarged, and possibly incompatible, configuration), and the tensor A accounts for the elastic deformations required to satisfy external constraints and correct for any growth-induced incompatibilities. Later refinements of this framework have separately accounted for growth, remodelling and morphogenesis; for details see [32,38,39] and references therein.

One common approach to modelling growth-driven tissue deformation is to describe thin tissue layers such as epithelia using von Kármán's equations for thin plates [40,41]. The theory is derived from nonlinear elasticity via a series of assumptions upon the magnitude of each of the stress/strain components, primarily that the magnitudes of transverse stresses and strains are at least $O(\varepsilon)$ smaller than those of the in-plane components, for aspect ratio $\varepsilon = h^*/L^* \ll 1$ (for plate thickness h^*, width $2L^*$). See Appendix A or [42] for further details. The resulting theory holds for transverse displacements of $O(h^*)$; we term this theory 'weakly nonlinear'. Hannezo et al. [29] deployed von Kármán theory in their model of the intestinal epithelium, presenting a three-layered model in which the epithelium rests upon a basement membrane mounted on an elastic stroma. Through tuning of the mechanical properties of the elastic stroma, and coupling of the epithelial growth rate to membrane curvature, the authors were able to attain buckled configurations which replicated the geometric differences between the large and small intestine (in which villi also protrude into the lumen).

An alternative approach is to regard the tissue layer as a thin, nonlinear shell, and to derive governing equations via a 'balance of forces' formulation [43–46]. While the use of nonlinear shell models is advantageous in facilitating study of larger-amplitude deformations, this approach presents additional complication in terms of selecting and justifying the necessary constitutive assumptions, often from a choice of many possibilities [45].

The model of Dervaux & Ben Amar [47] coupled a von Kármán description of an epidermal layer of skin tissue to a linearly elastic basement membrane, assessing the role of localised tissue growth in pattern selection. Comparing their full model to a 1D reduction in which growth is uni-directional, the authors concluded that under uni-directional growth the profile of the growing tissue is determined entirely by the net growth, becoming independent of the local growth field expression. However, in the full 2D problem, stronger energetic constraints on bending and stretching in two directions restrict attainable patterns, allowing patterned growth to play a stronger role in pattern selection. We revisit this claim here to assess the strength of the conclusions of [30], in which 1D models indicated that patterned growth was not sufficient to control crypt distributions.

In this paper, we extend the 1D models of [30] to two spatial dimensions. Motivated by the experimental formulation therein, we address the question of how a tissue engineer might best manipulate the cell environment *in vitro* toward the goal of generating intestinal epithelia which display the required crypt geometry. Considering a typical cell culture substrate as a thin plate, we present an extension to the standard von Kármán equations to incorporate (i) surface stresses induced by proliferating cells upon the substrate's upper surface, and a supporting foundation below, and (ii) spatial variations in the plate's mechanical properties. We present the configurations attained by a homogeneous plate buckling under the influence of a uniformly growing cell layer, and show how these configurations are affected by attachment to a supporting elastic foundation, localised softening of the substrate, and localised cellular growth.

2. Model

We consider a square cell culture substrate clamped between fixed supports along its four edges. Upon the upper surface of the substrate rests a confluent monolayer of epithelial cells (see Fig. 1). Continued proliferation causes the cells to exert sufficient force against the fixed boundaries to deform the substrate and

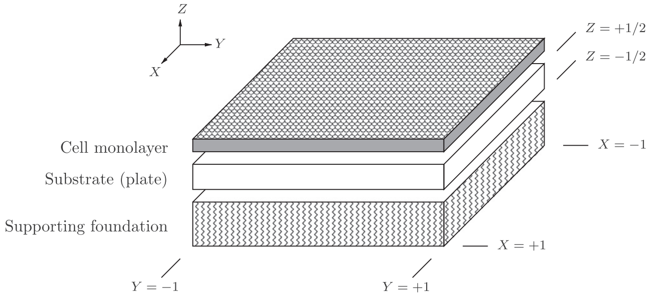

Fig. 1. Geometry of the modelled problem in the undeformed configuration. A square cell culture substrate, held between clamps along all four edges, is modelled as a thin plate. A growing cell monolayer rests upon this substrate, which in turn may rest upon a supporting viscous or elastic foundation. Expansion of the cell layer against fixed boundaries may drive deformations of the plate, with the supporting foundation playing a role in shape selection.