Colorectal crypt formation *in vitro* & *in silico*: elucidating the role of cellular growth upon tissue-scale buckling

M.R. Nelson[1,†], D. Howard[2], O.E. Jensen[1], J.R. King[1], F.R.A.J. Rose[2] & S.L. Waters[3]

† *Corresponding author: martin.nelson@nottingham.ac.uk*

[1] *Centre for Mathematical Medicine & Biology, School of Mathematical Sciences, University of Nottingham, UK*
[2] *Division of Advanced Drug Delivery & Tissue Engineering, School of Pharmacy, University of Nottingham, UK*
[3] *Oxford Centre for Industrial & Applied Mathematics, Mathematical Institute, University of Oxford, UK*

The structure and function of the digestive system have become increasingly well-studied in recent years, both experimentally and mathematically. With cancer of the large intestine (or colon) prevailing as the second-greatest cause of cancer-related mortality in developed countries (Stewart & Kleihues, 2003), much focus has been placed upon understanding the cellular dynamics of the colon and, in particular, the epithelial cell layer which forms its internal lining. In its capacity as a self-renewing tissue housing a 'stem cell niche', the colon is of great interest to stem cell biologists, while the growing benefits of transplant surgery motivate the interest of tissue engineers in recreating the tissue in the laboratory. Replication of intestinal tissue *in vitro* also has great potential in aiding understanding of the underlying biology and for such clinical applications as drug testing and gene therapy.

The colon's epithelial lining exhibits a number of invaginations into the underlying tissue, called the crypts of Lieberkühn. Housing stem cells at their bases, these crypts play an essential role in the maintenance of the epithelium, facilitating a complete renewal every 5–6 days (Ross *et al.*, 2003). Formation of the crypts is known to occur approximately seven days after birth in mice (Crosnier *et al.*, 2006; Barker *et al.*, 2008); prior to this the intestinal wall is smooth. However, the processes which underlie colorectal crypt formation are not conclusively understood. This study combines *in-vitro* experiments with biomechanical models simulated *in silico* to validate one potential mechanism: that in the developing epithelium, proliferation and growth on the cellular scale cause a build up of compressive stresses at the tissue scale, resulting in buckling instabilities which initiate crypt formation.

To examine the proposed mechanism *in vitro*, intestinal epithelial cells were cultured to confluence upon a flexible substrate. Since these cells exhibit contact inhibition,

their proliferation would usually cease once the cells have occupied all of the available space. However, this study demonstrates that cellular proliferation can generate sufficient force to deform a flexible substrate, increasing its surface area and facilitating continued proliferation.

We present a number of analogous biomechanical models in which a confluent cell monolayer adheres to a thin, compressible, elastic foundation. Cellular growth in the upper layer generates compressive stresses that are transmitted to the foundation, driving deformations via a series of buckling instabilities. Modelling growth parametrically as a sequence of equilibrium configurations, we use a simple one-dimensional model to demonstrate that spatial variations in cell–substrate adhesion and inhomogeneity of cellular growth have minimal influence upon configurations. Compressibility of the substrate is shown to be important only in separating bifurcation points; large-amplitude shapes are accurately approximated by incompressible solutions.

Comparing this model with an extension to a previous model by Edwards & Chapman (2007), we demonstrate that competition between lateral supports and stromal adhesion determines buckling wavelength. We show how non-equilibrium relaxation of tethering forces affects post-buckled shapes, and illustrate that variations in the mechanical properties of the stroma can have a much more significant effect upon buckled configurations than does inhomogeneity of cellular growth.

These models are then extended to two spatial dimensions through a modification of von Kármán plate theory, revealing the diverse range of tissue-scale buckling patterns attainable under various regimes of cellular growth, stromal adhesion and mechanical inhomogeneity.

References

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