Morpho-elasticity of intestinal villi

V. Balbi[a], P. Ciarletta[a]

a. *Institut Jean le Rond d'Alembert, CNRS UMR 7190, Université Pierre et Marie Curie - Paris 6*

Résumé :

Dans le contexte des processus morphogenètiques des tissus mous, on présente un modèle morpho-élastique sur le développement embryonnaire des villi intestinaux. Les villi commencent à se former à partir de certains motifs à deux dimensions, qui développent ensuite à la croissance et à la présence des contraintes résiduelles à l'intérieur de la muqueuse intestinale. On utilise un modèle à géométrie cylindrique avec une loi constitutive hyper-élastique, anisotrope pour décrire le comportement de la muqueuse, qui croit sous certaines contraintes géométriques. La formation du motif à deux dimensions est étudiée avec une analyse de stabilité linéaire, qui fonde ses bases sur la théorie des déformations incrémentales superposées à des grandes déformations. Les résultats obtenus en considérant divers scenarios de croissance, sont comparés avec ceux expérimentaux, en démontrant que la formation des villi intestinaux à l'état embryonnaire est guidée par l'anisotropie de la croissance parmi les diverses couches de la muqueuse, ainsi que par les caractéristiques géométriques et mécaniques du tissu.

Abstract :

In the context of morphogenetic processes of soft tissues, we present a morpho-elastic model of the embrionic development of the intestinal villi. Since the first stages of their development, a bidimensional pattern emerges on the epithelial layer of the mucosa, induced by growth and residual stresses inside the tissue. It's from this pattern that villi start to elongate. We use a cylindrical geometry and a constitutive hyper-elastic and anisotropic model in order to describe the non-linear behavior of the mucosa, which grows under geometrical constraints. In order to model the occurrence of the bi-dimensional ondulated pattern, we perform a linear stability analysis using the method of incremental deformations superposed on finite deformations. The numerical results are presented for different growth scenarios and compared with the experimental data, showing that the formation of intestinal villi in embryos is driven by a differential growth between the different layers of the intestinal mucosa, as well as the geometrical and mechanical properties of the tissue.

Mots clefs : **morphoelasticity ; villi morphogenesis ; elastic stability**

1 Introduction

Intestinal villi are finger-like structures lying on the inner layer of the intestinal wall. They increase the surface area of the intestine, allowing the absorbtion of liquids and nutrients from food. Many studies have demonstrated that the morphogenetic processes leading to the formation of the intestinal villi, follow similar development stages in various living organisms, but different initiation mechanisms. In all vertebrates, villi originate from the embryonic development of a soft tissue, the mucosa, that covers the internal part of the gastro-intestinal tract and it is made of three layers : the outer layer called *epithelium*, the *lamina propria*, which includes connective tissue, lymph nodes and blood-vessels, and the *muscularis mucosae*, a continuous thin sheet of smooth muscle cells (see Figure 1). Three stages have been identified during the villi morphogenesis : elevation of previllous ridges, delineation of villus bases and outgrowth of the definitive villi [6]. All along this morphogenetic process, the growing mucosa is geometrically confined by its surrounding tissues and residual stresses can arise as a consequence of the geometrical incompatibilities during growth [10, 5, 4].
In this work, we aim at modelling the formation of the bi-dimensional undulated patterns in the

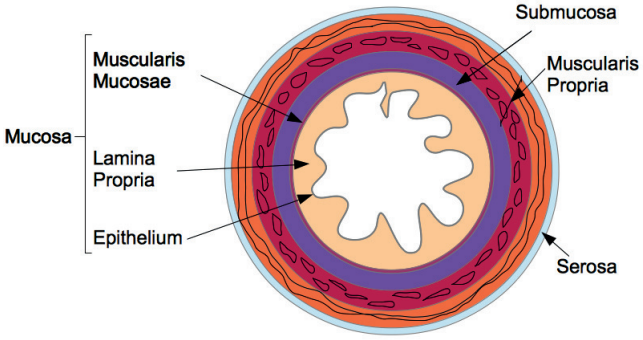

FIGURE 1 – Schematic structure of the intestinal wall : the inner layer called mucosa (in which concur the epithelium, the lamina propria and the muscularis mucosae), the submucosa (made of dense irregular connective tissue), the muscularis propria (oriented smooth muscles) and the outer serosa layer are evidenced.

embryonic intestinal mucosa, which characterize the initial stages of villi morphogenesis. A geometrical description of the model is given in Section 2 and the volumetric growth of the mucosa is described using the multiplicative decomposition of the deformation gradient. An hyperelastic constitutive model defines the mechanical behavior of the mucosa accounting for the anisotropy of the tissue in terms of the stiffness and the orientation of the collagen fibers distributed under the epithelial layer. In Section 3, we outline the equilibrium problem for the basic axial-symmetric solution and we perform a linear stability analysis for studying the emergence of the bi-dimensional patterns. Finally, the growth thresholds of instability, calculated for different growth scenarios and material properties, are presented in Section 4 and compared with experimental data.

2 Geometrical and constitutive model

In this section, the geometrical model and the constitutive hypothesis on the elastic behavior of the mucosa are introduced. The mucosa is modeled as a one-layer cylinder and the morpho-elastic deformation is identified by the mapping χ, defined as :

$$\mathbf{x} = \chi(\mathbf{X}), \qquad \chi : \mathcal{B}_0 \to \mathcal{B}_a \tag{1}$$

where $\mathbf{X}$, $\mathbf{x}$ are the position vectors in the reference and actual configurations $\mathcal{B}_0$ and $\mathcal{B}_a$, respectively, as depicted in Figure 2. The outer radius is fixed during the process, since the surrounding tissues are much stiffer than the mucosa, the annular surfaces cannot slide longitudinally and the internal surface is free of traction, because the inner intestinal pressure is negligible in embryos. As proposed by Rodriguez et al. [7], the deformation gradient $\mathbf{F} = \mathrm{Grad}\,\mathbf{x} = \dfrac{\partial \chi(\mathbf{X})}{\partial \mathbf{X}}$, associated to the volumetric growth of the mucosa, can be split into two components, as follows :

$$\mathbf{F} = \mathbf{F}_e \mathbf{F}_g. \tag{2}$$

where $\mathbf{F}_g = \mathrm{diag}(g_r, g_r, g_z)$ represents the pure homogeneous volumetric growth, with growth rates in the radial and longitudinal directions g_r and g_z respectively, and $\mathbf{F}_e$ is the elastic deformation tensor. The growth deformation defines a natural grown state in which the geometry of the tissue is not necessarily compatible with the constraint. The elastic deformation restores the compatibility of the deformation, introducing residual stresses inside the tissue.
The mucosa is assumed having a hyper-elastic behavior and it is modeled as an homogeneous, incompressible tissue, composed by a cross-ply continuous distribution of collagen and elastin fibers (anisotropic component), immersed into a homogeneous ground substance (isotropic component). Therefore,

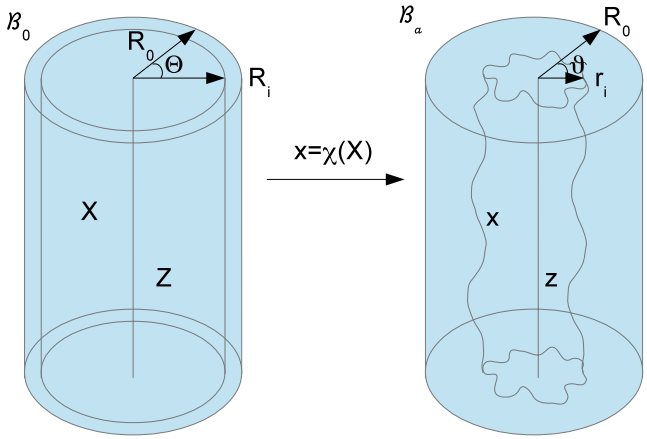

FIGURE 2 – Geometrical model of the mucosa growth process : the mapping χ transforms the point $\mathbf{X}$ from the reference configuration $\mathcal{B}_0$ into the point $\mathbf{x}$ in the actual configuration $\mathcal{B}_a$.

the strain energy function can be expressed as a sum of two term :

$$\Psi(\mathbf{C}_e, \mathbf{m}_{\pm\alpha}) = \Psi_{Iso}(\mathbf{C}_e) + \Psi_{Aniso}(\mathbf{C}_e, \mathbf{m}_{\pm\alpha}) - p(\det \mathbf{C}_e - 1) \tag{3}$$

where Ψ_{Iso} is the isotropic component, a scalar function of the right Cauchy-Green tensor $\mathbf{C}_e$; and Ψ_{Aniso} is the anisotropic component, which also depends on the orientation of the fibers through the vectors $\mathbf{m}_{\pm\alpha}$, with α being the cross-ply fiber angle with respect to the longitudinal direction ; and p is the Lagrange multiplier to ensure the incompressibility. In absence of body forces, the equilibrium equations for the mucosa read :

$$\mathrm{Div}\, \mathbf{S} = 0, \quad \mathrm{div}\, \boldsymbol{\sigma} = 0 \tag{4}$$

where $\mathbf{S} = \dfrac{\partial \Psi}{\partial \mathbf{F}_e} - p\mathbf{F}_e^{-1}$ is the nominal stress tensor, $\boldsymbol{\sigma} = \mathbf{F}_e\mathbf{S}$ is the Cauchy stress tensor and Div, div are the divergence operators in the reference and actual configurations, respectively. To Eqs. (4), we associate the following boundary conditions :

$$\mathbf{S}^T \cdot \mathbf{N} = 0 \quad \text{on} \quad R = R_i, \qquad \boldsymbol{\sigma} \cdot \mathbf{n} = 0 \quad \text{on} \quad r = r_i \tag{5}$$

where $\mathbf{N}$ and $\mathbf{n}$ are the outer normal units in the reference and actual configurations, respectively.

3 Linear stability analysis

The method of incremental deformations superposed on finite deformations is usually employed in order to study the growth instabilities that can occur in elastic tissues. The fundamental idea is to consider an infinitesimal perturbation of a basic elastic solution in order to perform a linear stability analysis. Therefore, we consider an axial-symmetric solution of the following form :

$$\begin{cases} r(R, \Theta, Z) = \sqrt{g_r^2 g_z R^2 + a} \\ \theta(R, \Theta, Z) = \Theta \\ z(R, \Theta, Z) = Z, \end{cases} \tag{6}$$

and we solve the equilibrium problem in Eqs. (4, 5), in order to get the spatial distribution of the residual stresses inside the mucosal tissue as a funciton of the growth rates. Then, we define an infinitesimal perturbation of the basic grown position $\mathbf{x}^{(0)} = \chi^{(0)}(\mathbf{X})$, as

$$\chi(\mathbf{X}) = \chi^{(0)}(\mathbf{X}) + \varepsilon\chi^{(1)}(\mathbf{x}^{(0)}) \tag{7}$$

where $|\varepsilon| \ll 1$, so that the term $\chi^{(1)}(\mathbf{x}^{(0)})$ can be regarded as a first-order incremental displacement with respect to the axial-symmetric configuration. We rewrite the constitutive relations after the pertubation, as :

$$\mathbf{S} = \underbrace{\mathbf{S}^{(0)}}_{\text{zero-order term}} + \underbrace{\varepsilon\dot{\mathbf{S}}}_{\text{first-order term}} \tag{8}$$

where the zero-order term $\mathbf{S}^{(0)}$ is the nominal stress calculated for the axial-symmetric configuration and $\dot{\mathbf{S}}$ is its increment. Therefore, the incremental equilibrium problem rewrites as :

$$\operatorname{div} \dot{\mathbf{S}}_0 = 0 \tag{9}$$

where $\dot{\mathbf{S}}_0 = \mathbf{F}_e^{(0)}\dot{\mathbf{S}}$ is the push forward of $\dot{\mathbf{S}}$ in the perturbed configuration. We choose the incremental deformation with components in the form :

$$\chi^{(1)}(r,\theta,z) = (U(r), V(r), W(r))e^{i(m\theta + k_z z)} \tag{10}$$

where m (resp. $k_z = \frac{2n\pi}{L}$) is the circumferential (resp. longitudinal) mode of the perturbation, with m and n positive integers, and i is the imaginary unit. Using the perturbation defined in Eq. (10), the deformed mucosa is depicted in Figure 3, showing the characteristic bi-dimensional undulated pattern at the inner surface emerging at the initial stages of intestinal villi formation. We impose the extintion

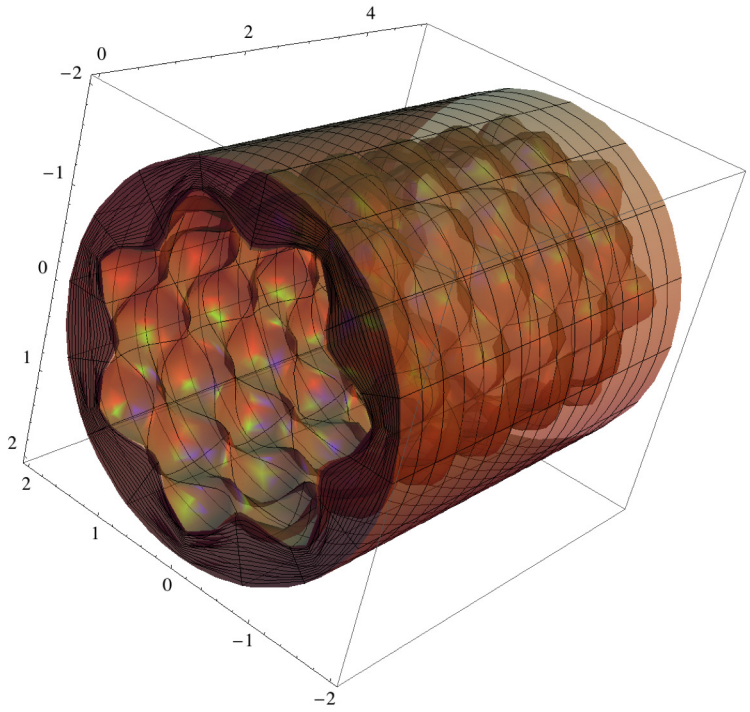

FIGURE 3 – Morphology of the intestinal mucosa after imposing a perturbation of the axial-symmetric solution of the elastic problem, having the form of Eq. (10). The geometrical parameters are $r_0 = 2$, $r_i = 1.5$, $L = 5$, $m = 7$, $k_z = 5$ and $\epsilon = 0.15$.

of the perturbation at the outer surface and a no-traction condition on the inner surface, then we reformulate the incremental equilibrium problem in Eq. (9), using the Stroh formulation [9]. This procedure allow us to transform the system of partial differential equations in Eq.(9) into a system of ordinary differential equations of first order. Then, we numerically solve it using a particular numerical scheme based on the determinantal method [3, 1] and we find the values of a control parameter for which there is a solution in the form of Eq. (10). Since the growth has been assumed homogeneous, the growth rates g_r and g_z can be considered as the control parameters of the elastic instability of the tissue.

4 Results

The results of the linear stability analysis performed in Section 3, show the growth thresholds at which a bifurcation occurs in function of the inital aspect ratio $H = R_0/R_i$, for different perturbation modes and different growth scenarios : isotropic growth ($g_r = g_z$) and anisotropic growth ($g_r \neq g_z$) processes. The marginal stability curves in Figure 4 confirm the occurrence of a surface instability mechanism : for high values of m, k_z, the growth thresholds converge to a single curve in the case of thick tissues ($H > 1.5$), while a large variability on the perturbation modes appears for thin tissues. We also take into account the material anisotropy of the mucosa. The instability growth thresholds

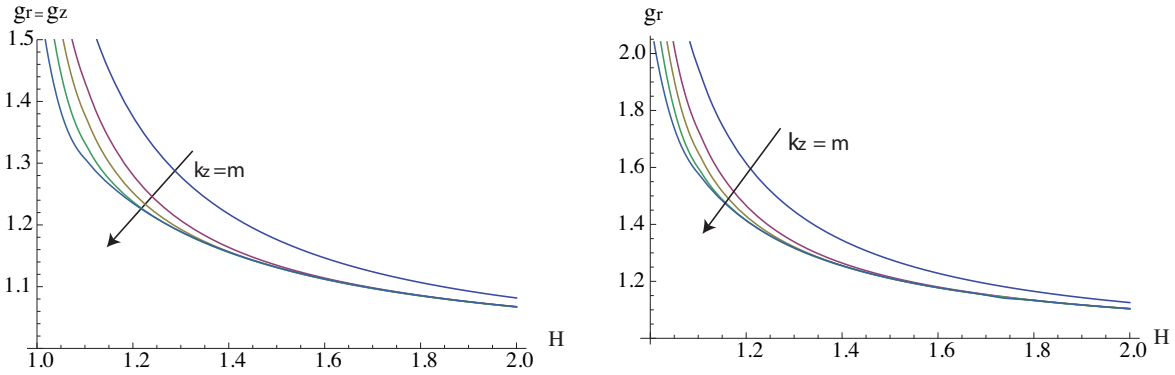

FIGURE 4 – Marginal stability curves showing the isotropic growth tresholds $g_r = g_z$ (left) and the anisotropic radial growth thresholds g_r (right, setting $g_z = 1$), calculated at different modes $m = k_z = 2, 5, 7, 10, 15$.

are shown for different cross-ply fiber angles α (Figure 5, right) and for different material anisotropy ratios k_1/μ, (Figure 5, left). The results in Figures 5 highlight how an increase of both the cross-ply angle and the stiffness of the reinforcing fibers induces an increase of the growth instability thresholds and demonstrates that the material anisotropy is a stabilizing factor. Finally, we compare the growth

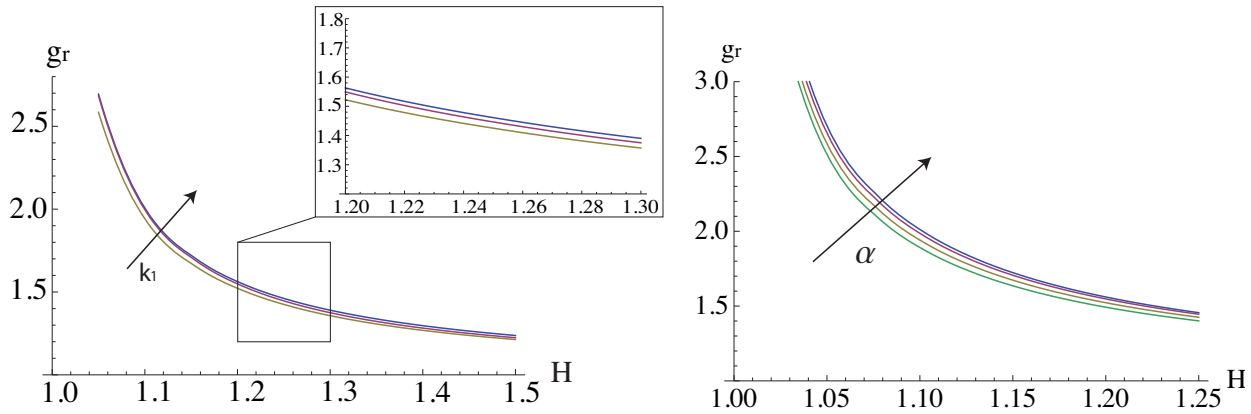

FIGURE 5 – Marginal stability curves for anisotropic growth ($g_z = 1$) showing the radial growth thresholds g_r, for different material anisotropy ratio $k_1/\mu = 0.1, 1, 10$ (left), and different cross-ply fiber angles $\alpha = (0, \pi/6, \pi/4, \pi/3)$ (right).

thresholds for the bidimensional ondulated pattern with those in [2] for both circumferential folding and longitudinal segmentation. The marginal stability curves in Figure 6 show how the instability thresholds for the bidimensional pattern, when considering thin mucosa, are smaller than those for folding and segmentation, both for isotropic and anisotropic growth scenarios, while for thick tissues the curves overlap. These results are in agreement with the experimental data collected in [8] on mouse embryos predicting an occurrence of the bi-dimensional pattern during the days between 12 and 16, when the value of the critical volume increase is around 1.3.

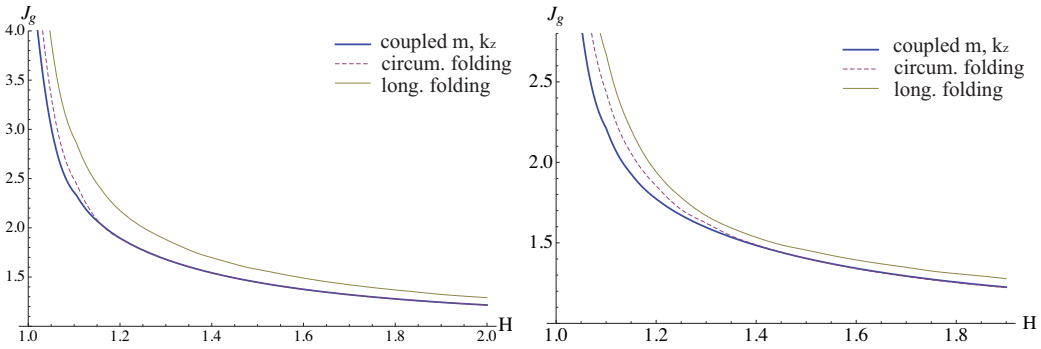

FIGURE 6 – Instability thresholds in terms of volume increase due to isotropic (left) and anisotropic (right, $g_r = 1$) growth processes. The solid curves referring to the circumferential and longitudinal folding are taken from [2].

5 Conclusions

In conclusion, the morpho-elastic model presented in this work, confirms that a differential volumetric growth between the epithelial and the mesenchymal layers of the mucosa cand driven the emergence of intestinal villi in embryos. As reported by experimental observations in mouse embryos, our study has demonstrated that the villi morphogenesis can start from a bi-dimensional undulation of the mucosa, or after a circumferential folding, followed by a zig-zag pattern. Our theoretical analysis predicts that the initial aspect ratio of the tissue plays an important role on the selection of the previllous structure, with thinner tubes not requiring any preceding mucosal folding. Finally, we have demonstrated that both the geometrical and the mechanical properties of the mucosa strongly influence the formation of previllous structures in embryos.

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