CURCIO, EVAN JOSEPH. Mathematical Modeling of Stomach Morphogenesis and the Biomechanics of Pattern in Notochords. (Under the direction of Sharon Lubkin).

This dissertation presents three treatments of mathematical modeling to two relevant areas of biological application. We apply foam physics to the morphometry of cell packings of notochords, elastic membrane physics to notochord biomechanics, and the theory of morphoelasticity to morphogenesis in the developing stomach.

The notochord is the defining feature of chordates, and during development it lengthens the embryo, provides structural support, and in many organisms acts as a template for spine development. It consists of inner, vacuolated chordocytes surrounded by an epithelial sheath of chordoblasts. Previous foam models have investigated how geometric and physical ratios might control chordocyte packing within the notochord. We examine the interplay between these ratios in the two lowest order regular patterns, determine a functional relationship between them, and compare our results to the previous work. Further, we employ an elastic membrane model to probe how these two lowest order patterns might affect overall biomechanics of the structure, providing evidence for the structural importance of both packing pattern and pattern orientation.

During development, the embryonic stomach anlage undergoes asymmetric radial cell rearrangement of the left mesoderm, giving rise to the curvature found in the mature organ. This rearrangement might occur by a number of mechanisms; in particular, we model two hypotheses - radial thinning combined with volume-preserving, equal axial and circumferential elongation, and radial thinning combined with volume preserving axial elongation - using a morphoelastic framework. Morphoelasticity is an area of continuum mechanics which decomposes deformations into growth and elasticity components, allowing an investigation of large strains. Under an assumption that the time scale of growth is longer than that of residual stress, this technique can be iterated.
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Mathematical Modeling of Stomach Morphogenesis and the Biomechanics of Pattern in Notochords

by

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To Jenna.
BIOGRAPHY

Evan Joseph Curcio was born in Tacoma, Washington on November 30, 1990 (354 ppm CO₂). He graduated in 2012 (394 ppm CO₂) with a B.A. in Mathematics from the University of Washington, after which he graduated in 2014 (399 ppm CO₂) with an M.A. in Statistics and Applied Mathematics from the City University of New York - Hunter College. After a few years in industry, he joined the Biomathematics Graduate Program at North Carolina State University in 2018 (409 ppm CO₂), graduating with a Ph.D. in 2023 (418 ppm CO₂).
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Figure 4.8 (Continued) Test 4, time-dependent study of transversely isotropic differential cell rearrangement. In each, $G$ is applied to the central left segment of the initial geometry (first column, blue stripes). Residual stress dissipates between time steps. Top rows: 3D ventral view. Middle rows: 2D midline slices show relative thinning of left and right sides. Since the anterior end is modeled as remaining planar but the posterior end is free, the cut posterior surface (red, dashed line) develops curvature (red, dashed curve) in response to growth above. Bottom row: 2D top-down view of anterior surface. Outlines (black curves) show segments of the initial geometry of each time step. Von Mises stress is shown in arbitrary units.

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Figure A.1 Initial geometry for a notochord in bamboo configuration with $N = 2$, with labels shown for vertices, edges, faces, and bodies. Note that Face VI is defined by traversing edges in the counterclockwise direction when describing Body 1, but is traversed in the clockwise direction when defining Body 2, in order to ensure the normal vector is outward with respect to the body to which it is being attributed.

Figure A.2 Initial geometry for a notochord in bamboo configuration with $N = 2$, with labels shown for vertices, edges, and faces. Vertex values are circled, Edge values are boxed, and Face values are both boxed and signed, based on the direction of the vector normal to their respective surface.
1.1 Background

The theory of epigenesis - that an organism develops from previous stages, rather than being preformed - can be attributed to Aristotle as described in Historia Animalium. He argued that some ‘vital cause’ initiated development from an embryonic state to maturity. Some of the earliest work relating form to geometry and mechanics came from Galileo Galilei (1). Modern study of morphogenesis - the biological development of shape - as studied through the lens of the physical and mathematical sciences, began in large part with Wilhelm His (2) and D’Arcy Thompson (3). One of His’s most famous experiments involved the manipulations of an elastic rubber tube and its comparison to the morphogenesis of tubular structures, including the gut - more recent work using morphoelastic modeling has corroborated His’s observation of these mechanical similarities (2,4). In On Growth and Form, Thompson provided an explanation of growth and form via physical forces and mathematical descriptions. To this day it remains one of the most cited works in morphogenesis and inspired a framework for modern biomechanics. With this historical context in mind, we can now introduce the specific systems, and physical or mathematical techniques, which will be detailed in later chapters.

This dissertation will examine two biological systems from three modeling perspectives. The first system we will discuss is the notochord, which is the defining structure of chordates.
It serves to elongate the embryo, acts as a center for morphogen signaling and in vertebrates is the precursor to the spinal cord (5). It consists of packed internal, vacuolated cells called chordocytes, surrounded by external sheath cells called chordoblasts, forming a cylindrical rod. This brings us to two questions that this dissertation will address in Chapters 2 and 3, respectively:

- How does cortical tension control the morphometry of the notochord?
- How is flexural rigidity affected by internal packing patterns of chordocytes?

In Chapter 4, we examine the primordial stomach (*anlage*) which starts as a tubular component of the gut. During development, it breaks symmetry, developing the greater and lesser curvatures found in the mature organ. (We note that the anatomical descriptions ‘greater’ and ‘lesser’ curvature are the opposite naming convention under a mathematical description of curvature.) The possible explanations for how and for what purpose this symmetry breaking occurs are numerous and dependent on the system in question. Some explanations may be teleological - that is, that the symmetry-breaking must occur in service of some future function. Other explanations could be genetic/evolutionary, morphological, or mechanical. In this dissertation, we will be largely concerned with the morphological and mechanical perspectives on this question and will investigate these via mathematical modeling. This motivates the third question which is addressed in Chapter 4:

- Which types of differential growth of the stomach *anlage* are sufficient to generate the curvature seen *in vivo*?

We restrict development to specific embryonic stages of the *X. laevis* stomach primordium (*anlage*) and *D. rerio* notochord, although it could be argued that development of an organism could span from the first combination of novel genetic material to the time of its eventual demise. We note that ‘left’ and ‘right’ are generally meant in the anatomical sense, thus when displaying figures of the embryonic stomach from a ventral perspective, the left side will display to the reader’s right, per anatomical convention.

1.2 Modeling the Notochord

1.2.1 Modeling Approaches

Every model is a simplification of reality, which is too complex to simulate in its entirety at the present time. Models offer us a methodological way to simplify phenomena into more manageable components and variables, ignoring some, but controlling for others, offering
insight into how the included variables affect one another. A model that is too simple will ignore variables or processes germane to the investigation at hand - a model that is too complex risks complicating the analysis, preventing the modeler from elucidating critical relationships.

A variety of modeling approaches can be used to describe physical systems. Phenomenological models describe empirical relationships between phenomena. For example, curve fitting can be used to approximate trends in data, without incorporating underlying theory. Machine learning models which use data-driven, statistical methods are also empirical and so fall into this category as well. Mechanistic models, on the other hand, derive these relationships from first principles, incorporating mathematical, physical, or biochemical laws assumed to be at the heart of the relationships in question. In this case, less data would be needed to initialize the model, but eventually, data should be used to verify outcomes.

Agent-based Models

Agent-based models are generally used by assigning attributes to individual components (e.g. cells) which then interact with each other, following rules which can be stochastic, deterministic, heuristic, and so on. The first computational models of growth used cellular automata, an on-lattice model in which a domain is subdivided into lattice sites containing cells - a famous early example of which is Conway’s Game of Life, popularized by Gardner in (6). Cellular Potts models are another popular technique in which cells can occupy more than one lattice site and can move or deform into adjacent sites. This method utilizes a process which minimizes a pseudo-energy function to determine how the cells move. However, lattice methods are constrained by the geometry of the lattice (1). To combat this, a modeler could choose to use an off-lattice method, using, for example, vertex models. In vertex models, cells are polyhedral, and interactions between cells are represented by vertex movements. While this can be useful in situations where cells are closely packed, new rules and parameters must be added to govern the myriad behaviors of cells interacting with their neighbors, which increases model complexity, and makes it more difficult to align these discrete models with macro-level behavior (1,7).

Continuum Models

An alternative to vertex models is to assume that the multi-cellular structure, or tissue, is space-filling. Individual cells which comprise the tissue have properties which average over small neighborhoods. This situation, which we encounter in all models presented in this dissertation, is called continuum modeling.

Rather than tracking interactions of individual cells, continuum models treat space-filling sections of tissue in bulk, utilizing differential equations such as conservation equations and
material constitutive equations to describe the behavior of materials. Physical quantities such as stress and strain are represented by tensors, which are defined independent of coordinate basis, facilitating the ability to describe deformation throughout the continuum as it deforms regardless of a particular choice of coordinate system.

When addressing the notochord, we simplify the system under the assumption that surface phenomena dominate - that is, that the components of the notochord having an outsized contribution to its morphometry or flexural rigidity can be modeled as surfaces with relatively small thickness. These will be meso-scale models - models of intermediate resolution, which take into account individual cells comprising the notochord, without explicitly modeling subcellular or external structures’ contributions. The sheath is treated as a tissue continuum, but individual chordocytes are modeled in order to investigate the effect of cellular packing. Chordocytes are considered to be closely-packed and thus space-filling, with their membrane tension properties consistent over the entire surface. In contrast, when addressing the stomach, we instead treat differential growth as a volume phenomenon. In this model, the stomach tissue is treated as a continuum, with properties being averaged over local neighborhoods of points instead of being attributed to discrete cells. We will provide rationale for our modeling choices beginning with the notochord.

1.2.2 Notochord Anatomy and Model Assumptions

The notochord is a defining feature of chordates. During development, the notochord elongates and provides structural support for the organism, and acts as a signaling center important in left-right differentiation (5). Some chordates maintain a notochord throughout their lifespan (e.g. amphioxus, lamprey), while in others (e.g. mammals) it is a template for the spine. Thus, the interruption of mechanisms which form the notochord or its constituent substructures can lead to defects of the spine, such as idiopathic scoliosis (8).

The notochord is a tubular structure running anteroposteriorly along the length of the developing chordate. It is composed of large inner vacuolated cells, called chordocytes, surrounded by a thin epithelial sheet of cells called chordoblasts (9–11). It is connected to external structures via an extracellular matrix (ECM), which we do not explicitly model here. However, one could take the perspective that, by modeling the mechanics of the epithelial sheath to which the ECM provides structural support, we therefore include its influence implicitly. The material properties of a tissue differ from those of individual cells which comprise it. Many studies have shown that multi-cellular environments develop emergent behavior, such as tissue jamming (12,13). Additionally, due to the fragility of the structure and the difficulty of experimentation, the material properties of chordoblasts, chordocytes, and the emergent properties
of the notochord itself remain speculative. Packed chordocytes have thin membranes relative to their fluid-filled vacuoles. Chordocyte mechanics are simplified to the internal pressure of their vacuoles and cortical tension of their membranes in our models, and are assumed to be closely packed. Chordoblasts are modeled as a thin, continuous surface, dominated by surface tension. As such, the notochord is modeled as a dry foam contained in a thin, flexible tube. While chordocyte packing patterns could be modeled using a variety of techniques (e.g. Cellular Potts), the use of continuum modeling facilitates the use of continuum mechanics to investigate bending and flexural rigidity in later simulations.

1.2.3 Modeling the Notochord as a Foam

Foams are collections of pockets of trapped fluid. These pockets (bubbles) can be monodisperse (having equal volume to each other) or polydisperse (having a variety of volumes), and can have substantial liquid volume separating each other (wet foam), or be directly in contact via a thin interface (dry foam). de Gennes, Kraynik, Hutzler, Pittet, and Weaire have pioneered the study of the physics of foams and soft matter (14–16). Of particular interest, as it relates to modeling the notochord, are experiments and simulations on bubbles packed in cylinders (17–19).

A key finding from these works is that the patterning of bubbles in these containers is controlled by \( \lambda \), a geometric ratio which is interpreted as the number of bubbles per unit length. \( \lambda \equiv D/d \), where \( D \) is the diameter of the tube, and \( d \) is the diameter of a sphere with the same volume as a bubble. Previous work has shown that while there are bifurcations in the \( \lambda \)-space between patterns, there are also regions where patterns can overlap (17,19). In the case of monodisperse foams in regular containers, \( \lambda \) will be equal everywhere. In polydisperse foams or containers whose diameter \( D \) changes, \( \lambda \) will vary throughout, thus in this case, the packing pattern may also vary.

The types of patterns that form and the transitions between them have been studied in great detail in regular cylinders (20), mechanical models of notochords as gel beads in silicone tubes and statistical analysis of notochords (21), and recent simulations of infinite notochords as liquid foams (e.g. soap films) (19). Equivalent patterns have been found in hard-sphere and soft-sphere models (22–26). Many of these patterns are now named, and in particular we will focus on two of the lowest-order patterns which are commonplace in foams contained in a tube, and most statistically representative of packing of chordocytes in the notochord. The pattern in which bubbles stack directly on top of one another, with each cell having one neighbor on each side, is called ‘bamboo’; bubbles which stack in an alternating left-right or dorsal-ventral direction, with each bubble having between two and four adjacent neighbors, is
called ‘staircase’ or ‘zig-zag’ (Figure 1.1).

In dry foams, the transition between these patterns has been found to occur due to a bifurcation in system energy (27). So, our model will take into account, at minimum, the energy of the system, chordocyte packing patterns, chordocyte internal pressure, and surface tension of both chordoblast and chordocyte surfaces. The physical ratio of chordoblast-to-chordocyte membrane tension is denoted by $\Gamma$.

![Figure 1.1: A 2D schematic of bamboo and staircase patterning.]

\[ H = \frac{1}{2} (\kappa_1 + \kappa_2), \]

where $\kappa_1$ and $\kappa_2$ are measured as reciprocals of the radii of osculating circles.

1.2.4 Physical and Computational Background

There are a few critical phenomena involved in foam physics which we must consider. The first are Plateau's Laws (28), which describe the structure of liquid foams. These can be summarized as follows:

- Soap films are composed of smooth, unbroken surfaces.
- The mean curvature on a section of soap film is constant. Mean curvature $H$ is defined as the average of principal curvatures of a surface, i.e. $H = \frac{1}{2}(\kappa_1 + \kappa_2)$, where $\kappa_1$ and $\kappa_2$ are measured as reciprocals of the radii of osculating circles.
- Soap films meet in threes at a Plateau border with an angle of 120 degrees, and these borders meet in fours at a vertex with a tetrahedral angle of approximately 109.5 degrees.
The curvature of a surface separating two bubbles relates to the pressure difference between them by the Young-Laplace equation, which states:

$$\Delta p = \gamma \left( \frac{1}{R_1} + \frac{1}{R_2} \right)$$  \hspace{1cm} (1.1)

where $\Delta p$ is the pressure difference (interior minus exterior), $\gamma$ is the membrane tension, and $R_1, R_2$ are the principal radii of curvature. Therefore, the pressure difference is proportional to mean curvature.

We model the finite notochord in Chapter 2 as a monodisperse soap film, with each bubble, or cell, having the same volume. Previous work modeled regular two-cell patterns, and $\lambda$ was imposed (19). Toroidal boundary conditions were used to map the bottom surface of cells to their own upper surface, effectively modeling two-cell patterns which would occur sufficiently far away from notochord ends, or, an ‘infinite’ notochord. Since we explicitly model $N$ cells, $\lambda$ is not imposed but instead is a consequence of the assignment of surface tensions via ratio $\Gamma$. Further, we are not constrained to choose toroidal boundary conditions.

We simulate these finite notochords with *The Surface Evolver* (29), a finite element program which minimizes energy of a structure by calculating force at each vertex as the gradient of total energy and moving the vertices iteratively by a choice of gradient descent, conjugate gradient, or stochastic descent method. We determined a relationship between $\lambda$ and $\Gamma$ in this context.

To generate the initial geometries of finite notochords in bamboo and staircase configurations (Figs. 1.2, 1.3), MATLAB code was written to output the .txt files read by the Surface Evolver. These programs are provided in the Appendix. Initial geometries in these finite notochords are composed of stacked prisms, which lie directly on top of each other in the case of bamboo packing, or offset in the left-right direction in the case of staircase packing.

The results of these simulations are discussed in Chapter 2 (30).
Figure 1.2: Creating notochord initial geometries in Surface Evolver. 

A. Two vertices (labeled 1 and 2, blue) are connected by an edge (labeled 1, orange). Edges are defined by two vertices and an orientation - in this case, edge 1 is defined in the direction from vertex 1 to vertex 2 (orange arrow). 

B. Adding two more vertices (labeled 3 and 4, blue) and three more edges (labeled 2, 3, and 4, orange) creates a closed polygon - a face. Faces are defined by traversing edges (green arrow) such that the right-hand rule specifies a direction of normal vector to the face (purple arrow). Here, edges are already defined so that traversing Edge 1 to 4 in order produces an upward normal for this face. 

C. Adding two more vertices (labeled 5 and 6, blue) and three more edges (labeled 5, 6, and 7, orange) creates a second face. Since faces I and II (green roman numerals) share Edge 3, if one needed to define an upward normal for both faces, Face II would need to traverse Edge 3 backward from how it was created. Thus, Face 1 is defined by Edges 1, 2, 3, 4, while Face 2 is defined by edges 5, 6, 7, -3. 

D. Attaching six faces to make a closed cube creates a Body. Faces on the boundary of a body must have outward normals; thus, edges defining Faces I-VI are shown traversed (green arrows) so that the right-hand rule produces only outward normals.
Figure 1.2: (Continued) When two bodies share a face, the edges defining this face must be traversed in opposite directions, depending on which Body is being described. **E.** In this example where two cubes are stacked on top of each other in bamboo pattern (separated to visually show the shared face, magenta, with red dashed lines representing shared vertices), the shared face (Face VI) must be traversed (green arrows) counterclockwise with respect to Body 1, but clockwise with respect to Body 2. For example, if Face VI is originally constructed with an upward normal, then we would reference ‘-6’ when assigning it to Body 2. **F.** A vertex-edge-face-body schematic for two prisms in bamboo configuration. **G.** Here, two prisms in staircase pattern share Face VI (separated to show shared face, magenta, with red dashed lines representing shared vertices). **H.** A vertex-edge-face-body schematic for two prisms in staircase configuration.
Each prism in our model is defined by eight vertices, each of which is denoted by an identifying number and unique coordinates. Edges are then defined, identified by the two endpoints they connect in the order they are to be traversed - however, if needed, edges can be traversed backwards by negating their identifying number. Edges can be assigned properties like tension. Facets are then defined by the edges which define their boundary; it is important to note that these edges can be referenced in either the clockwise or counterclockwise direction with respect to the definition of a certain face; the edge traversal direction dictates the orientation (inward or outward) of the normal vector to the face, which is important when assigning properties like pressure, and in defining bodies, where faces should be defined with their normal vector outward with respect to the body they enclose. Finally, bodies are comprised of surrounding facets all having a normal oriented the same way with respect to the body they surround. Bodies can be assigned properties like a constant internal volume or pressure, but not both. From an initial geometry via reading a .txt file with all vertices, edges, facets (2D), and bodies (3D) defined, the Surface Evolver evolves the initial geometry via conjugate gradient method by minimizing the energy of the surface. Total energy \( E_{\text{tot}} \) in the finite notochords discussed in Chapter 2 is given by:

\[
E_{\text{tot}} = \sum_i P_i V_i + \sum_j \gamma_j A_j.
\]
The first term represents the total body energy, which is the sum over all bodies of their prescribed volume $V_0$ multiplied by their respective pressures, where pressure is calculated to preserve the prescribed volume. The second term represents the total surface energy, which is the sum over all facets of their respective surface areas multiplied by their prescribed surface tensions, dependent on whether the surface denotes a cell-cell membrane or sheath-cell membrane. The Surface Evolver also tracks energy due to line tension of edges, though we do not consider line tension in this model.

### 1.2.5 Implementation

This model was implemented using the finite element program, *The Surface Evolver*. The initial geometry was constructed using a *MATLAB* program to create a .txt file read by *Surface Evolver*. The process is described and the code is included in the Appendix. Statistical analysis was performed in *JMP* and *Mathematica*. The results of this study are discussed in detail in Chapter 2.

### 1.2.6 Modeling the Notochord as an Elastic Membrane

To address how flexural rigidity of notochords are affected by their internal cell packing, we model them as composed of pressurized elastic membranes, which have a zero-stress configuration, rather than as liquid foam, which does not support bending. Also, without volume or pressure constraints, a liquid foam would have a minimal-energy configuration with a surface area of zero.

It is an unfortunate example of a ‘jargon homonym’ that we use *mechanical* membranes to model *biological* membranes. Elastic membranes, like shells, are 3D plane stress elements which can deform in both in-plane and out-of-plane directions. Unlike shells, membrane elements do not have bending stiffness. Since we assume that cell cortices are thin relative to their surface area, the *physical* membrane model is numerically better posed than a shell formulation. So, pressurized membrane elements are well suited to represent cell cortices, which produce tension beneath *cell* membranes.

In Chapter 3, we will consider a constant pressure constraint, with cell volumes not constrained to be equal. We model membranes as having a linear elastic constitutive law. Following an approximation of the notochord structure by truncated spheres as described in (30) (Appendix), we create these notochords as collections of truncated spheres separated by ‘septa’, membranes which are cell-cell interfaces. Sheath-cell membranes are pressurized, representing the internal vacuole pressure of chordocytes, and their Young's Moduli $E$ differ between sheath-cell ($s_c$) and cell-cell surfaces ($c_c$) by a constant stiffness ratio $\beta \equiv E_{sc}/E_{cc}$. In contrast
to Chapter 2, $\Gamma$ is not constrained; instead, it is calculated post-hoc by multiplying average stress $\bar{\sigma}$ and average thickness $\bar{t}$ over sheath-cell and cell-cell surfaces - that is, $\Gamma = (\bar{\sigma} \bar{t})_{sc}/(\bar{\sigma} \bar{t})_{cc}$. Varying internal pressure $p$ and stiffness ratio $\beta$, then, will vary $\Gamma$, and a relationship between these is determined. Further, implementing boundary conditions consistent with three-point bending allows for a bending study in which internal pressure $p$, stiffness ratio $\beta$, and force $F$ are varied. The maximum displacements $\delta_{\text{max}}$ can then be used to calculate flexural rigidity $EI$ via Euler-Bernoulli beam theory (31).

1.2.7 Implementation
Modeling of linear elastic membranes was implemented in COMSOL Multiphysics Membrane interface, found in the Structural Mechanics branch. The results of these pressure and bending studies are detailed in Chapter 3.

1.3 Modeling Stomach Morphogenesis

1.3.1 Biological Background
In vertebrates, the stomach anlage originates from the posterior foregut, adjacent to the esophagus, liver, pancreas, and intestine. Two mesenteries - dorsal and ventral - connect the gut to the abdominal wall on the respective sides. The dorsal mesentery is attached along the entire gut, while the ventral mesentery ends at the foregut. A number of signals - including WNT, FGF, BMP, and Retinoic Acid, among others - are responsible for segmentation of the foregut into sites which will become organs of the gut (32). Cilia in left-right organizer (LRO) regions create a directional fluid flow, which causes local differences in gene expression throughout the organism, establishing the left-right (LR) axis. This leads to the expression of growth factor Nodal, and ultimately an expression of transcription factor Pitx2 on the left side of the gut (33). Though the foregut is initially symmetric, these mechanisms cause it to develop left-right asymmetry. Failure to develop this asymmetry (heterotaxy) leads to myriad malformations or disorders, such as situs inversus, intestinal malrotation, and asplenia/polysplenia, among others (34–37).

In typical development, the stomach develops ‘greater’ and ‘lesser’ curvature, which are necessary for proper digestive function. The greater curvature is named for the longer curve on the left side of the stomach, and the lesser curvature is named for the smaller curve on the right - we note in an unfortunate instance of ‘jargon homonyms’ that, were one to use a naming convention in line with the mathematical definition of curvature, these would be opposite.
There are two prevailing hypotheses to how these curvatures develop. The first model posits that a rotation of the stomach about its dorsoventral axis creates the greater curvature from the dorsal wall, and the lesser curvature from the ventral wall (38). The mechanism behind this rotation has not yet been explained. The second model posits that differential growth is responsible for the development of stomach curvature. A recent study has shown that stomach curvature is independent of rotation, and documented radial thinning and rearrangement on the left side (39). This agrees with early theories of an asymmetric growth mechanism (40). In Chapter 4, we will model various types of growth to determine what types of rearrangement in particular could drive the morphology seen in vivo. To determine which types of rearrangement correspond to that of a typical developing stomach, we inspect simulations for the following morphological attributes:

- Radial thinning of the left side relative to the right.
- Increasing anteroposterior length.
- Migration of the LR midline towards the right.
- Generation of the greater curvature on the left, and lesser curvature on the right.
- Bulging out from the greater curvature, i.e., a widening of the stomach to form a larger cavity.

### 1.3.2 Modeling Stomach Growth

To model growth, we need to keep track of deformations in space and time. We can describe relative contraction or expansion of material between two points via stretch ratios. Stretch ratios are given by

\[ \lambda = \frac{L}{L_0} \]

where \( L \) is the distance between two points in the deformed configuration and \( L_0 \) is the distance between those same points in the initial configuration.

In homogeneous growth, these stretch ratios are applied over the entire domain of interest. Inhomogeneous growth, therefore, is when these stretch ratios are only applied to sections of the domain of interest. In isotropic homogeneous growth, stretch ratios are equivalent in all directions and applied to the entire domain; this does not cause shape change when material stiffness is isotropic, since ratios of dimensions of the body are preserved. In anisotropic growth, stretch ratios will vary in different directions, leading to shape change. Thus, assuming material stiffness is isotropic, differential growth (growth causing shape change) can occur via anisotropic growth, inhomogeneous growth, or some combination of the two.
Following the observations of (39), we will model the stomach as an initially straight cylinder with a hollow elliptic lumen (Figure 1.4). We further assume that as the embryo grows, the stomach grows equally on the left and right sides volumetrically, but that the left side also undergoes radial intercalation.

There are two scenarios we examine, based on results from previous work (41): first, that the change in stomach shape is due to a difference in types of growth between the left and right stomach. While both the left and right sides are growing, the left side is also undergoing rearrangement via radial thinning and compensatory axial and circumferential elongation, called transversely isotropic radial intercalation. In the second scenario, rearrangement is similar, but any radial thinning is compensated by axial elongation only. The key component of this analysis is a time-dependent study, in which growth is iteratively applied to achieve larger total strains than can be realized in just one step; however, due to the intensive nature of the iteration process, investigating many different types of growth regimes, as in (41), quickly becomes unwieldy. As such, we are able to discern between different possibilities of growth regimes, corresponding to a variety of biological mechanisms (e.g. hypertrophy, oriented cell division, intercalation in different directions, etc.). This provides novel insight into the ‘asymmetric growth’ hypothesis of stomach curvature generation.

Models of biological growth in which shape change occurs must include the effect of residual stress; that is, the stress field that may exist in a body when unloaded (1). Modeling deformations directly, without incorporating a constitutive law, can lead to geometric incompatibilities such as gaps or superpositions. Growth and stress must therefore be considered together. This interplay has been famously investigated through the mechanical loading of bones (e.g. Wolff’s Law), and soft tissues (e.g. Davis's Law, Fung's experiments on arteries) alike (1,42).

Among the many potential methods of modeling biological growth (e.g. lattice or off-lattice methods, agent-based methods, mixture models, etc. (1,43)), we will utilize the theory of morphoelasticity, which decomposes deformations into their mechanical and growth components, in order to incorporate both growth and the accompanying residual stress. This theory is based on continuum mechanics, a framework which mathematically describes deformations and their resultant stresses (or vice versa). This framework is well-suited for incorporating growth and elastic stress in soft tissues which can undergo large strains.

1.3.3 Continuum Mechanics and Morphoelasticity

Bodies undergoing shape change are considered to have three possible configurations - the initial configuration, the final deformed (current) configuration, and an intermediate virtual
Figure 1.4: The boundary conditions and the initial geometry of the stomach model. The anteriormost surface (blue) is constrained to have no $z$-displacement. Two points on this surface are prescribed displacement to prevent rotation. The point on the apical (lumen) surface (red) is constrained to move only in $y$, while the point on the basal surface (green) is constrained to move only in $x$. 
configuration. The virtual configuration is a purely mathematical construct rarely realized biologically, as it is stress-free. A position vector describing a point on the body in the initial configuration \((X)\) is mapped to a position vector describing the position of that point in the deformed configuration \((x)\). In three dimensions, separated by component:

\[
\begin{align*}
x_1 &= X_1 + u_1 \\
x_2 &= X_2 + u_2 \\
x_3 &= X_3 + u_3
\end{align*}
\]  

(1.4)

(1.5)

(1.6)

Here, \(u_i\) are components of the displacement vector \(u = x - X\). Then the map from the initial to deformed configuration can be written as:

\[
F_{ij} = \frac{\partial x_i}{\partial X_j}
\]

(1.7)

where \(F\) is called the deformation gradient tensor. Upon differentiating with respect to \(x_1\), \(x_2\), and \(x_3\), we can rewrite the above equation as:

\[
F_{ij} = \delta_{ij} + \frac{\partial u_i}{\partial X_j}
\]

(1.8)

where \(\delta_{ij}\) is the Kronecker delta, and the second term are components of \(\nabla u\). Therefore, \(F\) contains information on local deformations of a body via partial derivatives from the undeformed to deformed configurations.

The modern theory of morphoelasticity was initially described by Rodriguez et. al. (44). Similar descriptions to the following can be found in related work (1,41). Following (1,41,44) \(F\) is multiplicatively decomposed, using finite strains, into \(A\), the elastic deformation tensor, and \(G\), the growth deformation tensor; so, we can write \(F = AG\). Deforming the initial body by only considering \(G\) can sometimes result in geometric incompatibilities, such as self-overlapping. This is the stress-free, intermediate virtual configuration introduced earlier. \(A\) then maps the virtual configuration to the final, current configuration, resolving geometric incompatibilities while introducing residual stresses and strains. The decomposition of \(F\) into \(AG\) is due to the polar decomposition theorem, which states that for a second order tensor \(F\) with \(\det(F) \geq 0\), there exist unique, symmetric, positive definite tensors \(U\) and \(V\), and a unique proper orthogonal tensor \(R\) such that:

\[
F = RU = VR
\]

(1.9)

\(U\) and \(V\) are the left and right stretch tensors, and \(R\) is the rotational component of the defor-
Their squares relate \( \mathbf{F} \) to the Cauchy-Green tensors:

\[
\mathbf{F}^T \mathbf{F} = \mathbf{U}^2 = \mathbf{C} \quad \text{(1.10)}
\]

\[
\mathbf{F} \mathbf{F}^T = \mathbf{V}^2 = \mathbf{B} \quad \text{(1.11)}
\]

where \( \mathbf{C} \) and \( \mathbf{B} \) are the right and left Cauchy-Green tensors, respectively. The principal invariants of \( \mathbf{C} \) are involved in various strain-energy density functions \( W \). They are:

\[
I_1 = \text{trace}(\mathbf{C}) \quad \text{(1.12)}
\]

\[
I_2 = \frac{1}{2} (I_1^2 - \text{trace}(\mathbf{C}^2)) \quad \text{(1.13)}
\]

\[
I_3 = \text{det}(\mathbf{C}) \quad \text{(1.14)}
\]

Similarly, \( \mathbf{G} \) can also be decomposed as \( \mathbf{G} = \mathbf{R}_G \mathbf{U}_G \) where the tensor \( \mathbf{U}_G \) is symmetric, and the tensor \( \mathbf{R}_G \) is orthogonal. For our model of stomach growth, we assume tissue material properties are isotropic. It follows that the strain-energy density function \( W \) satisfies \( W(\mathbf{A}) = W(\mathbf{AR}) \) for a proper orthogonal tensor \( \mathbf{R} \) - that is, a tensor with \( \text{det}(\mathbf{R}) = 1 \) and with \( \mathbf{R}^T = \mathbf{R}^{-1} \). Since \( \mathbf{G} \) can be decomposed, it follows that:

\[
W(\mathbf{A}) = W(\mathbf{FU}_G) \quad \text{(1.15)}
\]

Thus, the strain-energy density function \( W \) depends only on the symmetric component of \( \mathbf{G} \) under our assumption of isotropic material properties. The components of \( \mathbf{G} \) are growth stretch ratios \( \lambda \) defined by their relationship with strains \( \epsilon \) in the \( i \) \( j \) direction by \( \epsilon_{ij} = \lambda_{ij} - 1 \). Therefore, \( \mathbf{G} \) can be expressed as:

\[
\mathbf{G} = \begin{bmatrix}
\lambda_{aa} & 0 & 0 \\
0 & \lambda_{bb} & 0 \\
0 & 0 & \lambda_{cc}
\end{bmatrix} \quad \text{(1.16)}
\]

where \( a, b, \) and \( c \) are coordinate directions of an orthogonal local coordinate system, described in the implementation section.

The Cauchy stress tensor \( \mathbf{T} = \hat{n} \sigma \), with \( \hat{n} \) the normal to the surface, contains components of stress \( \sigma_{ij} \) for each coordinate basis, in each direction. We utilize the equation of motion:

\[
\nabla \cdot \mathbf{T} + \rho \mathbf{b} = \rho \mathbf{v} \quad \text{(1.17)}
\]

and since we assume body forces are zero \( (\mathbf{b} = 0) \) and movement is only a result of growth or
rearrangement ($v = 0$), this reduces to:

$$\nabla \cdot T = 0. \quad (1.18)$$

The Cauchy stress tensor used in this model is symmetric since we do not consider moments at equilibrium. It relates to the elastic deformation tensor and strain-energy density function as follows:

$$T = T^T = J^{-1}_A A \frac{\partial W}{\partial A} - p I \quad (1.19)$$

where $J_A = \text{det}(A) = 1$. Since we assume stomach tissue does not change volume due to an elastic response, we assume the tissue to be incompressible.

**Neo-Hookean Hyperelasticity**

Due to our assumption of incompressibility, along with the observation that the stress-strain relationship in developing tissue is linear for moderate ($\approx 30\%$) stretch regimes (45), we chose to use the neo-Hookean hyperelastic model. Materials are considered hyperelastic if they continue to respond elastically after large deformations, common in materials like rubber or foam, and often used for modeling biological materials. Specifically, hyperelastic materials have strain-energy density functions whose derivative with respect to a strain component determines the corresponding stress component. In the case of incompressible materials, the strain-energy density function for the neo-Hookean formulation is:

$$W = \frac{\mu}{2} (I_1 - 3) \quad (1.20)$$

where $\mu$ is a Lamé coefficient corresponding to the shear modulus in the context of continuum mechanics, and $3\mu$ is the Young's modulus.

**Morphoelastic Framework Description**

We can summarize the previously described concepts and equations under a morphoelastic framework used in Chapter 4.

$$F = AG$$, the deformation gradient tensor \quad (1.21)

$$\frac{\Delta V}{V} = \text{det}(G)$$, the volume change equation \quad (1.22)

$$\nabla \cdot T = 0$$, the equation of motion \quad (1.23)
\[ T = T^T = J^{-1} A \frac{\partial W}{\partial A} - p I, \] the constitutive law \hspace{1cm} (1.24)

\[ W = \frac{\mu}{2} (I_1 - 3), \] neo-Hookean strain-energy density function \hspace{1cm} (1.25)

The implementation of this framework is discussed in more detail in the following section.

1.3.4 Local Coordinates

As the stomach anlage deforms, implementing growth and boundary conditions in anatomically relevant directions is not possible in Cartesian or cylindrical coordinates. In fact, using Cartesian or cylindrical coordinates is burdensome from the outset, since we begin with an initially elliptical cylindrical lumen.

Instead, we define a bespoke local coordinate system for each implementation of growth (discussed in the following section). By doing so, we can diagonalize \( \mathbf{G} \), expressed in these local coordinates. In COMSOL, this is achieved through the creation of Curvilinear Coordinates (Figure 1.5). First, COMSOL solves the Laplace equation \( \nabla^2 q = 0 \) on the domain. The Laplace equation arises in many contexts; here, we consider \( q \) to be the potential of a velocity field of an incompressible fluid with irrotational flow, so \( q \) can be thought of in this context as pressure.

To create this potential, we define two surfaces with constant pressures; an inlet \( (q = 1) \), and an outlet \( (q = 0) \), without loss of generality. The remaining surfaces are ‘walls’ where there is no flux \( (\hat{n} \cdot \nabla q = 0) \). The resulting vector field \( -\nabla q \) describes the direction of a gradient which follows curves (streamlines) through the domain; this is the first local coordinate direction. Vectors in this resultant vector field are the first basis vector in the local system, \( v_1 \). Vectors \( v_2 \) in the second coordinate direction, which is user-defined, can be calculated in a number of ways. Equipotential lines are lines on which \( q \) is constant, and these lines are always orthogonal to the direction of flow. So, by choosing a vector in the direction of constant potential at some point on a streamline, we assign this as the second basis vector at that point, orthogonal to the first. The third direction must be orthogonal to the first two, and is calculated via cross product. Therefore, the basis vectors \( (e_1, e_2, e_3) \) in the local coordinate system can be calculated from vectors \( v_1 \) and \( v_2 \) as follows:

\[ e_1 = v_1 \] \hspace{1cm} (1.26)

\[ e_2 = v_2 - (v_2 \cdot e_1) e_1 \] \hspace{1cm} (1.27)

\[ e_3 = e_1 \times e_2 \] \hspace{1cm} (1.28)

An example curvilinear coordinate system is shown below.
1.3.5 Growth as Thermal Expansion

Once a local coordinate system is created, each of the three local coordinate directions at some point describes a more anatomically relevant direction in the deformed geometry at each step in the time-dependent problem, which we can utilize to apply growth and boundary conditions. We use thermal expansion in COMSOL as a proxy for growth, as these are mathematically analogous. Growth is then implemented as:

\[ \mathbf{G} = \mathbf{I} + \mathbf{\epsilon}_{\text{th}} = \begin{bmatrix} 1 + \alpha_1(T - T_{\text{ref}}) & 0 & 0 \\ 0 & 1 + \alpha_2(T - T_{\text{ref}}) & 0 \\ 0 & 0 & 1 + \alpha_3(T - T_{\text{ref}}) \end{bmatrix} \]  

(1.29)

where \( \mathbf{I} \) is the identity tensor, \( \mathbf{\epsilon}_{\text{th}} \) is the thermal strain tensor, \( \alpha_i \) are the growth strains corresponding to each local coordinate direction, and \( (T - T_{\text{ref}}) = 1 \). Recall that \( \lambda_i = 1 + \epsilon_i \), so these inputs correspond to stretch ratios in each coordinate direction. To this point, we have been following the framework first implemented in (41). We extend this procedure by remeshing the deformed geometry, thus can continue implementing \( \mathbf{G} \) as many times as allowed by our choice of mesh and convergence threshold. This allows for modeling phenomena as in Chapter 4, where \( \mathbf{G} \) is iteratively applied, which generates much larger strains than could be performed in a single time step. However, one could model biological processes in which \( \mathbf{G} \) could be
applied to different domains throughout the process, for example in the case of modeling the stomach and surrounding structures, or where \( \mathbf{G} \) itself is updated as cell behaviors inducing growth of the tissue change over successive time steps. Thus, this sustained-growth modeling framework can be applied in many more ways than could be done previously.

For example, in the time-dependent model discussed in Chapter 4, \( \mathbf{G} \) is applied on the anatomical left side of the domain to study the asymmetric growth hypothesis. We took \( \lambda_r = 0.9 \) there, and since we modeled tissue rearrangement separately from isotropic growth, \( \det(\mathbf{G}) = \lambda_r \lambda_\theta \lambda_z = 1 \). In the case of radial thinning with volume-conserving axial elongation as shown in Chapter 4, \( \lambda_r = 0.9, \lambda_\theta = 1, \) and \( \lambda_z = 1/\lambda_r = 1.1 \). \( \mathbf{G} \) was applied ten times for total stretch ratios of \( \lambda_r = 0.9^{10} \approx 0.35, \) and \( \lambda_z = 1.1^{10} \approx 2.87 \). Another example presented in Chapter 4 is \( \lambda_\theta = \lambda_z = 1/\sqrt{0.9} \approx 1.054 \) in the case of radial thinning with volume-conserving, equivalent circumferential and axial elongation. Here, \( \mathbf{G} \) was applied eighteen times, for total stretch ratios of \( \lambda_r = 0.9^{18} \approx 0.15, \) and \( \lambda_\theta = \lambda_z = 1/\sqrt{0.9^{18}} \approx 2.58. \)

### 1.3.6 Boundary Conditions

The anteriormost surface was given a roller condition - it was fixed from moving along the anteroposterior axis, but allowed to expand or contract in the transverse plane, either dorsoventrally or left-right. Further, one point on the lumen on this same surface was chosen to only move along the left-right axis, while one additional point - also on the anterior-most surface, on the opposite side of the lumen - was prevented from moving in the dorsoventral direction. This combination of conditions achieved two things: first, the anterior-most section of gut tube is assumed to be fixed, representing the esophagus of the organism. Second, prescribing displacement of two additional points in the described way fully determines the system of equations.

### 1.3.7 Time Dependence and Residual Stress

Once growth is implemented, the resultant deformed geometry is remeshed and used as the initial geometry for the following study. In doing so, stress fully dissipates between iterations. This assumes that the time scale for growth is slower relative to the time scale for stress dissipation. This is in accordance with the hyper-restoration principle, which has been observed experimentally (46–49). In brief, the hyper-restoration principle states that biological tissue responds to a change in stress by generating forces which restore, then overshoot, the original (target) stress. The results for this time-dependent study of stomach morphogenesis are discussed in detail in Chapter 4.
1.3.8 Implementation

The stomach model was implemented using the Nonlinear Structural Materials module and the Structural Mechanics module of the finite element solver, COMSOL Multiphysics.
1.4 References


CHAPTER

2

MODELING NOTOCHORD MORPHOMETRY

The following work, coauthored with Dr. Sharon R Lubkin, has been published in *Cells & Development* under the following citation:

**Title**
Physical models of notochord cell packing reveal how tension ratios determine morphometry

Physical models of notochord cell packing reveal how tension ratios determine morphometry

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1. Introduction

Chordates are defined by their notochord, an axial hydrokeleton (Fig. 1A). In vertebrates, the notochord functions to elongate the embryo and template spine development. Disruption of normal notochord development can lead to severe functional deficits such as scoliosis (Stemple, 2005; Corallo et al., 2015; Bagnat and Gray, 2020; Ellis et al., 2013a,b). In zebrafish, after convergent extension, the notochord consists of interior cells (chordocytes) containing a large vacuole, surrounded by a sheath composed of a squamous simple epithelium (chordoblasts) and an extracellular matrix (ECM) (Stemple, 2005; Waddington and Perry, 1962; Witten and Hall, 2022).

It has been previously shown that the vacuolated cells in a zebrafish notochord pack in a small number of stereotypical patterns, which are statistically dominated by low-order patterns, commonly called "bamboo" and "staircase" (Norman et al., 2018) (Fig. 1B). In wild-type (WT), bamboo packing was most common at the tail end, and staircase packing dominated away from the tail end, the staircase cells alternating positions laterally. Disruption of this characteristic packing pattern, by mutation, ablation, or other means, has been shown to be disruptive to downstream development (Ellis et al., 2013a,b).

The cell packing patterns in WT and mutant notochords have been shown to be governed by the geometric ratio λ, defined as cells per unit length (Fig. 1C). This dimensionless ratio also governs the regular packing patterns of bubbles in a tube (Hutzler et al., 1997; Mann and Stephens, 1953; Pittet et al., 1996; Reinelt et al., 2001; Tobin et al., 2011; Weaire and Hutzler, 2001) and the irregular packing patterns of gel beads in rigid and flexible tubes, which are statistically indistinguishable from notochord cell packing patterns (Norman et al., 2018). Bamboo patterns are only observed at the lowest range of λ in simulations and experiments, whether in gel beads, soap bubbles, or notochords. Staircase patterns, in all these systems, are observed over a higher λ range; other patterns are only observed for still higher λ values (Tobin et al., 2011; Sorrell, 2019; Sorrell and Lubkin, 2022).

The staircase pattern is associated with an ovaling of the transverse cross-section; other packing patterns exhibit a round transverse cross-section (Norman et al., 2018). The relationship between transverse eccentricity (Fig. 1D) and the staircase pattern is seen in both notochords and gel bead systems. Gel beads in transversely ovalled rigid tubes were found to pack in staircase pattern at lower λ values than in round tubes. Conversely, packing gel beads in flexible silicone tubes at λ values consistent with staircase patternning causes the flexible tube to become correspondingly oval in transverse cross-section (Norman et al., 2018).

Encouraged by the agreement between the observations in zebrafish notochords, bubble-in-tube systems, and gel-bead-in-tube systems, Sorrell et al. (Sorrell and Lubkin, 2022) developed a suite of models of the physics of cell packing in the notochord. These models considered the notochord to be infinitely long, with a homogeneous sheath, and with
interior cells, of fixed volume and surface tension, packed in regular low-dimensional patterns. The three models differed in the treatment of the sheet, ranging from rigid to flexible. These models confirmed the bidirectional relationship between staircase pattern and eccentricity of the transverse cross-section. They quantified the relationship between the eccentricity resulting from staircase pattern and the model parameters, and found that eccentricity was independent of $\lambda$, but depended significantly on the ratio $F$ of surface tensions of the sheet ($\gamma_s$) and interior cells ($\gamma_c$).

The previous models of cell packing in the notochord (Sorrell and Lubkin, 2022) imposed the ratios $\lambda$ (cells per unit length), and $F$ ($=\gamma_s/\gamma_c$). However, imposing $\lambda$ was only necessary because the notochord was modeled as infinitely long. A natural notochord, or a finitely long flexible tube of gel beads, would have $\lambda$ determined by the tension ratio $F$; alternatively, fixing $\lambda$ would determine the tensions and the tension ratio.

In this paper, in order to establish the relationship between the tension ratio $F$ and the length ratio $\lambda$, we model the notochord as containing a finite number of cells, packed in regular patterns, in a flexible but uniform sheet. This finite-length framework allows us to study properties, such as pressure, eccentricity (or aspect ratio), and taper, which vary along the length of the notochord.

2. Methods

2.1. Model structure

Our model of cell packing in the notochord neglects subcellular details, and focuses on surface mechanical forces governing cell shape. It treats cells in a tissue as physically equivalent to bubbles in a foam, following long-established laws governing force balances, internal angles, etc. (Plateau, 1873). The model uses the following simplifying assumptions:

- The notochord consists of a sheath and interior cells, with negligible space between them. This is the cellular equivalent of a dry foam.
- The notochord is of finite length and contains $N$ vacuolated interior cells (chondrocytes).
- Interior cells have uniform constant volume $V$ and variable surface area, and are packed in topologically regular patterns (‘bamboo’ or ‘staircase’) (Fig. 1B).
- Internal vacuolated cell mechanics are governed by uniform net surface tension $\gamma_s$ (Brodland et al., 2009) (Fig. 1E). Other cellular details are neglected.
- The sheath is thin relative to the diameter of the notochord. Individual sheath cells (chondroblasts) and ECM features are neglected, and the sheath is considered to be a continuum, with negligible thickness, and uniform surface tension $\gamma_c$ (Fig. 1E).

These assumptions correspond to the infinite flexible sheet model developed in Sorrell and Lubkin (2022), with two differences:

- The infinite (toroidal) boundary condition is omitted
- $\lambda$ is now a free variable, rather than an imposed parameter.

As in Sorrell and Lubkin (2022), because adjacent surfaces’ tensions act in parallel, each cell-cell interface of area $a$ has surface energy $2\gamma_s a$, and each cell-sheath interface of area $a$, has surface energy $(\gamma_s + \gamma_c) a$. The flexible model from Sorrell and Lubkin (2022) demonstrated relationships between the regular packing patterns and key dimensionless ratios:

- number of cells per unit length, $\lambda \equiv D/d$, where $d$ is the diameter of a sphere of the same volume as the cell $V = (4/3)\pi d^3/2^3$, and $D$ is the

![Fig. 1. A. 2D illustration of a 48 hpf zebrafish, showing anatomical position of notochord (magenta). B. Lowest-order regular cell patterns, “bamboo” (left) and “staircase” (right). Top: 2D schematic; bottom: 3D shapes of model pairs of interior vacuolated cells. Cell-cell boundaries (magenta) and sheath-cell boundaries (cyan) are modeled as single surfaces with respective surface tensions $2\gamma_s$ and $\gamma_s + \gamma_c$.

C. A key nondimensional ratio is $\gamma_s/\gamma_c$ defined as the ratio of the major ($M$) to minor ($m$) axis lengths. The eccentricity $e$ of this ellipse is given by $e = \sqrt{1 - a^2}$, assuming $a \geq 1$. Right: Eccentricity $e$ increases from 0 (circle) and approaches 1 (becoming flatter). E. Another key nondimensional ratio is $\Gamma = \gamma_s/\gamma_c$, the ratio of sheath tension to inner cell tension. Unequal surface tensions at a cell-cell junction are shown in a force diagram. The contact angle $\theta$ satisfies the Young-Dupré equation (Schrader, 1995).](image-url)
diameter of a cylinder with cell volume $V$ and height $h$ (centroid-centroid distance) (Fig. 1C).
- shear aspect ratio $\alpha$ or eccentricity $e = \sqrt{1 - \alpha^{-2}}$ (Fig. 1D).
- surface tension ratio $\Gamma = \rho_0/\rho_1$ (Fig. 1E).

Our extension of the flexible model (Sorrell and Lubkin, 2022) fixes $\Gamma$, but not $\alpha$; thus, $\Gamma$ fully determines the resulting $\alpha$ and $e$ for the bamboo and staircase patterns experimentally measured in Norman et al. (2018), and mechanically modeled in Sorrell and Lubkin (2022).

We vary the parameters for cell counts $N = 20, 50, 70, 100,$ and 150, and tension ratio $\Gamma = 0.5, 1, 2, 3.5, 7,$ and 10, for each of the two patterns (bamboo and staircase).

An alternative model was also investigated, constraining cell pressures rather than volumes. However, that model displayed an instability, with the most distal cell volumes sequentially shrinking to zero, a consequence of the phenomenon outlined in the two-balloon experiment (Merritt and Weinhaus, 1978). Therefore, it was not studied in depth.

2.2. Computation of energy-minimized structures

Each instantiation of the model is calculated by minimizing surface and body energies subject to the constraints described in Section 2.1. We calculate the minimal surfaces using the finite element program Surface Evolver (Brakke, 1992). The volume constraint is enforced with Lagrange multipliers corresponding to the cell internal pressures.

Simulations are initialized with cells represented as prisms, via a custom MATLAB script. Initial prism-packaging geometry is either in series for the bamboo pattern or in a staggered arrangement for the staircase pattern (Sorrell, 2019). These arrangements are topologically equivalent to the respective patterns, but not geometrically at minimum energy.

The Surface Evolver minimizes energy by refining and adjusting the mesh, following a conjugate gradient process with noise (to avoid false minima). Each step in the iterative process involves calculating energy gradients on vertices, then calculating the projecting vectors according to body volume constraints, then moving the vertices down the energy gradient. The simulations terminate at a predetermined convergence criterion.

2.3. Analysis of model notochords

Geometry, pressure, energy, and volume data of minimal-energy model notochords were exported from Surface Evolver for further analysis. Energy is reported only for the entire structure, not for individual cell bodies. Cell number $n$ indexes successive cells along the anteroposterior axis. Pressure, volume, and geometry data were reported for each cell with index $n$ from 1 to $N$. Volumes were verified and cell centroids were calculated in Mathematica. This method has previously been verified against analytical solutions for related systems such as a bubble pair (Sorrell, 2019).

The length of the tapered ends, i.e. the taper length $L(x)$, was calculated by fitting local pressure curves $p(n)$, for each $\Gamma$ and pattern, to a function of the form

$$p(n) = p_{\text{end}} + (p_{\text{end}} - p_{\text{mid}})\exp(-cn/L(x)).$$

scaling the completeness of decay by the parameter $c$ (described in Section 2.3).

In contrast to the models previously developed (Sorrell and Lubkin, 2022), our model does not constrain eccentricity or $\alpha$; these are calculated post-hoc. Local aspect ratio $\alpha(n)$ was calculated by projecting a cell and its nearest neighbor(s) longitudinally onto the $x$-$y$ plane. Finding the maximum differences between all $y$-coordinates and all $x$-coordinates, respectively, yielded the major and minor axes $M(n)$ and $m(n)$, giving $\alpha(n) = M(n)/m(n)$. Eccentricity $e(n)$ is then calculated as $e(n) = \sqrt{1 - \alpha(n)^{-2}}$.

The length ratio $\lambda \equiv D/d$, cells per unit length, was calculated by several complementary methods:

- The overall length ratio is calculated as $\lambda_{\text{tot}}(N) = N/L$, where $L$ is the overall notochord length. The ratio varies with total cell count $N$, due to the tapering end zone, where cells pack differently from the rest of the length.
- A trimmed $\lambda$ for the entire structure was calculated similarly, using the length of the notochord remaining after removing $T$ cells comprising the taper at each end.
- Local $\lambda(n)$ was calculated based on cell volumes $V$ and centroid-centroid distances $h(n)$, relating the equivalent volumes of spheres and cylinders. For a sphere of diameter $d$ and a cylinder of height $h$, diameter $D$, and base area $A$, cell volume is $V = \pi d^2 h/6$. Since, for longitudinal coordinate $z$, $dV/dz = A - V/h$ where $h(n)$ is the local centroid-centroid distance in the $z$ direction. Therefore, at each cell, $\lambda(n)$ is found as $\sqrt{2d/3h}$ where $d = \sqrt{3}V/\pi h$. These $\lambda(n)$ values are then smoothed by central differences.

$$V = \left(\frac{4}{3}\pi d^2/2\right)^3 = Ah = \pi D/2y h$$

Since computed model outcome measures reached consistent values away from the end zones, independent of $N$, we developed models of simplified geometries, for purposes of estimating the relationships of these variables with the tension ratio $\Gamma$ (derivations in Appendix A).

Nondimensional pressure was estimated as

$$P_0(\Gamma) \equiv p_0/f_0 = (1 + \Gamma(2/\lambda + 3\lambda^2/(1 + \Gamma)))$$

for bamboo and staircase, respectively. Similarly, the cells per unit length were estimated as

$$\lambda_0(\Gamma) = \sqrt{2\lambda^2(3\lambda/2 + 3\lambda^2 + 1)^1/\lambda}$$

$$\lambda_1(\Gamma) = \left((\lambda^2/9\pi)(1 + \lambda^2)/\Gamma(\Gamma + 1)\right)^1/\lambda$$

where $I(\Gamma)$ is an integral expression that must be evaluated numerically (Appendix A). Finally, for the staircase pattern, aspect ratio and eccentricity were modeled as

$$a(\Gamma) = (\Gamma + 2)/(\Gamma + 1)$$

$$e(\Gamma) = \left(1 - ((\Gamma + 1)/(\Gamma + 2))^2\right)^{1/2}.$$
morphologies seen in hard-sphere packings (Fu et al., 2016) (Fig. 2), with \( \lambda \) approaching \( 1 / \sqrt{2} \approx 0.707 \) for bamboo and \( 1 / \sqrt{3} \approx 0.577 \) for staircase, and with staircase pattern approaching aspect ratio \( \alpha = 1 + 1 / \sqrt{2} \approx 1.71 \). As \( \Gamma \) approaches \( \infty \), that is, when the interior cell tension becomes negligible relative to the sheath tension \( (t_s \gg t_c) \), the finite notochord approaches a sphere, packed with flattened interior cells. The cell-cell boundaries are not completely flat, even in bamboo pattern, but are slightly concave towards the ends, indicating an increasing pressure as cells approach the ends (Fig. 2, \( \Gamma \approx 10 \) and inset).

Between those extreme tension ratios, simulations show (Fig. 2) that cell and notochord morphology are consistent in the middle region, away from the ends. The end cells are always special cases, regardless of \( \Gamma \) or pattern, due to their lack of neighbors. However, the cells near the end are also generally not of the same morphology as the cells in the middle; there is some tapering of the notochord, visible near the ends, accompanied by increasing concavity of the cell-cell interfaces as the end is approached (Fig. 2, inset). The length of this tapering zone (in cell count) can be seen to have a dependence on \( \Gamma \) (Section 3.3, Fig. 2) as well as on the pattern (bamboo vs. staircase).

### 3.2. Longitudinal variation

In contrast with the infinite-length flexible sheath notochord model in (Sorrell and Lubkin, 2022), the finite-length flexible sheath notochord model allows for local measurements of pressure, eccentricity (or aspect ratio), and \( \lambda \) along its length (Figs. 3A, B, C, 4A, B). Pressure \( p(n) \) and \( \lambda(n) \) both reach consistent asymptotic values \( p_{\text{end}} \approx p(N/2) \) and \( \lambda_{\text{end}} \approx \sqrt{N/2} \) away from the ends, with half-length profiles \( p(n) \) and \( \lambda(n) \) that do not depend on \( N \), except for the shortest notochords \( (N = 20) \) at the highest sheath tension \( \Gamma = 10 \) (Fig. 3B, C). Aspect ratio and eccentricity do not vary significantly along the length of the notochord (Fig. 4).

#### 3.3. Length of taper

Since pressure and \( \lambda \) both approach asymptotic values away from the ends, \( p(n) \) and \( \lambda(n) \) profiles can be used to quantify the number of cells which comprise the taper at each end of the notochord.

#### 3.3.1. Pressure

For equal volume cells, the highest pressure is in the cells closest to the ends, \( n = 1 \) and \( N \) (Fig. 3A, B). Nondimensional asymptotic pressure and \( \Gamma \) are related by the Young-Laplace equation (Young, 1805; Laplace, 1799; Gauss, 1830). The pressure profile reflects the morphology of the taper: cells at (and close to) the ends have a greater proportion of their surface area in contact with the sheath. Thus, for higher \( \Gamma \), a proportionally higher pressure is needed to enforce the equal-volume constraint (Fig. 3A). For cells packed in a staircase pattern, more of their surface area is composed of cell-cell interface than cell-sheath interface, and since for \( \Gamma > 1 \) the cell-cell interfaces will have lower surface tension than the cell-sheath interfaces, this will naturally correspond to lower pressure (and therefore energy) due to the Young-Laplace condition. Thus, when comparing notochords of equal \( N \) and \( \Gamma \), cells in staircase will have a lower pressure and energy than those in bamboo. As vacuole pressurization is essential for proper notochord development (Bagnat and Gray, 2020), the effect of \( \Gamma \) on pressure and the downstream biomechanical implications warrant further study.

In general, the mid-notochord pressure \( p(N/2) \approx p_{\text{mid}} \) does not vary with \( N \), but \( p(n) \) approaches \( p(N/2) \) asymptotically as \( n \to N/2 \). However, at higher values of \( \Gamma \), the mid-notochord pressure \( p(N/2) \) for the \( N = 20 \)-cell model does not agree with the pressure \( p(N/2) \) at higher cell...
numbers \( N \), indicating that 20 cells is too few for comparison. Therefore, it is excluded from later data summaries. For models with more cells (\( N > 20 \)), the number of cells comprising the taper is independent of the total number of cells (Fig. 3B).

For each taper and pattern, taper length \( T(p) \) was calculated by fitting \( p(n) \) to a function of the form

\[
p(n) = \frac{n}{N} + (p_{\text{end}} - p_{\text{mid}}) \exp(-cn/T),
\]

scaling by \( c \) (Fig. 3D). Larger \( c \) corresponds to a more tightly trimmed tapering region. Taper length \( T(p) \) is linear for each pattern \( R^2 > 0.95 \), corresponding roughly to the value of \( \Gamma \) for bamboo and \( 2 \Gamma \) for staircase (Fig. 3E). These statistical fits were best for \( \Gamma > 1 \), where more than 1 or 2 cells contribute to the taper.

### 3.3.2. Lambda

As with pressure \( p(n) \), \( \lambda(n) \) asymptotically approaches a constant value \( \lambda(N/2) \) away from the ends, but does not reach this value for the shortest notochords (\( N = 20 \)). For \( N > 20 \), \( \lambda_{\text{end}} \) is independent of \( N \), but dependent on \( \Gamma \) and pattern (Fig. 3C). However, because of computational noise in the calculation of centroids, which is magnified in calculations of the centroid-centroid distances \( \lambda \), local \( \lambda(n) \) was also noisy. Because of this, we did not attempt to quantify the taper length \( T(\Gamma) \) for \( \lambda(n) \) as we did for \( p(n) \), though the lengths are comparable.

### 3.4. Aspect ratio and eccentricity

Some studies use aspect ratio (Norman et al., 2018), and others use eccentricity (Sorrell and Lubkin, 2022); for purposes of comparison, we calculate both.

Eccentricity of the bamboo pattern is negligible, corresponding to an aspect ratio of 1, and is independent of \( \Gamma \) and \( N \) (Fig. 4). Since the bamboo pattern's transverse cross-section is circular unless constrained in a rigid container of a different shape (Tobin et al., 2011; Sorrell and Lubkin, 2022), these aspect ratios remain close to unity regardless of \( \Gamma \) (Fig. 4B). As with the local \( \lambda(n) \) values, there was substantial noise in \( \alpha(n) \) and \( \alpha_{\text{end}}(n) \) values (Fig. 4A, B). For bamboo, computed eccentricity shows much higher variability than does aspect ratio, because \( \varepsilon = \sqrt{1 - \alpha^{-2}} \) means that \( d\varepsilon/d\alpha \to \infty \) as \( \alpha \to 1 \), which magnifies errors from measuring \( \alpha \). The staircase pattern's aspect ratio and eccentricity have a negligible dependence on \( n \) (even for \( N = 20 \)) and a strong dependence on \( \Gamma \) (Fig. 4).

We screened nonlinear parametric models for staircase eccentricity \( \varepsilon(\Gamma) \), by both \( R^2 \) and information criteria (AIC and BIC). The best nonlinear model found was a biexponential of the form

\[
\varepsilon(\Gamma) = a \exp(-b \Gamma^c) + d \exp(-e \Gamma^f) + g,
\]

where \( a \) through \( g \) are parameters to be estimated.
Fits for eccentricity of the staircase pattern as a function of tension ratio, Table 1 shows more variability in eccentricity than in aspect ratio because of nonlinear relationship $e = \sqrt{1 - \sigma^2}$ between eccentricity and aspect ratio. Asymptotic eccentricity $\epsilon_{\text{asym}}$ (C) and aspect ratio $\alpha_{\text{asym}}$ (D) show a strong relationship with pattern and $\Gamma$ independent of $N$. Asymptotic values approximated by averaging the middle third of cells in the notochord; $N = 20$ excluded. Fitted curves for staircase pattern in (C): 4-parameter biexponential fit (red, solid) for finite notochord model data (red markers) and infinite notochord flexible (black, solid) and semi-flexible (black, dashed) models from (Sorrell and Lubkin, 2022) show close agreement. Star marks a prediction for $\Gamma = 7.9$ corresponding to WT eccentricity $e \approx 0.4$. Analytically derived formulas for $\epsilon(\Gamma)$ (C) and $\alpha(\Gamma)$ (D) for staircase pattern are shown as solid blue curves. Insets: comparison of simulated results against empirically derived values.

We also fitted aspect ratio and eccentricity data to two empirically derived formulas (Appendix A):

\[
e(\Gamma) = a \exp(-b \Gamma) + c \exp(-d \Gamma),
\]

where $a = 0.59 \pm 0.016$, $b = 0.048 \pm 0.0028$, $c = 0.25 \pm 0.013$, and $d = 0.55 \pm 0.046$, with $R^2 > 0.99$ and $p < 0.0001$ for all parameters (Fig. 4C, red curve). These parameters are in remarkable agreement with previous findings for two infinitely-long notochord models with flexible or semi-flexible sheaths (Sorrell and Lubkin, 2022) (Fig. 4C, black curves, Table 1).

We fitted aspect ratio and eccentricity data to two empirically derived formulas (Appendix A):

\[
\alpha(\Gamma) = \frac{(\Gamma + 2)/(\Gamma + 1)}{1 - ((\Gamma + 1)/(\Gamma + 2))^2},
\]

\[
e(\Gamma) = \sqrt{1 - ((\Gamma + 1)/(\Gamma + 2))^2},
\]

with $R^2 = 0.930$ and $R^2 = 0.953$, respectively (inset, Fig. 4C, D). These formulas are less accurate for lower values of $\Gamma$ (higher eccentricity and aspect ratio), since they are derived based on 2D geometry (as explained in Appendix A.2).

### 3.5. Lambda vs. Gamma

Like pressure $p(I)$, the asymptotic $\lambda_{\text{asym}}$ due to the noise (Fig. 3C), we estimated $\lambda_{\text{asym}}$ not by curve fitting $\lambda(n)$, but by using the trimmed $\lambda$ described in Section 2.3, excluding $N = 20$. In both bamboo and staircase patterns, $\lambda$ depends strongly on $\Gamma$ and is independent of $N$ (Fig. 5A). We fitted the data to two empirically derived formulas (Appendix A):

\[
\lambda_{\text{asym}}(\Gamma) = \sqrt{2/3}((3/2)^{1/2} + 3/2)^{1/2},
\]

\[
\lambda_{\text{asym}}(\Gamma) = \left((64/9 \sigma^2)(1 + \Gamma)^{1/4}\right)^{1/8},
\]

(Fig. 5A) with $R^2 = 0.997$ and $R^2 = 0.998$, respectively. $\lambda(I)$ is an integral which must be evaluated numerically.

### 3.6. Pressure vs. Gamma

Pressure values $p_{\text{mid}}$ (away from the taper) were consistent and independent of $N$. Thus, we can analyze the relationship between $I$ and pressure by choosing mid-notochord pressure values $p_{\text{mid}}$ for $N = 150$ for

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Semi-flexible</th>
<th>Flexible</th>
</tr>
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<tbody>
<tr>
<td>$a$</td>
<td>0.59 ± 0.016</td>
<td>0.53 ± 0.05</td>
</tr>
<tr>
<td>$b$</td>
<td>0.048 ± 0.0028</td>
<td>0.048 ± 0.01</td>
</tr>
<tr>
<td>$c$</td>
<td>0.25 ± 0.013</td>
<td>0.27 ± 0.04</td>
</tr>
<tr>
<td>$d$</td>
<td>0.55 ± 0.046</td>
<td>0.57 ± 0.13</td>
</tr>
</tbody>
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each \( \Gamma \). We modeled \( p_{\text{tot}}(\Gamma) \) with empirically derived formulas for each pattern

\[
P_0(\Gamma) = \frac{pd}{\sqrt{\lambda}} - (1 + \Gamma)(2/\lambda + 3\lambda^2/(1 + \Gamma))
\]

\[
P_1(\Gamma) = \frac{pd}{\sqrt{\lambda}} - (1 + \Gamma)(2/\lambda + 3/2\lambda^2/(1 + \Gamma))
\]

(Appendix A) which agreed well with the observed pressures (\( R^2 = 0.996 \)) (Fig. 5B). The data and the formulas show that for any given \( \Gamma \), nondimensional pressure is highest in the bamboo pattern, and lower in the higher-order staircase pattern.

### 3.7. Energy vs. Gamma

Surface Evolver reports total energy \( E \), which yields total energy per cell \( E_{\text{cell}} = E/N \). The energy per cell is composed of surface energy and volume energy, i.e., \( E_{\text{cell}} = E_{\text{surf}} + E_{\text{vol}} \). We obtain \( E_{\text{surf}} = PV \) from pressure data, since \( V \) is constant, and \( E_{\text{tot}} = E_{\text{surf}} + PV \). These are nondimensionalized as \( \Omega_{\text{surf}} = E_{\text{surf}}/(pV_0) \) etc. Nondimensional total, body, and surface energies per cell \( \Omega_{\text{tot}}, \Omega_{\text{body}}, \text{and } \Omega_{\text{surf}} \) are strongly dependent on \( \Gamma \) and independent of \( N \) (Fig. 5G). Because nondimensional volume energy per cell is \( \Omega_{\text{vol}} = E_{\text{vol}}/(pV_0^2) = PV/(pV_0^2) \), where \( d \) corresponds to the diameter of a sphere with volume \( V \), we write \( \Omega_{\text{vol}} = (V/6)pV_0^2(\Gamma)/(pV_0^2) \), where \( pV_0 \) is \( \Omega(\Gamma) \) corresponds to pattern-specific derived formulas:

\[
P_0(\Gamma) = \frac{pd}{\sqrt{\lambda}} - (1 + \Gamma)(2/\lambda + 3\lambda^2/(1 + \Gamma))
\]

\[
P_1(\Gamma) = \frac{pd}{\sqrt{\lambda}} - (1 + \Gamma)(2/\lambda + 3/2\lambda^2/(1 + \Gamma)).
\]

Modeling \( \Omega_{\text{surf}} = 4\Omega_{\text{surf}} \) and \( \Omega_{\text{tot}} = \Omega_{\text{surf}} + \Omega_{\text{vol}} = 2\Omega_{\text{surf}} \) gives a surprisingly consistent fit to the data (\( R^2 = 0.993 \)) (Fig. 5G).

### 4. Discussion

#### 4.1. Summary of findings

Previous models of notochord cell packing, idealizing the structure as infinitely long, established the mechanical significance of ratios for cells per unit length \( \lambda \), tension \( \Gamma \), and eccentricity \( \epsilon \), and their interactions with packing patterns (Sorrell and Lubkin, 2022). Our current study of model notochords of arbitrary length reveals surprisingly robust relationships between model inputs and outcomes, at both local and global levels.

Comparison of models with different cell counts \( N \) revealed, surprisingly, that many relationships are independent of the total number of cells \( N \), for \( N \) above a relatively small value (~20). Near the ends, the model notochords show a taper, with cells becoming narrower and longer, accompanied by a local increase in cell pressure. The length of this taper is determined by \( \Gamma \) and pattern, and is not significantly dependent on \( N \) (Fig. 3).

Pressure, aspect ratio, eccentricity, cells per unit length, surface energy per cell, and total energy per cell were all found to reach limiting values at sufficient distance from the tips, and all of these quantities were found to satisfy empirically derived formulas, for each packing pattern, relating them to the tension ratio \( \Gamma \) (Figs. 4, 5).

We confirm previous findings of the relationship between eccentricity \( \epsilon \) and tension ratio \( \Gamma \) for the staircase pattern (Fig. 4C). Notably, the fit of \( \epsilon(\Gamma) \) agrees remarkably well with that found in flexible and semi-flexible models (Sorrell and Lubkin, 2022), which had assumed an infinitely long notochord.

Because our finite model did not impose the number of cells per unit length \( \lambda \), we were able to determine a relationship between \( \lambda \) and tension ratio \( \Gamma \) (Fig. 5A). For any given \( \Gamma \) and pattern, we have different resulting \( \lambda(\Gamma) \), given by an empirically derived formula (Appendix A). For any given \( \Gamma \), \( \lambda(\Gamma) \) is larger for the staircase pattern than for the...
bamboo pattern, consistent with staircase being a lower-energy packing pattern (Fig. 5C).

Dimensional total, body, and surface energy per cell are lower for staircase than for bamboo (Fig. 5C). Previous experiments (Piotet et al., 1995) and models (Tobin et al., 2011; Sorrell and Lubkin, 2022) showed a bifurcation where, below a critical value of \( \lambda \), bamboo is a lower-energy pattern than staircase. The bifurcation is not seen in our finite models, because unlike in all these previous experiments and models, \( \lambda \) is not imposed, but is a consequence of the imposed tension ratio \( \Gamma \). The minimum possible \( \lambda (\Gamma) - \lambda (0) \) is \( \sqrt{2/3} \approx 0.816 \), and the minimum possible \( \lambda (\Gamma) - \lambda (0) \) is \( 4/3 \approx 1.33 \), which is higher than the bifurcation value of \( \lambda^* \approx 1.13 \) found in all three models from (Sorrell and Lubkin, 2022). Thus, when \( \lambda \) is imposed, there is a bifurcation at \( \lambda^* = 1.13 \); when \( \lambda \) is not imposed, there is no bifurcation, since the staircase pattern never freely attains \( \lambda = 1.13 \).

The finite-length notochord model analyzed in this paper is generally consistent with the infinite-length notochord (Sorrell and Lubkin, 2022), and has additionally explained, with explicit formulas, how the tension ratio \( \Gamma \) determines nondimensional pressures \( P_k(\Gamma) \) and \( P_k(\Gamma) \), staircase aspect ratio \( a(\Gamma) \), and eccentricity \( e(\Gamma) \), cells per unit length \( \lambda(\Gamma) \) and \( \lambda(\Gamma) \), length of taper \( \Gamma(T) \) and \( \Gamma(T) \), and nondimensional volume, surface, and total energies per cell \( \Gamma V_a(\Gamma) \), \( \Omega_a(\Gamma) \), \( \Omega_a(\Gamma) \), \( \Omega_a(\Gamma) \), \( \Omega_a(\Gamma) \), \( \Omega_a(\Gamma) \), \( \Omega_a(\Gamma) \), \( \Omega_a(\Gamma) \), and \( \Omega_a(\Gamma) \). Almost all of these formulas were based on derived relationships from simplified geometric models, which nonetheless fit the data with impressive accuracy. Only the taper lengths and surface-to-volume energy ratios used parameter estimation.

4.2. Formulas provide biomechanical estimates

It is substantially easier to measure cell lengths, widths, and volumes than cell pressures and tensions. Most of the formulas established in this paper can be inverted, to potentially estimate an upper bound of the aspect ratio \( \Gamma \) from quantities that are easier to measure in the lab, such as aspect ratio and cells per unit length. Two of these have straightforward formulas:

\[
\Gamma(a) = (2 - a)/(a - 1)
\]

\[
\Gamma(a) = \frac{1}{\sqrt{1 + \frac{a}{2}} - 1}
\]

There is no simple formula for \( \Gamma(\lambda) \), but for any measured value of \( \lambda(\Gamma) \), \( \Gamma(\lambda) \) can be read off of the graph of \( \lambda(\Gamma) \) (Fig. 5A). The formula for \( \Gamma(\lambda) \) requires \( a > 1 \), and gives a better estimate for lower aspect ratios (higher \( \Gamma \)).

The practical application of these formulas, however, requires some subtlety. There are few published notochord morphology studies that include both cell morphology (Andrews et al., 2021) and packing pattern (Norman et al., 2018). It is important to note that the \( \Gamma \) estimation formulas are specific to each packing pattern, and will not be valid for regions of a notochord packed in other patterns.

A recent paper (Norman et al., 2018) reported pattern, aspect ratio \( \alpha \), and \( \lambda \) in notochords of 40 hpf wild-type zebrafish (\( \lambda = 1.33 \pm 0.14 \)), and in mutants with fewer internal cells and more sheath cells (Notch-enhanced, NICD, \( \lambda = 0.8 \)), or with more internal cells and more sheath cells (Su(H)-inhibited, Su(H)DN, \( \lambda = 1.6 \)). The Su(H)-DN mutants' high \( \lambda \) corresponded to packing patterns more complex and irregular than staircase and bamboo, so our \( \Gamma \) estimation formulas would not be applicable. The NICD mutants showed only bamboo packing, so we can estimate \( \Gamma(\alpha) \) for them. It is interesting that the lowest possible value, \( \Gamma = 0 \), corresponds to \( \lambda(\Gamma) = \sqrt{2/3} \approx 0.816 \), which is extremely close to the reported \( \lambda \), suggesting that, for the NICD mutants, \( \Gamma = 0 \), i.e., \( \lambda(\Gamma) = 1.33 \). As for the WT, the mean \( \lambda \) = 1.33 corresponds to \( \Gamma = 2.34 \) for bamboo packing, and \( \Gamma = 0.41 \) for staircase (the predominant pattern). It is, however, hard to use these estimates from a mean value of \( \lambda \) to infer the corresponding \( \Gamma \), because there is substantial variability in the patterns within an individual. Nonetheless, these estimates suggest a range for \( \Gamma \) of approximately 0.4–2.3, with the higher values corresponding to bamboo packing, which was more commonly seen near the ends of the notochords.

In theory, the aspect ratio can also be used to estimate \( \Gamma \). The WT notochords, which were predominantly in the staircase pattern, had a reported mean \( \epsilon = 0.4 \), corresponding to mean aspect ratio \( \alpha = 1.07 \). This corresponds to \( \Gamma = 7.9 \) using the four-parameter biexponential fit described in Table 1, or \( \Gamma = 9.9 \) using the empirical formula for \( e(\Gamma) \). Estimation using this mean aspect ratio or eccentricity suggests that \( \Gamma = 8–10 \), which is substantially higher than the estimate of 0.4–2.3 based on mean \( \lambda \). It is possible that the discrepancy is due to error in measuring the aspect ratio, which is sensitive to where in the sheath the measurement falls, and/or the high variability in the measured aspect ratio. The same paper reported the same \( \lambda = 1.33 \) for gel beads in a flexible silicone tube, packed in ~40% staircase pattern, and an aspect ratio of \( \alpha = 1.15 \). The aspect ratio suggests that that system has \( \Gamma = 3 \), but the \( \lambda \) value suggests it has \( \Gamma = 0.4 \). It is important to note that neither of these systems are organized entirely in staircase pattern, and all other patterns are associated with an aspect ratio of 1. We therefore suggest that our formulas for estimating \( \Gamma \) be used only locally, on consistently patterned sections of notochord.

4.3. Limitations and open questions

All models are approximations of reality; as such, they are predicated on simplifying assumptions. These assumptions may not correspond to the full complexity of a specific system, but the power of a highly restrictive set of assumptions is in its general applicability, beyond a specific system. Some simplifying assumptions, such as omission of sheath details (individual chordoblasts, thickness, ECM components) (Bocina and Saraga-Babić, 2006), or adjacent tissues, could be adjusted, for models targeting different aspects of the system.

For example, our model assumes a straight A-P axis of the notochord, which allowed us to infer general mechanical and geometric properties. However, insights gained from this and other biomechanical studies of straight notochords could apply to the question of notochord straightening from an initially bent shape - straightforwardly.

Notochord cells (Witten and Hall, 2022) are clearly not constrained to equal volumes, but assuming so allowed us to see that (a) internal pressure will vary positionally, and (b) the taper of a notochord is in part a consequence of the sheath's tension relative to the chordocytes' tension. Similarly, it is unlikely that the tension ratio \( \Gamma \) is constant along the length of a notochord. However, many of the results of our study are applicable on a local basis, to sections of a notochord, where properties may be close to uniform.

A significant limitation of this study relates to the balance between pressure, volume, and tension. Modeling cell biomechanics with the physics of bubbles requires fixing tension and either volume or pressure - but not both. In this study, we fixed chordocyte volumes, because fixing chordocyte pressures led to a biologically unrealistic phenomenon of disappearing end cells. Neither constant pressure nor constant volume are realistic; they are simply a practical modeling simplification. There is an interplay between volume and pressure via mechanosensing,
explored elsewhere (Seleit et al., 2020; Adams et al., 1990; Baghat et al., 2022; Garcia et al., 2017), and it would be very reasonable to assume that tensions are also under dynamic control. Cells may be regulating tensions locally at different stages of development, to stabilize against external pressures, or in order to create a pressure gradient to drive cytoplasmic flow (Baghat et al., 2022). It would be of great interest, in future work, to explicitly include mechanisms regulating pressure, volume, and tension.

Little is known of the role of mechanosensing in enforcing eccentricity or stabilizing pressure, and it is not known to what extent the surrounding tissues, which we neglect, serve to stabilize or guide the resulting notochord configuration. It has been noted that vacuole internal pressure and pressure gradients are necessary for proper AP axis development (Baghat et al., 2022; McLaren and Steventon, 2021), and the generation of fluid flows which, when interrupted, can contribute to developmental defects such as idiopathic scoliosis (Grimes et al., 2016).

Sheath cells (chordoblasts) will migrate to the interior to replace damaged vacuolated cells (chordocytes) (Seleit et al., 2020; Garcia et al., 2017), suggesting an interplay between mechanosensing and volume control in the notochord.

Although our model partially explains the origin of the taper in zebrafish notochords, it is not capable of explaining why these tapering ends in WT are typically packed in bamboo pattern, when the middle zone is typically in staircase. The model used an assumption of consistent patterning within a notochord. Under those assumptions, bamboo always had higher pressure and higher energy than staircase. Future models, with relaxed packing assumptions, may show a lower overall energy, potentially explaining the physical basis of the bamboo-packed ends.

Many limitations of our model reflect gaps in the knowledge of the material properties of these structures, such as how stiffness of ECM, sheath cells, or inner vacuolated cells may vary locally, or how these biomaterials respond to growth and pressure. Notably, there is very little known about the directional material properties of notochord sheaths, though they are biomechanically important (Adams et al., 1990; Koehl et al., 2000; Folg Clark and Cowey, 1958; Rier, 2012; Wainwright, 2013; Wainwright, 2000).

Omission of surrounding tissues from our model may particularly affect relationships involving eccentricity, which in mechanically isolated systems is only associated with the staircase pattern. In zebrafish notochords, the WT, predominantly packed in staircase, is wider in the lateral direction, with dorsoventral flattening. Other teleost fishes (Baghat and Gray, 2020; Seleit et al., 2020; Grotmol et al., 2006; Yanuska, 2020) have notochords flattened in the lateral direction, and taller dorsoventrally, the disruption of which is associated with downstream developmental defects. These variations suggest that while notochord flattening is developmentally significant in teleost fish, its orientation is governed by mechanisms we have not yet considered. Interestingly, the NICD zebrafish mutants, packed in bamboo pattern, had flattened notochords (Norman et al., 2018), which suggests that a force external to the notochord is causing that compression.

This paper and its predecessors (Norman et al., 2018; Sorrell and Lubkin, 2022) focused on a particular developmental stage of a particular chordate, and on specific low-dimensional packing patterns which have been shown to be developmentally significant. In principle, most of the modeling assumptions used in this paper could be applied to other chordates, stages, and patterns, such as the more complex packing patterns and sometimes nonconvex cell shapes seen in amphioxus (Andrews et al., 2021).

4.4. Conclusions

Despite different assumptions, agreement between our models and previous ones (Sorrell and Lubkin, 2022) reinforces the importance of the tension ratio as an essential governing characteristic of many aspects of the developing notochord, and provides clues as to how local pattern, tension, volume, and pressure variations could drive morphological changes and developmental disorders. What our finite model has added is a quantitative explanation for the relationships among model variables.

Although the notochord is substantially more complex than it has been presented here, simplified modeling has provided a foundation of mechanical insight for interpreting observations and guiding further studies, both in vivo and in silico.

CRediT authorship contribution statement

SRL and EJC designed the study. EJC developed the computational models and ran simulations. SRL developed the simplified geometric models used in curve fitting. SRL and EJC performed the statistical analysis, created the figures, and wrote the paper.

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Appendix A. Derivations of estimated functional relationships

A.1. Introduction to simplified geometry

Although the 3D shapes of the minimal-energy bamboo- or staircase-packed cells do not have analytical expressions, we can use approximations of these shapes for the purposes of estimating functional relationships between the input model parameter $t^*$ (tension ratio) and the outcome variables aspect ratio $a$, eccentricity $e$, length ratio $l$ and nondimensional pressure $P$.

For these purposes, we model cells as spheres of radius $R$, clipped by planes. Angles at the triple junctions, where cell pairs meet the sheath, are governed by force balances (Fig. A1). These angles are given by

$$\cos \theta = \frac{\nu_1}{\nu_1 + \nu_2} = 1/(1 + \nu)$$

For the bamboo pattern, the cells are radially symmetric, barrel-shaped, and coaxial (Fig. A1A). Their anterior and posterior cell-cell interfaces are disks. The staircase pattern, the overall geometry for replacing the pairs of diagonal cell-cell interfaces zigzagging across the midplane with a single flat face on the midplane (Fig. A1B). The anterior and posterior faces of the staircase cells are thus modeled as disks that are clipped by the midplane (Figs. A1C, A2).
For each simplified cell geometry, we calculate the cell volume $V$. We have other expressions for cell volume that are based on modeling the cell as a sphere or a cylinder. For a sphere of diameter $d$, $V = \frac{4}{3} \pi (d/2)^3$, giving $d = (6V/\pi)^{1/3}$. For a cylinder of height $h$, diameter $D$, and base area $A$, cell volume $V = Ah = \pi (D/2)^2 h$. That is,

$$V = \frac{4}{3} \pi (d/2)^3 = Ah = \pi (D/2)^2 h.$$

Since $h = V/A = \frac{4}{3} \pi (d/2)^3 / \left(\frac{\pi (D/2)^2}{2}\right)$ and $J = D/d$, we obtain the cell heights as $h = 2d/(3J^2)$.

### A.2. Staircase pattern aspect ratio $a(I')$, eccentricity $e(I'$), and tension ratio metric $\gamma(I')$

The bamboo pattern is radially symmetric, so its transverse sections have aspect ratio $a = 1$. The staircase pattern can be approximated as a 2D pair of bubbles (Fig. A1C) which has transverse aspect ratio $a = (R + c)/R - 1 + c/R$. Since $c/R = \cos \theta = \rho/(\rho_1 + \rho_2) = 1/(1 + I)$,

this approximates the staircase aspect ratio as

$$a(I') = 1 + \frac{1}{(I' + 2)^{1/2}} (I' + 1).$$

We see for this formula that as $I' \to \infty$, $a(I') \to 1$, which is correct. In the other limit, as $I' \to 0$, $a(I') \to 2$, which is not actually observed, since in 3D the limiting aspect ratio is $a(0) = 1 + 1/\sqrt{2} = 1.71 < 2$. Nonetheless, because of its simplicity, we will use the simple formula

$$a(I') = (I' + 2)/(I' + 1)$$

for purposes of curve fitting. Converting to eccentricity via $e = \sqrt{1 - a^{-2}}$ gives

$$e(I') = \sqrt{1 - (1/(I' + 1))(I' + 1)^2}$$

showing that as $I' \to 0$, $e(I') \to 0$. As with the approximation for aspect ratio, there is an error in this formula for eccentricity as $I' \to 0$, which gives $e(0) = \sqrt{3/4} \approx 0.87$ rather than the true value $e(0) = \sqrt{1 - (1 + 1/\sqrt{2})^{-2}} \approx 0.81$.

Despite these errors for small $I'$, these simple formulas provide an easy way of measuring $I'$, by measuring aspect ratio under the microscope. The equation for $a(I')$ can be inverted, yielding a tension ratio metric:

$$\Gamma(I') = (1/a - 1) - 1 - (2 - a)/(a - 1).$$

This will be most useful in the middle range of $I'$, far from the limiting cases of $I' = 0$ (where the formula does not fit well) and $a = 1$ (where it is sensitive to measurement errors).

### A.3. Cell packing ratio $\lambda(I')$ and tension ratio metric $\gamma(I')$

To estimate the relationship between cells per unit length $\lambda = D/d$ and tension ratio $\lambda = \gamma/I'\rho$, we model cells in bamboo or staircase patterns as spheres of radius $R$, cut by planes (Fig. A2). We therefore assume a uniform curvature $1/R$ in both directions. The dimensional ratios of the cells are determined by the contact angles, and these ratios determine the calculated volume of the truncated sphere of radius $R$. Setting this volume equal to the cell volume $V$ gives the desired relation $\lambda(I')$ for each pattern.

For bamboo, with sphere radius $R$ and cell height $h$ (Fig. A3A), the cell volume is easily calculated to be $V = \pi (R^3h - R^2h/12)$. The trigonometric ratios give the relationship $2Rh = 1 + I'$. Equating the model volume with the volume of a sphere of radius $R$ if $\gamma = (d/h)^2 = (3(I' + 1)^2 - 1)/2$. Since $x^2 = 2d/3h$, we obtain

$$\lambda(I) = \sqrt{\frac{2}{3} (3I'^2 + 3I' + 1)}^{1/3}$$

which can be inverted to yield a convenient way of determining $I'$ from morphometry:

$$\Gamma(I) = \sqrt{\frac{2}{3} (3I'^2 + 3I' + 1)}^{1/3} - 1.$$

For staircase, cells are modeled with sphere radius $R$ and cell height $2h$, since $h$ represents the length of notochord occupied by one cell, and in the staircase pattern, each anterior or posterior neighbor’s centroid is a distance $2h$ away (Fig. A1C). The distance between the midplane and the center of the sphere is given by $c$. The trigonometric relations give
where

\[ \cos \theta = \frac{\tau_e}{(\tau_e + \tau_s)} - \frac{1}{(1 + \Gamma)}. \]

Writing \( X = x/R, Y = y/R, z = z/R \), the sphere can be written in scaled Cartesian coordinates as \( (X^2 + (1 + \Gamma)Y^2 + Z^2 - 1) \). This gives an expression for the cell volume as \( 4\pi h \int_{0}^{1} \Gamma(R, x, y) \Gamma(R, x, y) \int_{-1}^{1} (1 - Z^2 - (X - \frac{1}{1 + \Gamma})^2) \frac{\sqrt{1 - Z^2}}{\sqrt{1 - (1 - Z^2)}} dZ. \)

This integral can be calculated numerically for each value of \( \Gamma \). Setting \( h = R/(1 + \Gamma) - \frac{1}{4}d/2^2 \) gives the volume of a cell as

\[
V = \left( \frac{\pi}{2} \right)^2 \frac{d^3}{R^3(1 + \Gamma)^3} - 4R^2(1 + \Gamma).
\]

Solving this for \( \lambda \) gives

\[
\lambda(\Gamma) = \left( \frac{6d}{8\pi(1 + \Gamma)^3} \right)^{1/6}.
\]

Unlike the simple formula for bamboo, this cannot be inverted to give a simple expression for \( \Gamma(\lambda) \), however it can be used numerically to infer \( \Gamma \) from a measured \( \lambda \).

### A.4. Nondimensional pressure \( P(\Gamma) \)

The Young-Laplace condition

\[ p = \gamma(\kappa_1 + \kappa_2) \]

relates pressure, tension, and mean curvature. For our model notochords, we can use this to estimate the relationship between asymptotic pressure \( p_{\text{max}} \), and geometric and physical model parameters.

The relevant tension for this estimate is \( (\tau_e + \tau_s) \), the net tension on the external surfaces. Thus the Young-Laplace condition can be rewritten as

\[ p = \gamma(\kappa_1 + \kappa_2) = \gamma(\kappa_1 + \kappa_2)(\kappa_1 + \kappa_2) = \gamma(1 + \Gamma)(\kappa_1 + \kappa_2). \]

It remains to estimate the two curvatures \( \kappa_1 \) and \( \kappa_2 \). The curvature of a transverse cross-section is estimated from the cross-sectional diameter as

\[ \kappa_1 = \lambda^{-1} = \left( \frac{D}{2} \right)^{-1} - 2/\pi D. \]

The curvature of a longitudinal cross-section is estimated from trigonometric relations (Fig. A1).

For the bamboo pattern, the radius of curvature \( r_2 \) is given by

\[ \cos \theta = (h/2)/r_2 \]

where \( h \) is the cell length, measured for bamboo as centroid-centroid distance. We have the same angle in terms of the tensions:

\[ \cos \theta = \tau_e/(\tau_e + \tau_s) - 1/(1 + \Gamma) \]

giving

\[ \kappa_2 = (2 \cos \theta)/h = 2/\lambda((1 + \Gamma)). \]

Thus

\[ p = \gamma(1 + \Gamma)/2(\lambda(1 + \Gamma)). \]

Rearranging slightly gives

\[ p_d/\gamma = (1 + \Gamma)/(2(1 + \Gamma)(\lambda + 2d)/h). \]

Since \( \lambda = d/4 \) and \( h = (2/3)d/2^3 \), we obtain our estimate for the asymptotic nondimensional pressure

\[ P_s = p_d/\gamma = (1 + \Gamma)
\]

For the staircase pattern, the derivation is the same, but in \( \kappa_3 \), \( h \) is replaced by \( 2h \), since the nearest neighbors are staggered. This gives our estimate of the asymptotic nondimensional pressure in staircase as

\[ P_s = p_d/\gamma = (1 + \Gamma)/(2\lambda + 3(2/3)^2)/(1 + \Gamma). \]
Fig. A1. Trigonometric relations used to estimate force balances and geometric ratios in (A) bamboo and (B, C) staircase patterns. Cells are modeled as spheres of radius $R$, clipped by planes. Angles at triple-junctions where cell pairs meet sheath are governed by force balances and determine geometric ratios of cells. Cell-cell interfaces: magenta; cell-sheath interfaces: teal; similar triangles yellow. A. Bamboo cells are modeled as radially symmetric barrel shapes, parallel to and sharing the notochord axis, with centroid-centroid distances $h$. B. Staircase cells are modeled as barrel shapes oriented parallel to the notochord axis, centered either left or right of the midline, and cut by the plane separating left and right sides of the notochord. C. For staircase, centroid-centroid distance $h$ is half the cell height because of staggered arrangement. Similar triangles show that $\cos \theta = c/R = h/R = 1/(1 + \Gamma)$. Limiting case occurs when $\theta = \pi/4$, i.e. when $\Gamma = \sqrt{2} - 1 \approx 0.41$, when cell-cell contacts become complete disks (dotted teal curve).

Fig. A2. Computed shapes for simplified geometry of staircase cells. Volumes not to scale. For $\Gamma > \sqrt{2} - 1 \approx 0.41$, cell-cell interfaces meet on two edges. For $\Gamma = \sqrt{2} - 1 \approx 0.41$, these interfaces meet at two points. For $\Gamma < \sqrt{2} - 1 \approx 0.41$, cell-cell interfaces do not meet.

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CHAPTER 3

MODELING NOTOCHORD BIOMECHANICS

A version of the following has been accepted by *Cells & Development*.

**Title**
Flexural rigidity of pressurized model notochords in regular packing patterns

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3.1 Abstract

The biomechanics of embryonic notochords are studied using an elastic membrane model. An initial study varying internal pressure and stiffness ratio determines tension and geometric ratios as a function of internal pressure, membrane stiffness ratio, and cell packing pattern. A subsequent three-point bending study determines flexural rigidity as a function of internal pressure, configuration, and orientation. Flexural rigidity is found to be independent of membrane stiffness ratio. Controlling for number and volume of cells and their internal pressure, the eccentric staircase pattern of cell packing has more than double the flexural rigidity of the radially symmetric bamboo pattern. Moreover, the eccentric staircase pattern is found to be more than twice as stiff in lateral bending than in dorsoventral bending. This suggests a mechanical advantage to the eccentric WT staircase pattern of the embryonic notochord, over patterns with round cross-section.

3.2 Introduction

The notochord is the defining structure of chordates, serving to stiffen and elongate the embryo and template spine development. It is composed of two cell types: internal vacuolated cells (chordocytes), surrounded by external sheath cells (chordoblasts) (1,2). Chordocytes initially are in a ‘stack of coins’ arrangement, until the formation and enlargement of their vacuoles lengthens the notochord (3). Hydrostatic pressure drives and maintains this elongation, and is crucial in many aspects of chordate axial development (4). If vacuolated cells are ablated, causing a drop in pressure, the pressure is recovered via chordoblast invasion and differentiation into chordocytes (5). Disruption of the normal sequence of events can lead to developmental defects such as idiopathic scoliosis (6–8).

Once vacuolization occurs, zebrafish (*D. rerio*) chordocytes are packed in a variety of patterns, dominated by the two topologically lowest-order patterns: “bamboo”, with cell centroids on a line, and “staircase”, where cell centroids zigzag, alternating sides of the AP axis (9). These packing patterns were found to be governed by a length ratio $\lambda$, a nondimensional geometric parameter which is a measure of the number of cells per unit length, based on a standardized cell volume (10). This length ratio also governs the packing patterns of bubbles in a tube (10–15),
and related problems of packing of hard spheres (16,17) and soft spheres (18–20) under various types of containment.

Proper patterning is necessary for correct development (21). In previous work, it was found that the staircase packing pattern is tied bidirectionally to notochord eccentricity (9). In a variety of mechanical and computational models, staircase patterning has been found to cause ovaling of the sheath, and conversely, imposing an eccentric sheath cross-section leads to staircase patterning by lowering packing density threshold (9,10). An eccentric cross-section has biomechanical and functional consequences, notably for motility and rigidity (22). Cross-sectional morphology of the staircase packing pattern is modulated by the nondimensional tension ratio \( \Gamma \), which is a measure of the relative tensions of sheath and inner cells. A previous study (10) imposed combinations of \( \lambda \) and \( \Gamma \), yielding an explanation for eccentricity in staircase as a function of \( \Gamma \). In another model, only \( \Gamma \) was imposed, determining both eccentricity and \( \lambda \) as a consequence of \( \Gamma \) (23). For model notochords packed in staircase configuration, increasing \( \Gamma \) decreases eccentricity (23).

Notochords have been previously modeled using the assumption that their mechanics are dominated by surface tension of a continuous sheath surrounding cellular ‘bubbles’ filled with passive fluid, making the structure equivalent to a dry liquid foam (10,23). Modeling a finite notochord with \( N \) distinct cells gave rise to a natural tapering at the ends of the notochord (23), which has been observed \textit{in vivo} (9). The \( N \)-cell notochord model imposed \( \Gamma \) under an assumption of constant volume, and it was determined that beyond a certain minimum taper length, pressures and cell shapes become uniform away from the ends of the notochord, independent of total cell count \( N \) (23). An alternative model with constant internal pressure could not be investigated in these liquid foam models due to a physical instability similar to that of the two-balloon experiment (24).

The notochord’s early function as a hydrostatic skeleton elongates the embryo and provides structural integrity (3,4), suggesting that the material properties of the structure are important. Axial stiffness has been shown to affect swimming efficiency and control (25). These facts lead us to ask how relative stiffness of sheath and cell surfaces, internal pressure and packing patterns affect the mechanical function of the notochord. We therefore develop a model designed to answer these questions.

Because previous notochord models (10,23) were based on the mechanics of liquid foams, which do not support bending, they are unsuitable for studying the notochord’s flexural rigidity. In this paper, we instead model the notochord as a pneumatic elastic structure, more like a closed-cell foam than a liquid foam.

The mechanics of pressurized cylinders has been of interest in both engineering and biology. What were likely the first buckling tests on thin-walled cylinders subjected to bending moments
were undertaken in the mid-1800’s for the construction of the Britannia and Conwy Railway bridges (26,27). With few exceptions, engineering studies of pressurized tubes are on simple vessels with one lumen (28–32). Biomechanical studies do consider pressurized cylinders (elliptical and circular in cross-section) with internal structure (33–38) but few if any consider cylinders composed of variable internal structural patterning. Other work has focused on buckling of packings of hard (39,40) and soft spheres (41), and in one study (42), bending stiffness was explicitly studied for hard spheres arranged around a cylindrical rod.

Due to the importance of notochord mechanics, and the lack of studies on pressurized tubes with internal structure relevant to notochord structure, we aim to better understand the control of notochord biomechanics by developing and analyzing pressurized model zebrafish notochords under bending. We perform two computational studies. In an initial pressurization study, we establish relationships between tension ratio $\Gamma$, internal pressure $p$, sheath-to-cell stiffness ratio $\beta$, and cell packing pattern. A subsequent three-point bending study of these pressurized model notochords determines flexural rigidity $EI$ as a function of internal pressure, stiffness ratio, and cell packing pattern.

### 3.3 Methods

#### 3.3.1 Model description

All models are simplifications of the system they represent. We choose which aspects of the system to represent in greatest detail and fidelity, and impose simplifying assumptions on those aspects which are of less significance or relevance to the questions of interest. Because we are here interested in the mechanical significance of cell packing pattern, pattern and other notochord features are modeled using simplifying assumptions on geometry, biomechanics, surrounding tissues, physiology, and time.

**Geometry**

For consistency with previous models (10,23), we use many of the same assumptions on notochord anatomy. Ignoring details of sheath structure, we assume that notochords consist of a simple uniform sheath of negligible thickness, surrounding interior cells (chordocytes), with negligible intercellular space. These notochords are initially straight and of finite length, containing $N$ vacuolated interior cells, which are packed in uniform patterns. We consider only the lowest-order topologically regular patterns, either “bamboo” or “staircase” configuration. Staircase is considered to be oriented such that cells alternate in the LR direction, as is seen in *D. rerio* (9). We ignore all structures external to the notochord sheath and all structures internal
to the chordocytes and sheath, modeling the entire structure as a collection of surfaces under internal pressure.

In (23) (Appendix), we showed that approximations of the computed minimal-energy surfaces, based on clipped spheres, led to remarkable functional fits of the relationship between $\Gamma$ and $\lambda$. Consequently, rather than import a computed minimal-energy liquid foam geometry as the base for an elastic model for use in bending tests, the initial geometries for the current study are constructed using sphere primitives with radius $R$ intersected by planes at each cell-cell junction, with sphere centers placed approximating centroid positions from the simplified analytical model of (23) (Fig. 3.1A). Constructed this way, the initial geometry had $\lambda_B \approx 0.95$ and $\lambda_S \approx 1.32$; using the functional fits described in (23) (Appendix), both correspond to an initial tension ratio of $\Gamma \approx 0.5$. Surfaces comprising cell-cell and cell-sheath interfaces are initialized with thickness $t = R/70$.

Figure 3.1: Initial geometries and boundary conditions for pressurization and three-point bending studies. A. Left: Pair of energy-minimized notochords in bamboo and staircase configuration ($N = 6$ and $N = 8$, respectively) with $\Gamma = 1$, calculated in Surface Evolver. Sheath-cell boundaries: cyan, cell-cell boundaries: magenta. Right: Initial geometries of bamboo ($N = 6$) and staircase ($N = 7$), displayed in COMSOL. Cell centroids (orange) lie directly in line in bamboo configuration. Following the truncated sphere model from (23) A2, they form an equilateral triangle in the constructed staircase geometry when $\Gamma = 1$. 
**Biomechanics**

A mechanical model must make constitutive assumptions. The constitutive laws of chordocytes and sheath are unknown. Previous notochord models have used mechanics equivalent to soap films under tension (10,23). This assumption is not suitable for our current bending study, because a liquid foam does not have a unique zero-stress configuration. Therefore, our current model of notochord biomechanics utilizes elastic membrane theory (43). Note that “elastic membrane” is used here as an engineering term, not as a cell biology term; the elasticity of the cell membrane is negligible relative to that of the cortex and ECM. In this context of solid mechanics, membranes are similar to shells in that their thickness dimension is at least an order of magnitude smaller than other dimensions - however, membranes have no bending stiffness. Membranes also do not support compression.

**Model assumptions**

The model thus consists of the following assumptions:

- Model notochords are composed of $N$ vacuolated interior cells (chordocytes), arranged in a uniform pattern of either Bamboo or Staircase configuration. We neglect subcellular details.

- The sheath is treated as a thin continuum, neglecting individual sheath cells.

- We neglect mechanical factors and structures external to the sheath, such as surrounding tissues, which are assumed to be soft relative to the notochord.

- Prior to bending, the longitudinal axis is straight.

- Because of assumed thinness and lack of interstitial space, the adjacent surfaces of chordocytes can be considered as a single cell-cell interface, and the sheath and its adjacent cell surfaces can be considered as a single sheath-cell interface.

- Mechanics are governed by elastic membrane physics (43) (no bending stiffness). We do not separately account for cell-cell adhesion, which acts mechanically to reduce tension, hence can be considered as simply a parameter adjustment in our elastic membrane model.

- Interior cells have constant net internal pressure $p$ applied to sheath-cell interfaces, while cell volumes $V$ and surface area are variable. Because of equal intracellular pressures, there is zero net pressure on cell-cell interfaces.

- The most distal surfaces are considered part of the sheath.
• We assume interfaces to have a linear isotropic elastic stress-strain relationship.

• Poisson’s ratio $\nu = 0.45$, as an approximation of incompressibility.

• Young’s Modulus $E$ differs between sheath-cell (sc) and cell-cell surfaces (cc) by a constant stiffness ratio $\beta \equiv E_{sc}/E_{cc}$.

• The system is considered quasi-static, since at this early developmental stage, bending dynamics are not significant. Thus, any potential viscoelastic components are neglected.

This elastic model is parameterized by the stiffness ratio $\beta$ and internal pressure $p$. The resulting tension ratio $\Gamma$ is not fixed as in (10,23), but is calculated from simulation results, for each $\beta$, $p$, and configuration.

### 3.3.2 Pressure study

To investigate the geometric and biomechanical consequences of varying internal pressure and stiffness ratio, minimal boundary conditions were prescribed, as shown in Figure 3.1B. These boundary conditions were selected so as to ensure unique solutions, without introducing stresses due to the boundary conditions themselves.
Figure 3.1: (Continued) B. Boundary conditions for pressurization study. Sheath-cell boundaries pressurized. Lower axis (blue) constrained to only expand or contract along its length. Upper axis (green with roller symbol) is constrained to expand/contract along its length and to move toward or away from the lower axis. Curved edges (green with plane symbol) constrained to the plane perpendicular to the upper axis to prevent twisting.

The volume of each individual cell was calculated via surface integrals. Volume, displacement, stress, and strain data were exported from computed results for statistical analysis. \( \lambda \) was calculated as \( \lambda \equiv D/d \), where \( d \) is the diameter of a sphere with volume \( V = \frac{4}{3} \pi (\frac{d}{2})^3 \) of a single cell at the middle of the notochord, and \( D \) is the diameter of a cylinder with cell volume \( V = \pi (\frac{D}{2})^2 h \) and height \( h \) measured by adjacent centroid-centroid distance. Since all cells between pivot axes have the same volume \( V \) and equal centroid-centroid distances \( h \) both in the initial geometry and after pressurization, the \( \lambda \) values shown for a notochord under specific values of \( p \) and \( \beta \) are analogous to trimmed \( \lambda \) from (23).

For the purposes of nondimensionalization, we similarly calculate a volume based diameter \( d_{\text{init}} \) where each configuration is nondimensionalized according to its central cells’ initial (unpressurized) volumes.

In contrast to this previous work (23), \( \Gamma \) is not constrained, but is calculated from exported average stress (\( \bar{\sigma} \)) and average deformed thickness (\( \bar{t} \)) values over the sheath (\( s_h \)) and septa (\( s_e \)) respectively, as \( (\bar{\sigma} \bar{t})_{s_h} / (\bar{\sigma} \bar{t})_{s_e} \). Pressure is nondimensionalized as: \( p_{nd} \equiv (p \cdot (d_{init}/2)) / (E_{cc} \cdot t) \). Using round numbers, suppose we have cells of diameter \( d_{init} = 70 \, \mu m \), giving “membrane”
thickness 0.5 \( \mu \)m, and suppose the equivalent Young’s modulus \( E_{cc} \) is 2 kPa. This gives \( E_{cc} \cdot t = 1 \) kPa-\( \mu \)m, so a \( p_{ND} \) of 1 would correspond to about 30 Pa. Nondimensional pressure values \( p_{ND} \) in our study ranged from 0 to 0.7. Nondimensional bulk modulus \( K_{ND} = V(d p_{ND}/d V) \) was approximated via a finite difference method.

### 3.3.3 Bending study

For each configuration of the pressure study, a bending load was added. The bending load was imposed as an edge load distributed across the notochord, as shown in Figure 3.1C. Since bamboo configuration is symmetric about the longitudinal axis and we consider only isotropic material properties, force need only be applied in one direction (Fig 3.1C, left). However, staircase configuration is not rotationally symmetric, therefore force is applied in either dorsal-ventral (DV) or left-right (LR) directions (Fig 3.1C, center and right).

Figure 3.1: (Continued) C. Boundary conditions for three-point bending study. Upper left: schematic of three-point bending of a two-dimensional beam. Force (red arrow) applied perpendicular to the beam at its midpoint. The left end’s position is fixed (blue triangle) but allowed to rotate, while the right end (green roller) is allowed to move left/right along the line of the beam’s initial length. Notochords are bent analogously in three dimensions, inheriting boundary conditions from the pressurization study for comparison. A total force is distributed along arcs halfway between pivot axes. Staircase configuration is bent in either dorsal-ventral or left-right directions.
Nondimensional bending load is given by $F_{ND} \equiv F / ((6\pi E_{cc} d_{init} t) \cdot (d_{init}/L)^3)$. Following the same estimation procedure as pressure above (§3.2.2), suppose there are 150 cells in a notochord. For bamboo, this gives $L \approx 150 \cdot d_{init}$; for staircase, $L \approx 75 \cdot d_{init}$. Since $F_{ND} = F / ((6\pi E_{cc} d_{init} t) \cdot (d_{init}/L)^3)$, a $F_{ND}$ of 1 corresponds to about 0.4 pN for bamboo and 3 pN for staircase. Nondimensional force values ranged from 0 to 3.3.

Bending displacements were isolated from displacements due to pressurization by subtraction, and position data was exported from computed results for statistical analysis. Nondimensional displacement is given by $\delta_{ND} \equiv \delta / d_{init}$. Using the standard result from classical beam theory for 3-point bending, $\delta_{max} = FL^3/(48EI)$ (44), we solve for observed flexural rigidity in terms of displacements: $EI = FL^3/(48\delta_{max})$. Nondimensional flexural rigidity $(EI)_{ND}$ is given by $EI/(E_0 I_0) = FL^3/(48\delta_{max} E_0 I_0)$, where $E_0 = E_{cc}$ and $I_0 = \pi (d_{init}/2)^3 t$.

### 3.3.4 Computation of structures

Solutions were computed using the finite element method (FEM) in the COMSOL Multiphysics Membrane module, including the full nonlinear strain formulation. All surfaces are assigned a user-defined material using the assumptions in §3.3.1. Since membranes do not support compression, simulations are initialized with a small pre-stress, to avoid numerical slackness. A convergence criterion was set for a relative tolerance of 0.001. Parameter ranges were chosen to cover the largest range of biologically reasonable estimates for which simulations would converge.

### 3.3.5 Statistical analysis and visualization

Nonlinear fitting was used to model axial strain $\varepsilon_z$, volumetric strain $\varepsilon_V$, and tension ratio $\Gamma$. We compared displacements along the longitudinal axis of the notochord against Euler-Bernoulli beam theory (44):

$$
\delta(z) = \begin{cases} 
Fz(3L^2 - 4z^2)/(48EI) & \text{for } 0 \leq z \leq L/2 \\
F(z - L)(L^2 - 8Lz + 4z^2)/(48EI) & \text{for } L/2 < z \leq L
\end{cases}
$$

Statistical analysis was performed in JMP, and data visualization was performed in JMP, COMSOL, and Python.
3.4 Results

3.4.1 Pressure Study

To determine the influence of stiffness ratio and internal pressure on notochords of varying pattern and cell count, we analyzed stresses, strains, and displacements. Figure 3.2A illustrates example strain distributions; stresses are similarly distributed. Simulations varied nondimensional internal pressure, and the stiffness ratio ranged from 0.5 to 1.5 with a step size of 0.1. Multiple values of total cell number $N$ were considered; for testing and illustrative purposes we began with $N = 6$ for bamboo and $N = 7$ for staircase. Since these lengths were insufficient for comparison with beam theory, we collected both pressure and bending data for longer notochords of $N = 20, 34,$ and 50 for bamboo and $N = 21, 35,$ and 51 for staircase.

Figure 3.2: Inflation study: geometric changes. A. Strain plots on sample notochords. Left: Bamboo configuration ($N = 6$) with nondimensional pressure 0.36 and stiffness ratio 0.5. Right: Staircase configuration ($N = 7$) with nondimensional pressure 0.62 and stiffness ratio 1.3. B. Axial ($z$) and transverse ($y$) strain against lateral ($x$) strain for both configurations. C. Axial ($z$) strain as a function of pressure and stiffness ratio for both configurations. $t e x t b f C'$: Comparison of statistical fit of axial strain vs. the simulation results, for both bamboo (blue markers) and staircase (red markers). D. Lambda ($\lambda$) depends on configuration only, and is independent of $p$ and $N$. Best fits: Bamboo, $\lambda = 0.947$ (blue); staircase, $\lambda = 1.324$ (red). E. Nondimensional bulk modulus depends strongly on $\beta$ and configuration, but only weakly on $p$, and is independent of $N$. 

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**Bulk strains and aspect ratio**

We calculated bulk strains in the longitudinal (z), lateral (x), and dorsoventral (y) directions based on changes in overall longitudinal length and transverse lengths. For both configurations, strains were isotropic (Fig 3.2B). Volumetric stretch ratio was confirmed to be the cube of linear stretch ratio, and likewise surface area stretch ratio was confirmed to be the square of the linear stretch ratio.

Axial (longitudinal) strain $\varepsilon_z$ was found to be linear in internal pressure $p_{ND}$, and nonlinear in stiffness ratio $\beta$ (Fig 2C). We fit these results with a function (Fig 3.2C'): $\varepsilon_z = b_{B,S} \beta^{-m_{B,S}} p_{ND}$ where $m_B = 0.801 \pm 0.002$, $m_S = 0.661 \pm 0.002$, $b_B = 0.3409 \pm 0.0002$, $b_S = 0.3605 \pm 0.0003$. (Fits listed as parameter value ± SE; $R^2 > 0.999$ and $p < 0.001$ for all parameters.) Since strains are isotropic, $\varepsilon_x$ and $\varepsilon_y$ are equal to $\varepsilon_z$. Note that while $b_B$ and $b_S$ are similar, their difference is statistically significant, and setting them equal leaves clear trends in the residuals. Similarly, volumetric strain $\varepsilon_V$ was also found to be linear in $p_{nd}$ and nonlinear in $\beta$, which we fit with the function: $\varepsilon_V = c_{B,S} \beta^{-w_{B,S}} p_{ND}$ where $w_B = 0.903 \pm 0.003$, $w_S = 0.72 \pm 0.01$, $c_B = 1.166 \pm 0.003$, $c_S = 1.242 \pm 0.004$ (Fits listed as parameter value ± SE; $R^2 > 0.996$ and $p < 0.001$ for all parameters). Aspect ratios of the cross section were calculated based on maximum coordinates in the transverse $x$ and $y$ directions. The aspect ratio for bamboo is consistently 1.0, and the aspect ratio for staircase is consistently 1.7, regardless of pressure, stiffness, or cell count $N$.

**Geometric ratio $\lambda$ is constant for each pattern**

Cell linear density $\lambda$ depended only on configuration (Fig 3.2D); pressure, stiffness ratio, and total cell count $N$ had no significant impact on the results.

**Bulk Modulus is strongly dependent on stiffness ratio and configuration**

Bulk modulus was found to be dependent on stiffness ratio and configuration, and only weakly dependent on pressure. Total cell count $N$ was found to be independent of the results. These data are shown in Figure 3.2E.

**Tension ratio $\Gamma$ is dependent on stiffness ratio and pressure**

As with strain, stress is uniform on septa and sheath, respectively, with minor local variations near the triple junctions (Fig 3.3A). We therefore calculate the tension ratio $\Gamma$ as described in §3.3.2.

While $\Gamma$ is approximately linear with stiffness ratio $\beta$ (Fig 3.3B), it varies nonlinearly with pressure (Fig 3.3C). For $\beta$ close to 0.7, $\Gamma$ is constant regardless of configuration. For $\beta$ above that value, $\Gamma$ is concave down; below that value, $\Gamma$ is concave up. Determining slope values for the $\Gamma(\beta)$ lines for fixed pressure, and using these as inputs of an exponential function, suggested a
functional form which fit the data well for $\beta > 0.5$ and $p_{ND} > 0.00045$ (Figure 3.3C'). This nonlinear fit was of the form: $\Gamma - \Gamma_{B,S} = k_{B,S}(\beta - \beta_0) p_{ND}^n$ where $k_B = 0.444 \pm 0.003$, $k_S = 0.343 \pm 0.003$, $n = 0.344 \pm 0.005$, $\beta_0 = 0.694 \pm 0.005$, $\Gamma_B = 0.699 \pm 0.001$, and $\Gamma_S = 0.679 \pm 0.001$ (parameter value ± SE; $R^2 = 0.99$ and $p < 0.001$ for this fit). Alternatively, using $\Gamma - \Gamma_0 = k_{B,S}(\beta - \beta_0) p_{ND}^n$, where parameter $\Gamma_0$ is shared between the configurations, yields a comparable fit ($k_B = 0.434 \pm 0.003$, $k_S = 0.298 \pm 0.003$, $n = 0.306 \pm 0.005$, $\beta_0 = 0.641 \pm 0.005$, and $\Gamma_0 = 0.679 \pm 0.001$, also with $R^2 = 0.99$ and $p < 0.001$) using one less fitting parameter.

Figure 3.3: Pressure study: resulting tensions. A. Stress plots on sample notochords. Left: Bamboo configuration ($N = 6$) with nondimensional pressure 0.67 and stiffness ratio 1.1. Right: Staircase configuration ($N = 7$) with nondimensional pressure 0.57 and stiffness ratio 1.3. B. For a fixed pressure, tension ratio $\Gamma$ is independent of the number of cells $N$, and linearly dependent on stiffness ratio. C. $\Gamma$ increases or decreases nonlinearly with pressure, depending on stiffness ratio. C’. comparison of statistical fit against the simulation results.
3.4.2 Bending Study

To investigate the effect of internal pressure, membrane stiffness, total cell count, and packing pattern on the mechanical properties of these pressurized structures, notochords were subjected to three-point bending. Low pressures or stiffness ratios far from 1 resulted in poor or non-convergence where the membrane slackens (28). Therefore, we chose to range over higher pressure values than in the pressure study: 0.36 to 0.67 in increments of 0.045, with stiffness ratios restricted to $\beta = 0.8, 1, 1.2$. Each combination of packing pattern and cell count $N$ was tested with six force values. An example study result for each orientation (bamboo, staircase dorsal-ventral, and staircase left-right) is shown in Fig 3.4.

![Fig 3.4: Examples of three-point bending. Lateral and/or transverse displacements are nondimensionalized, dividing by initial cell dimension $d_{\text{init}}$. Bamboo parameters: $p_{ND} = 0.67$, $F_{ND} = 2.4$, $\beta = 1.2$. Staircase parameters: $p_{ND} = 0.53$, $F_{ND} = 0.91$, $\beta = 1.2$. Wireframes show initial geometry.](image)

Comparison to Euler-Bernoulli Beam Theory

We tracked displacements of the centroids of each septum. We verified that lateral or
transverse displacements match the expected centerline displacements under Euler-Bernoulli beam theory (§3.3.5) (44). An example comparison is shown in Fig 3.5A.

![Figure 3.5: Notochord mechanics under three-point bending. A. Nondimensional lateral displacements by nondimensional longitudinal position. Longitudinal position is normalized by length between boundary conditions post-bending. Simulation lateral displacements are nondimensionalized, dividing by initial diameter $d_{\text{init}}$. Comparison of simulation results (markers) to classical Euler-Bernoulli beam theory (curve) using one fitting parameter. Example shown: 34 cells bamboo configuration, $p_{\text{ND}} = 0.36$, $F_{\text{ND}} = 1.6$, and $\beta = 1.2$. B. Flexural rigidity as a function of internal pressure. Notochord is more rigid in staircase configuration, especially when force is applied in LR direction. Data for each orientation (markers), power-law fits (solid curves; exponent 1.29 for all) and comparison with flexural rigidity ratios derived for analogous elliptical and round hollow cylinders (Appendix; dotted curves; staircase at $\alpha = 1.7$). BB: Bamboo, blue; DV: staircase, dorsoventral bending, red; LR: staircase, left-right bending, green. Values for low pressures not calculated.]

**Flexural Rigidity ($EI$)**

Flexural rigidity $EI$ was calculated as described in §3.3.3. $EI$ as a function of pressure was estimated via both linear fits and a power law approach (Fig 3.5B). Flexural rigidity was found to be nonlinear in pressure, with a coefficient depending on the configuration/orientation (bamboo, staircase DV, and staircase LR), with negligible dependence on stiffness ratio and $N$. This nonlinear fit is of the form: $(EI)_{\text{nd}} = b_{BB,DV,LR} p_{\text{ND}}^a$, where coefficient $b$ depends upon configuration/orientation, but the exponent $a$ does not. Here, $b_{BB} = 2.73 \pm 0.02$, $b_{DV} = 6.58 \pm 0.04$, $b_{LR} = 14.20 \pm 0.11$, and $a = 1.29 \pm 0.01$. The coefficients $b_{LR}$, $b_{DV}$, and $b_{BB}$ are in a 5.2 : 2.4 : 1 ratio (Fig 5B). For comparison, a linear fit was tried, also with intercept constrained to
the origin, which had fitted slopes in a similar 5 : 2.3 : 1 ratio. The linear fit, however, showed a clear trend in the residuals, which is absent from the nonlinear fit. We can compare these ratios to those found from geometric approximations (Fig 5B, Appendix). The derived ratio for the same aspect ratio of 1.7 as in our staircase models is 4.26 : 1.97 : 1. This is close to the ratio 5.2 : 2.4 : 1 seen in our bending model, but underpredicts it by about 20%.

3.5 Discussion

3.5.1 Summary of findings

We have presented a notochord modeled as a beam for an appropriate comparison to beam theory, identifying some factors contributing to flexural rigidity. Using an elastic membrane model - rather than a soap-film model, as in earlier work - allowed for further study of the mechanics of pressurized notochords with different internal packing patterns.

An initial pressurization study was conducted, which confirmed a strong dependence of the notochord’s geometric and physical properties on its internal pressures, membrane stiffness ratios, and chordocyte packing pattern (Fig. 3.2). Shape was unaffected by pressure or stiffness ratios. Notochord size, tension ratio \( \Gamma \), and bulk modulus were affected by pressure, stiffness ratio, and pattern (Figs. 3.2 and 3.3).

We then conducted the bending study. Deflections were simulated and compared to Euler-Bernoulli beam theory with remarkable precision (Fig. 3.5A). We have shown flexural rigidity to have a strong dependence on internal pressure, cell packing configuration, and in the case of staircase configuration, the direction of bending. Flexural rigidity was found to have a nonlinear, concave up relationship with internal pressure as seen in engineering studies of simple hydrostats (28). However, interestingly, flexural rigidity did not depend on sheath-to-membrane stiffness ratio (Fig. 3.5B), just as shear modulus was independent of network concentration in (45).

We developed an estimate to compare the flexural rigidity ratios of the different packing patterns and orientations against simple thin-walled round or eccentric tubes of comparable sizes and aspect ratios (Appendix). For a fixed number of cells of the same baseline volume, we expect a notochord packed in staircase pattern to be half the length of a notochord in bamboo packing, and to have twice its cross-sectional area (23). This immediately implies that the staircase-packed notochord, even at transverse aspect ratio 1, would have a flexural rigidity \( 2\sqrt{2} = 2.8 \) times that of a bamboo-packed notochord, due to its twice-larger cross-sectional area (Fig. 3.6 (A1)). However, we note that, depending on transverse aspect ratio, an elliptical cross-section would have greater flexural rigidity in bending against the long
axis than against the short axis. This ratio of flexural rigidities in the orthogonal directions of bending is an increasing function of transverse aspect ratio, which we estimate (Appendix). Notably, for the specific aspect ratio (1.7) of our simulated notochords, this flexural rigidity ratio was even larger than predicted by the estimate, as was the ratio comparing staircase to bamboo.

### 3.5.2 Comparison with alternative models

Previous studies have modeled notochords as dry liquid foams (10,23). An infinite-length model of notochords imposed volume, $\lambda$, and $\Gamma$, determining resultant packing patterns within that parameter space (10). It also found staircase aspect ratio as a function of tension ratio $\Gamma$. A finite-length model of notochord cell packing constrained cell volumes and allowed variations of internal pressure, imposing $\Gamma$ but not $\lambda$ (23). It found that, under a constraint of constant volume, internal pressure would increase towards the ends of the notochord. Due to a fluid instability, that study was limited to using pressure as a free variable, which was a significant limitation in understanding the balance between pressure, volume, and tension.

The current study, in order to test bending, uses an elastic rather than liquid foam model, and is thus able to impose pressure and determine volume as a consequence. It has a reference configuration at a fixed geometric ratio $\lambda$, internal pressure is constrained in each simulation, and volume and $\Gamma$ are free variables. Our Euler-Bernoulli beam model could be compared with other beam models, such as Timoshenko’s, with the addition of one more parameter, however the excellent agreement of our results with the Euler-Bernoulli model suggests that an additional parameter will not be helpful. Moreover, we observe transverse septa remaining transverse under bending, which also suggests that the Timoshenko model is unnecessary. As in (23), the relationships between model variables are robust to variation in the total cell count $N$ (beyond a small minimum cell count), in remarkable agreement with the treatment of notochords as infinitely long (10). The agreement of this finding under a variety of model assumptions lends credence to the idea that these properties are independent of organism size (beyond a small minimum cell count). Moreover, this implies that the relationships found by our models will apply locally to notochords with variations along their length.

The initial unpressurized geometries of our model had $\lambda_B \approx 0.95$ and $\lambda_S \approx 1.32$ (each corresponding to an imposed $\Gamma \approx 0.5$ from (23)). However, here $\lambda$ was imposed, and simulation values at low pressures converged to $\Gamma \approx 0.7$. Constant $\lambda$ dependent only on configuration is a consequence of our choice of initial geometry, and isotropic strain. As such, this differs from previous models and observations (9,22,23).
3.5.3 Biological implications

Hydrostatic pressure has been found to play multiple roles in notochord development, such as axis elongation, cell fate determination, and flexural stiffness (4,33). Our pressurization study offers insight into the vacuolization process of notochord development by investigating the impact of varying internal pressure on some fundamental geometric and biomechanical properties.

In our bending study, we modeled the notochord as uniform to ensure an accurate comparison to classical beam theory. In reality, a notochord tapers at the ends corresponding to bamboo cell packing (9), thus we might expect lower flexural rigidity and wider range of motion at these places; however, more needs to be understood regarding pressure differences and sheath uniformity (or lack thereof) along the length of the notochord to determine this fully.

Zebrafish and many teleost fishes have embryonic notochords flattened dorsoventrally; however, other teleost fishes have notochords flattened laterally (21,46–48). This change in orientation can be provided by ensuring proper staircase packing in a certain direction, and has a mechanical consequence modeled here. Specifically, orienting the notochord to be less flexurally rigid in the direction of intended bending may be of developmental importance.

For a fixed interior volume at equal pressure, an elliptical staircase tube will be stiffer than a circular bamboo tube, and will be stiffer still in the taller direction of its cross section. Further, the difference in flexural rigidity between staircase orientations is higher than a simple elliptic tube would predict (Fig 5B, Appendix). Other cell packing patterns that have been studied are not eccentric (9,10), so this mechanical disparity should only exist with the staircase packing pattern.

Because correct cell packing pattern is crucial to proper development (21) and function (6), this study is further evidence of the interplay between packing pattern, tension and volume regulation in the dynamic environment of the developing notochord. This underlines the importance to the resulting biomechanics of proper patterning as well as proper orientation of that patterning. These have immediate impacts in proper development and later in efficient motility.

Analysis of our model determined the relationship between internal pressure and flexural rigidity, and found it specifically to be superlinear (concave up), i.e. a certain increase in pressure increases flexural rigidity by a greater amount at higher pressures (Figure 5). There are very few extant measurements of embryonic notochord flexural rigidity to compare with this prediction. Studies of embryonic X. laevis (33,34) show that notochord diameter, internal pressure, and flexural rigidity all increase with developmental stage, but these studies do not provide enough measurements to test our predictions.
3.5.4 Limitations and open questions

While there are many fascinating differences between notochords of different clades (49,50), our focus here has been on the peculiar relationship between staircase cell packing and eccentric cross section, which has only been reported in 48 hpf zebrafish (9).

Our primary interest was in understanding the significance of the asymmetric staircase pattern, the only regular packing pattern not round in cross-section. While it is possible to study torsion using our model framework, torsional loads are likely insignificant in the developmental stages to which our model corresponds, where growth-induced stresses would dominate. Additionally, since torsion and compression are not asymmetric with respect to the cross-section, these loading modes are not relevant to the question of the unique asymmetry of the staircase configuration. Torsion could in principle be studied by similar methods, with some challenges in setting boundary conditions in staircase configuration. Similarly, we did not investigate tensile loading, which is inapplicable to the developing embryo. We did not investigate compressive loading, even though the job of the notochord at this stage is to lengthen the embryo, because at this stage, the notochord is already bent, hence a compressive load would correspond to the first buckling mode, rather than the straight-column unbuckled compressive mode.

For parsimony, our model ignores some factors which may be anatomically and physiologically significant, and which could be incorporated into future models if warranted:

- The modeled notochord lacks the signature tapering morphology at its ends.
- Chordocytes do not pack in distinct, uniform patterns, but rather in a variety of patterns which change along the length (9).
- Cell shapes are not uniform in the real notochord; examples of the diversity of cell shapes can be seen in amphioxus (49).
- The notochord is also intrinsically bent in its resting configuration (33,51), as opposed to the perfectly straight AP axis we have constructed here. Our model framework could be extended to account for spatial variations in tension, such as the ventral actomyosin contraction found in the Ciona embryo (51).
- We neglect the contributions of the surrounding tissues and cavity external to the notochord in order to isolate properties only contributed by the notochord. NICD zebrafish mutants with notochords in bamboo configuration were also flattened (9), suggesting that these external factors are candidates for additional study. Our model framework could
be extended to account for spatial variations in tension, such as the greater expansion of the adjacent dorsal epithelium in the *Ciona* embryo (52).

- The real notochord is anisotropic in many respects (53). Anisotropy of stiffness in the sheath due to varying orientation of fibers, for example, would affect cross-sectional eccentricity when vacuolization occurs, which might contribute to differences in the orientation of notochord flattening between teleosts (35,54,55).

The specific consistent values found for $\lambda$ and staircase aspect ratio are consequences of our elastic model and starting geometries; we expect these values to differ depending on initial geometry. This contrasts with our previous liquid foam model (23) where aspect ratio and $\lambda$ were both functions of $\Gamma$.

### 3.5.5 Conclusions

This study complements and expands on previous work (10,23), providing further context for the relationships between pressure, tension ratio, and resulting flexural rigidity. We note that embryonic zebrafish intracellular pressure has been recently measured *in situ* (56), providing a new technique which can provide cell-level data to the larger discussion of the importance of pressure regulation in development. While the notochord is a far more complex structure than has been modeled here, the theoretical framework revealing the interplay between the model variables may benefit future studies on pressure-volume regulation and mechanosensing in a variety of tissues.

Furthermore, our treatment of the notochord as an elastic sheath containing compartments arranged in two types of internal packing configurations is novel in the context of pneumatic structures. Therefore, this study provides a foundation for further study of pressure vessels, in the context of developmental biology or otherwise.

The model provides a basis for what has been observed experimentally in notochords (33), and builds a framework for understanding how geometry, initial conditions, packing configuration, stiffness ratio, and internal pressure interact to determine flexural rigidity. Orientation is crucial, since, depending on aspect ratio, flexural rigidity can vary by more than a factor of two in orthogonal directions of bending. Further, previous work showed that, of all low-order regular packing patterns, eccentric cross-section is unique to staircase configuration (9,10). Our findings on the unique mechanical consequences of the eccentric notochord, combined with previous results tying this eccentricity to a specific packing pattern, suggest further investigation into the origin and control of cell packing and notochord orientation.
3.6 Appendix

Flexural rigidity of comparable simple tubes
For beams of simple shapes, there are analytical expressions for flexural rigidity $EI$. It is helpful to compare our model notochord flexural rigidity with simple shapes for which we have exact results. For these comparison purposes, we use an unpressurized thin-walled cylinder of isotropic stiffness $E$ and wall thickness $t$, and a cross-section which is either circular (bamboo) or elliptical (staircase). Let the dimensions of the cross-sections be radius $r$ (circle), and let the semi-major (LR) and semi-minor (DV) axes of the ellipse be $A$ and $B$ respectively.

We take the thin-walled approximation to find the equivalent area moments $I_{LR}$ and $I_{DV}$ about the LR and DV axes respectively, for each configuration. For bamboo, $I_{LR} = I_{DV} = I_{BB} = \pi r^3 t$. For our thin-walled elliptical staircase approximation, $I_{DV} = I_{lat} = \pi (3AB^2 + B^3) t / 4$ for bending in the lateral direction (about the DV axis) and $I_{LR} = I_{flex} = \pi (3BA^2 + A^3) t / 4$ for bending (flexion or extension) in the DV direction (about the LR axis). When $A = B$, this reduces to $I_{flex} = I_{lat} = \pi A^3 t$ for a circular cross-section.

For a model notochord, the average cross-sectional area is the average volume per unit length. For a set number of cells of equal volume, the cross-sectional area of staircase configuration will then be twice that of bamboo, and its length half that of bamboo. Thus, $\pi AB = 2 \pi r^2$. We define aspect ratio $\alpha = A / B \geq 1$. Then $B = A\alpha$. Substituting into the area comparison equation gives $A = r \sqrt{2\alpha}$, and $B = r \sqrt{2\alpha}$. Substituting these into $I_{flex}$ and $I_{lat}$ gives

$$I_{flex} = \frac{\pi}{\sqrt{2}} r^3 t \sqrt{\frac{1}{\alpha} \left(3 + \frac{1}{\alpha}\right)}, \text{ and}$$

$$I_{lat} = \frac{\pi}{\sqrt{2}} r^3 t \sqrt{\alpha(3 + \alpha)}.$$

The ratios of these area moments provide a fair comparison for the equivalent ratios measured in our model notochords:

$$I_{lat}/I_{flex} = \alpha^2 (3 + \alpha) / (1 + 3\alpha),$$

$$I_{flex}/I_{BB} = \sqrt{\frac{1}{2\alpha} \left(3 + \frac{1}{\alpha}\right)}, \text{ and}$$

$$I_{lat}/I_{BB} = \sqrt{\frac{\alpha}{2} (3 + \alpha)}.$$

A plot of these ratios over a range of $\alpha$ is shown in Fig. 3.6 (A1). We note that the value of $\alpha = 1.7$ for staircase configuration, which was a consequence of our choice of initial geometry, corresponds to a flexural rigidity ratio staircase LR : staircase DV : bamboo of 4.26 : 1.97 : 1. This can be compared to the ratio calculated from our data in §3.4.2, plotted in Fig. 3.5B.
Figure 3.6: Ratios of equal-volume staircase:bamboo model area moments of inertia depend on staircase aspect ratio $\alpha$. Diagrams annotate their respective ratio curves, showing ellipses of $\alpha = 1.7$ and dotted lines marking bending axes. When $\alpha = 1$, $I_{\text{flex}}/I_{BB} = I_{\text{lat}}/I_{BB} = 2\sqrt{2} = 2.8$, since for equal-volume cells, staircase has twice the cross-sectional area of bamboo.
3.7 References

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**Title**
Morphoelastic models discriminate between different mechanisms of left-right asymmetric stomach morphogenesis

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4.1 Abstract

The mechanisms by which the vertebrate stomach undergoes its evolutionarily conserved leftward bending remain incompletely understood. Although the left and right sides of the organ are known to possess different gene expression patterns and undergo distinct morphogenetic events, the physical mechanisms by which these differences generate morphological asymmetry remain unclear. Here, we develop a continuum model of asymmetric stomach morphogenesis. Using a morphoelastic framework, we investigate the morphogenetic implications of a variety of hypothetical, tissue-level growth differences between the left and right sides of a simplified tubular organ. Simulations reveal that, of the various differential growth mechanisms tested, only one category is consistent with the leftward stomach curvature observed in wild-type embryos: equal left and right volumetric growth rates, coupled with transversely isotropic tissue thinning on the left side. Simulating this mechanism in a defined region of the model over a longer period of growth leads to mature stomach-like curvatures.

4.2 Introduction

The vertebrate stomach, like the heart, is one of the earliest organs to break the embryo's bilateral symmetry. The embryonic foregut tube, initially straight and symmetrical at the midline, bends so as to be concave on the right and convex on the left, displacing the nascent stomach leftward (Figure 4.1A). Early in development, cilia-mediated flows (1–5) establish
left-right (LR) asymmetric gene expression patterns in tissues that will give rise to the stomach (6–11) initiating LR differences in tissue morphology (e.g., disproportionate expansion of the left wall) that are likely to contribute to its asymmetric bending. What is not well established, however, is exactly how genetically-induced tissue-level asymmetries physically actuate the observed shape change. The classical and simplest explanation is that the stomach undergoes “differential growth” of the left and right sides, which compels the tubular structure to curve (12). Although conceptually straightforward, this simplified explanation obviously lacks critical morphogenetic and tissue-mechanical details.

Figure 4.1: Vertebrate embryonic stomach bending in vivo. A. The initially straight foregut tube is illustrated from the esophagus (esoph) to the proximal intestine (duodenum, duod), and divided into left (light blue) and right (dark blue) halves. During bending, the stomach region (stom) curves leftward, forming a concavity on the right and a convexity on the left. Simultaneously, the left stomach lengthens in the longitudinal direction ($z$); this change in length is indicated by $z$. B. A transverse cross section through the stomach of the *Xenopus laevis* embryo (posterior view) shows that, during bending, the left wall of the stomach (light blue) also expands in the circumferential ($\theta$) direction, indicated by $\theta$, and thins in the radial ($r$) direction, indicated by $r$. Simultaneously, the right wall (dark blue) flattens.
In general, metazoan morphogenesis leads to dramatic changes in the visible shape and size of cells, tissues, organs and organisms. Such changes are often described, at the gross morphological level, as due to “differential growth” but both terms - “growth” and “differential” - represent several geometrically and biologically distinct processes. Thus, the phrase “differential growth” is a broad term that covers too many diverse phenomena to be mechanistically informative. To truly understand the physical mechanisms that drive morphogenesis, it is necessary to break down geometrically complex, dynamic tissue shape changes into more defined processes and events that are amenable to quantitative modeling (13).

For example, in the context of growth, differential can mean different amounts in different locations (inhomogeneous), or it can mean different amounts in different directions (anisotropic), or both (Fig 4.2). These distinctions have significant biological implications, so any inferences on morphogenetic mechanisms must be based on reasoning which includes these distinctions. The class of mechanisms which can cause inhomogeneous growth (e.g. local differences in proliferation), is very different from those which can cause anisotropic growth (e.g. oriented cell divisions).
Figure 4.2: Distinct modes of growth, starting from idealized cylindrical shape. Cylinder represented in coordinates $r$ (apico-basal), $\theta$ (circumferential), and $z$ (anterior-posterior). In isotropic growth, distances change by the same stretch ratio in all directions ($r$, $\theta$, and $z$), changing tissue size while preserving exact shape. Differential growth, which causes shape change, can include distinct phenomena. In anisotropic growth, distances change by different ratios in different directions, leading to tissue-level shape changes such as elongation, thickening, and dilation. In inhomogeneous growth, growth ratios are different in different locations, leading to spatially heterogeneous shape changes. Inhomogeneous growth can be locally isotropic or anisotropic.

Likewise, in the context of shape change, growth can mean volume increase due to hyperplasia (more cells) or hypertrophy (larger cells). But there are also a variety of tissue shape changes that conserve volume, which can also be lumped into the term “growth”, such as cell rearrangements (e.g., intercalation). Cell rearrangements, likewise, cannot be characterized by just one descriptor, such as "radial intercalation" because we can't simply specify the direction that gets shorter; we must also consider which direction(s) gets longer, because that difference corresponds to a distinct change in tissue shape.

In this paper, we aim to clarify and refine the description of embryonic stomach bending
due to “differential growth”, trading that broad phrase for a rational, specific, and quantifiable explanation. Here, we present a set of physics-based morphoelastic models simulating specific hypotheses/mechanisms of how differential growth of a simplified tubular organ could lead to the generation of an asymmetric curved shape similar to that observed in the early vertebrate stomach.

4.3 Methods

4.3.1 Simplifying Assumptions

Because individual cells play an insignificant role in stomach morphogenesis, we model the embryonic stomach tissues as a continuum. In the interest of parsimony, we make no distinction between the different tissue layers (mesoderm and endoderm), and do not explicitly model chemical reactions or gene regulatory networks. The initial geometry of the stomach (the reference configuration) is modeled as a thick-walled straight elliptic cylinder, with bilateral symmetry, not in contact with any other tissues.

4.3.2 Residual Stress and Morphoelasticity

The conceptual basis for the different growth mechanisms that we decided to test herein is illustrated by the following thought experiment (Figure 4.3). If we consider the stomach to be a simple tube (Fig 4.3A), and if the entire tube undergoes uniform isotropic growth (that is, if there is no LR differential growth), the stomach simply enlarges, retaining the same bilaterally symmetrical shape (Fig 4.3B). If we were to sever the left and right halves, we would observe that they easily fit back together.

In contrast, if both sides grow isotropically, but the left side grows faster than the right (Test 1; Fig 4.3C), upon severing the halves, we would expect the separation to cause each half to change shape. If we attempt to put the left and right sides of the tube back together, we expect that it would take some force to bend and stretch the halves, so they can meet again where they were separated, since their intrinsic shapes have undergone differential growth and are now incompatible. Similarly, if there is isotropic growth on the left side and anisotropic growth patterns on the right (Tests 2 and 3; Fig 4.3D, 4.3E), it would also take some force to reunite the severed halves.
Figure 4.3: Growth and differential growth. A. Initially symmetric tube, in perspective and cross-section. Intrinsic growth of the left and right sides can be considered separately, and experimentally observed by cuts. B-E Labeling: +/-/0 designates left stretch ratio greater than/less than/equal to right stretch ratio in the same direction. B. Both sides grow isotropically by the same amount (homogeneous growth). \( \lambda_r \) and \( \lambda_\theta \) are equal and equal on left and right, so there is a change in size but not shape. C. Growth of both left and right sides is isotropic, but unequal (inhomogeneous). \( \lambda_r \) and \( \lambda_\theta \) are greater on the left than the right, so tissue volumes do not remain equal. D. Oriented cell divisions correspond to growth in only one direction. If tissue grows faster in one direction but the same in the other (anisotropic growth), its stretch ratios \( \lambda_r \) and \( \lambda_\theta \) are unequal. Here right \( \lambda_r \) and \( \lambda_\theta \) and left \( \lambda_r \) are equal, but left \( \lambda_\theta \) is greater, so volumetric growth is greater on the left than the right. E. Shape change by cell intercalation preserves volume. Anisotropic growth that preserves volume must balance elongation in one direction with shrinkage in another direction. \( \lambda_r \) is smaller on the left than on the right, and \( \lambda_\theta \) is greater on the left than on the right. F-I. Stress induced by growth. First principal stress (positive, tension, blue; negative, compression, red) shown in arbitrary units below their corresponding ‘cut’ schematics. F. Homogeneous growth does not create shape change or residual stress. C, D, E. Differential growth leads to incompatibility of left and right shapes, which no longer fit without additional modification. Internal (residual) stresses must be generated to bend and stretch tissue, to maintain tissue continuity across the LR boundary (G, H, I).
Early descriptions of surface and volumetric growth in the context of continuum mechanics described this issue of growth creating geometric incompatibility in the tissue (14–17). In the intact stomach tube, the incompatibility is resolved by a deformation of the tissue, which creates a hidden force, or residual stress (Fig 4.3G-I). To account for this stress, the principle of morphoelasticity decomposes observed shape changes into an intrinsic shape change (growth strain) and an elastic strain which ensures tissue continuity (18–23), in this case, LR connectivity.

Following the established principles of morphoelasticity (14,15), we represent tissue shape changes in space and time by the deformation gradient tensor $F$, which is multiplicatively decomposed, using finite strains, into a growth deformation $G$ and elastic deformation $A$. For simplicity, we use the incompressible neo-Hookean elasticity model for $A$. When we follow growth for a sufficiently short interval, the elastic stresses resulting from growth incompatibilities do not dissipate; hence, the tissue is modeled mechanically as elastic rather than plastic or viscoelastic. When we follow growth for a sufficiently long time period, the elastic stresses dissipate and become permanent, plastic deformations.

4.3.3 Time dependence

Growth was simulated over both short (Tests 1-3) and longer (Test 4) time scales, corresponding to small reversible deformations or large irreversible deformations, respectively. When modeling time-dependent growth of the stomach, we consider a sequence of short time-steps, corresponding to small geometric changes. At each time step, the intrinsic growth tensor $G$ represents the growth over that short time interval. Each time step begins with an assumption of no residual stresses. That is, we assume that on the time scale of growth, residual stresses dissipate, in accordance with the hyper-restoration principle (16–19), which has been observed in experiments (20,21).

4.3.4 Local coordinates

For simplicity, we use a bespoke coordinate system where the three coordinate directions - radial, circumferential, and axial - are oriented with respect to the curvature of the local geometry. This local orthogonal coordinate system allows us to diagonalize the intrinsic growth tensor $G$ to explicitly assign growth strains in each direction, using only 3 terms. We calculate this local coordinate system ($r, z, \theta$), corresponding to axial or longitudinal $z$ (anterior-posterior), radial $r$ (apico-basal, across stomach wall), and circumferential $\theta$ (hoop) coordinates by a Laplace solver, using level surfaces and gradient fields. For time-dependent growth, the local coordinate system is updated with each time step as the geometry changes.
4.3.5 Differential growth

Differential growth (Fig 4.2) is represented by left-right differences (inhomogeneity) in $G$ (Fig 4.3). In all cases, the right side is assumed to grow isotropically, that is, with equal stretch ratios in each direction ($r$, $z$, and $\theta$). For simplicity, the left side growth is modeled in ratios ($\lambda_r$, $\lambda_z$, and $\lambda_\theta$) relative to the baseline isotropic right side growth. The volumetric ratio of the growing left and right sides is given by the product of the left-right (LR) stretch ratios: $V_L / V_R = \lambda_r \lambda_z \lambda_\theta$. If $V_L / V_R = \lambda_r \lambda_z \lambda_\theta = 1$, the left and right sides are growing at an equal volumetric rate, and morphogenetic differences are instead due to anisotropic left-side growth mechanisms such as oriented cell divisions or cell rearrangements (intercalation) (Fig 4.3E, 4.3I). If $\lambda_r = \lambda_z = \lambda_\theta$, left-side growth is isotropic, like the right side (Fig 4.3C, 4.3G). If, moreover, $\lambda_r = \lambda_z = \lambda_\theta = 1$, then $V_L = V_R$, there is no differential growth of any kind, and the stomach will not bend (Fig 4.3B, 4.3F).

4.3.6 Boundary conditions

In the short-term tests (Tests 1-3), $G$ is imposed on the entire left side of the model stomach, which is initialized as a straight elliptic cylinder (Fig. 4.4, box). For computational simplicity, we take advantage of the domain's DV symmetry, only computing and displaying the dorsal half. A symmetry boundary condition is imposed on the cut surfaces, and one point on a cut surface is fixed in displacement and rotation. The other surfaces are traction-free.
Figure 4.4: Test 1, isotropic differential growth. Stomach is initially symmetric (box). Right side grows isotropically. Left side also grows isotropically, but more than the right side, with L:R stretch ratio $\lambda = 1.2$ in each direction, resulting in 70% greater volume on the left side than the right. Differential growth causes the stomach to bend, but not thin. Each row, left to right: anterior surface, dorsal surface, and ventral cutaway views.

In the sustained-growth tests (Test 4), at each time step, $\mathbf{G}$ is imposed on a central-left region of the model stomach, which again is initialized as a straight elliptic cylinder. The anterior-most surfaces are fixed in the AP direction, but allowed to deform in the orthogonal directions. Two points on the constrained surface are given prescribed displacement in orthogonal directions from each other, to prevent rotation.

4.3.7 Computation

Because the intrinsic growth $\mathbf{G}$ is imposed, the remaining quantities to be determined computationally are elastic strain, total strain, residual stress, and displacements. Elastic incompress-
ibility is modeled with Poisson ratio $\nu = 0.45$. Computations and visualizations are done in a finite element method (FEM) package, COMSOL. Computational accuracy was verified on test problems with analytical solutions.

### 4.4 Results

Stomach bending has been most extensively studied in embryos of the frog, *Xenopus laevis* (6,7,12), in which the left stomach lengthens and bends along its longitudinal axis, gradually forming a left convexity, while the right stomach wall tends to flatten, and eventually forms a concavity (Fig 4.1A). Simultaneously, the left stomach wall undergoes radial thinning (via intercalation of its component cells), and expands in the circumferential direction ((12); Fig 4.1B). Similar results are observed in mammals (12).

To explain these concomitant morphological changes, we tested three major categories of hypothetical LR asymmetric differential growth mechanisms over a short interval of simulated development: 1) greater volumetric growth (isotropic) in the left wall (Fig 4.3C), 2) greater volumetric growth of the left wall in specific directions (radial, circumferential or longitudinal; Fig 4.3D), and 3) growth of the left wall via volume-conserving anisotropic cell rearrangements (Fig 4.3E). We then chose the conditions which qualitatively best matched *in vivo* observations, and performed 4) iterative growth tests in an extended region of the corresponding models, correlating with known LR asymmetric gene expression patterns. Since the stomach curves leftward, model assumptions always differed in the behavior of the left side relative to the right side and in all cases, the right side was assumed to grow isotropically.

In all tests, residual strain and stress are observed in the vicinity of the maximal dorsal and ventral positions, near where there is a jump in growth deformations between left and right sides.

#### 4.4.1 Test 1

LR differences in tissue volume were first modeled as isotropic differential growth (Fig 4.3C, 4.3G) in which the left and right sides of the tubular organ both grow isotropically, with a faster volumetric increase on the left side (Fig 4.4). In this experiment, the left side elongates in the longitudinal and circumferential directions, while simultaneously thickening in the radial direction by the same ratio. The enforced connectivity between left and right sides causes the expanding left side to encroach past the centerline, which has the effect of flattening the right side as the left side bends.

This model captures the elongation and bending of the left stomach wall and the flattening
of the right wall, as seen in the *Xenopus* stomach, but not the thinning of the left side. Thus, mere LR differences in tissue volume can recapitulate some, but not all, aspects of stomach bending.

### 4.4.2 Test 2

We next modeled LR differences in oriented cell divisions (OCD) as anisotropic differential growth in which the left side grows faster (i.e. the volumetric increase is greater) than the right side, but in one direction only (Fig 4.3D, 4.3H), corresponding to an excess of OCD in one of three particular orientations (radial, circumferential, or longitudinal) (Fig 4.5). When the OCD are oriented radially (*r*+, *θ*0, *z*0; Fig 5A), the left side thickens relative to the right side, and bending of the stomach tube is not observed. When the OCD are oriented circumferentially (*r*0, *θ*+, *z*0; Fig 4.5B), the left side elongates and crosses the midline, and flattens the right side, but paradoxically the stomach tube starts to bend in the opposite direction (i.e., rightward) to that observed in vivo. Finally, when the OCD are oriented longitudinally (*r*0, *θ*0, *z*+; Fig 4.5C), the stomach bends leftward, but the left wall does not lengthen circumferentially (cross the midline) nor thin radially.
Figure 4.5: Test 2, anisotropic differential growth tests corresponding to oriented cell divisions. Stomach is initially symmetric (box). Right side grows isotropically. Left side grows anisotropically, with L:R stretch ratios $\lambda = 1, 1, \text{and} 1.2$ in each of three directions. Resulting tissue volume is 20% greater on the left side than the right. A. Faster left radial growth thickens left side. B. Faster left circumferential growth enlarges left side but does not lead to longitudinal bending. C. Faster left longitudinal growth leads to stomach bending, but not thinning. Each row, left to right: anterior surface, dorsal surface, and ventral cutaway views. Labeling: $+/0$ designate left length changes greater than/equal to right length change in the same direction. None of these permutations agrees with observed morphological changes in the bending stomach.

These simulations show that faster circumferential growth in the left stomach can lead to flattening of the right side, but it does not elicit thinning of the left wall nor appropriate leftward bending. Likewise, while faster longitudinal growth on the left can cause leftward bending, it does not lead to radial thinning of the left wall, nor does it flatten the right side. Thus,
proper stomach curvature cannot be captured by simply imposing one direction of anisotropic differential growth within the left stomach wall.

4.4.3 Test 3

In contrast to the differential volumetric increases in Tests 1 and 2, here we model volume-conserving morphogenetic events (e.g., cell rearrangements) in the left wall (Fig 4.3E, 4.3I). In this experiment, the left and right sides grow with equal volumetric increase, but the left side undergoes tissue-thinning cell rearrangements (simulating radial intercalation scenarios, Fig 4.6). Since the volume increase is equal on both sides, the volume ratio \( V_L / V_R = \lambda_r \lambda_z \lambda_\theta = 1 \), although the individual LR stretch ratios \( \lambda_r, \lambda_z, \) and \( \lambda_\theta \) may differ from 1. If one of the LR stretch ratios is greater than 1, then at least one of the others must be less than 1 to preserve the volume ratio.

![Diagram of volume-conserving tissue thinning cell rearrangement modes](image)

Figure 4.6: Volume-conserving tissue thinning cell rearrangement modes. A. Initial arrangement of cells, colored by layer. "Radial intercalation" (thick arrows) is ambiguous, and can result in distinct tissue shape changes depending on the interaction of the radial motion with average motion in the longitudinal and circumferential directions. B. In radio-longitudinal ("r z") intercalation, tissue thins in the radial direction (\( r_- \)), elongates in the longitudinal direction (\( z_+ \)), and remains the same in the circumferential direction (\( \theta_0 \)). C. In radio-circumferential ("r \( \theta \)”) intercalation, tissue thins in the radial direction (\( r_- \)), elongates equally in the circumferential (\( \theta_+ \)) and longitudinal (\( z_+ \)) directions. D. In transversely isotropic ("r z”) radial intercalation, tissue thins in the radial direction (\( r_- \)), and elongates equally in the circumferential (\( \theta_+ \)) and longitudinal (\( z_+ \)) directions.
In the 6 permutations of stretch ratios with one equal to 1, one less than 1, and one greater than 1 (Fig 4.7ABC, others not shown), some bend the tube to the left, some bend the tube to the right, some thin the left wall and some exhibit a circumferential expansion of the left wall that extends across the midline, but only one \((r_-, \theta_0, z_+)^{-}\) — combining longitudinal growth with radial thinning — reproduces all aspects of *in vivo* morphogenesis of the bending stomach (i.e., the stomach tube bending to the right, with the left side thinning and extending circumferentially to cross the midline towards the right side) (Fig 4.7C).

Figure 4.7: Test 3, anisotropic differential cell rearrangement tests. Stomach is initially symmetric (box). Left and right sides grow by the same volumetric ratio. Right side grows isotropically. Left side grows and engages in volume-preserving cell rearrangements with stretch ratios (A, B, C, one direction unchanged) \(\lambda = 1, 1.2, \text{ and } 1/1.2 = 0.83\) in each direction, or (D, E, F; transversely isotropic) \(\lambda = 1.2, 1/\sqrt{1.2} \text{ and } 1/\sqrt{1.2} = 0.91\). Each group, left to right: anterior surface, dorsal surface, and ventral cutaway views. Labeling: +/-/-0 designate left length changes greater than/less than/equal to right length change in the same direction. Only two combinations (C and F) show shape changes consistent with those observed *in vivo*. Other permutations not shown.
Likewise, in the 6 transversely isotropic permutations of stretch ratios with two ratios equal to each other and less than 1, and the third ratio being greater than 1, or vice-versa (Fig 7DEF, others not shown), only one case, with $\lambda_r < 1$, $\lambda_z > 1$, and $\lambda_\theta > 1$, shows shape changes consistent with what is seen in vivo (12). This scenario involves radial thinning with a combination of both longitudinal growth and circumferential growth ($r_-, \theta_+, z_+$) (Fig 4.7F).

These experiments suggest that, for proper stomach bending, the left side tissues must thin, but also lengthen in the longitudinal direction (Fig 4.7C), or in both the longitudinal and circumferential directions (Fig 4.7F).

### 4.4.4 Test 4

The above short-term experiments suggest that leftward stomach curvature may be best captured by volume-conserving cell rearrangements in which the left side thins in the radial direction and expands longitudinally (Fig 4.7C), or thins radially and expands both longitudinally and circumferentially (Fig 4.7F). Therefore, we next modeled sustained growth of these two radially thinning models from Test 3, within a defined region of the tubular organ (Fig 4.8), to mimic the stomach region of the larger foregut which exhibits left-sided expression of pitx2 and sim2 genes (7,12).

As with Test 3, the left and right sides grow with equal rates of volumetric increase, but here, growth deformation $G$ is limited to a central oblong portion of the left side. In contrast to the previous tests, the anterior surface is fixed in $z$, in order to mimic a connection to the firmly anchored esophagus. The anterior (esophageal) end is constrained to lie in a plane, but the posterior (intestinal) end is considered to be mechanically free. The volume ratio $V_L/V_R = \lambda_r \lambda_z \lambda_\theta = 1$. In particular, volume is conserved, but the radial thinning of the left side is balanced by compensatory circumferential and/or longitudinal expansion.

In the first simulation (Fig 4.8A), corresponding to Fig 4.7C, radial thinning is offset only in the longitudinal direction. At each time step, $\lambda_r = 0.9$, $\lambda_\theta = 1$, and $\lambda_z = 1.\bar{1}$ to preserve the volume ratio. These stretch ratios were iteratively applied ten times, with residual stress dissipating entirely between steps. After ten iterations, $\lambda_r = 0.9^{10} \approx 0.35$, and $\lambda_z = (1.\bar{1})^{10} \approx 2.9$. That is, while the left and right sides grow volumetrically in equal amounts, the left has thinned to 35% of the thickness of the right, and has elongated axially by 190% compared to the right, i.e. almost tripled in relative length. Morphologically, this sustained growth test showed that radial thinning offset only by longitudinal expansion does not replicate the prominent greater curvature typical of the vertebrate stomach (e.g., see Fig 4.1A).
Figure 4.8: Test 4, time-dependent study of transversely isotropic differential cell rearrangement. In each, \( \mathbf{G} \) is applied to the central left segment of the initial geometry (first column, blue stripes). Residual stress dissipates between time steps. Top rows: 3D ventral view. Middle rows: 2D midline slices show relative thinning of left and right sides. Since the anterior end is modeled as remaining planar but the posterior end is free, the cut posterior surface (red, dashed line) develops curvature (red, dashed curve) in response to growth above. Bottom row: 2D top-down view of anterior surface. Outlines (black curves) show segments of the initial geometry of each time step. Von Mises stress is shown in arbitrary units. A. Radial thinning with volume-preserving axial extension of the left stomach generates curvature, but overall morphology distinct from that seen in vivo, a consequence of \( \lambda_\theta = 1 \). Initial geometry \( (t = 0) \) deformed with incremental stretch ratios \( \lambda_r = 0.9 \) and \( \lambda_z = 1.1 \) over ten time steps, achieving total stretch ratios \( \lambda_r \approx 0.35 \) and \( \lambda_z \approx 2.9 \). Top row: Short-term residual stress is greatest near the boundary of the thinning patch, radiating locally. Middle row: Stress concentrates axially, more so at the midline of the patch than in (B). Bottom row: Growth causes slight distortion in shape, less so than in (B), without noticeably moving the midline.

In the second simulation (Fig 4.8B), corresponding to Fig 4.7F, radial thinning is offset equally in the longitudinal and circumferential directions. At each time step, \( \lambda_r = 0.9 \), and
\( \lambda_z = \lambda_\theta \approx 1.05 \) to preserve the volume ratio. These stretch ratios were iteratively applied eighteen times, with residual stress dissipating completely between each step. After eighteen iterations, \( \lambda_r = 0.9^{18} \approx 0.15 \), and \( \lambda_\theta = \lambda_z \approx 2.6 \). That is, while both sides are growing, the left has thinned radially to 15% the thickness of the right side, and expanded in the other two directions by 160% relative to the right side, i.e. more than doubling relative length in each direction.

\[ \lambda_r = 0.9^{18} \approx 0.15, \quad \lambda_\theta = \lambda_z \approx 2.6. \]

Figure 4.8: (Continued) Test 4, time-dependent study of transversely isotropic differential cell rearrangement. In each, \( G \) is applied to the central left segment of the initial geometry (first column, blue stripes). Residual stress dissipates between time steps. Top rows: 3D ventral view. Middle rows: 2D midline slices show relative thinning of left and right sides. Since the anterior end is modeled as remaining planar but the posterior end is free, the cut posterior surface (red, dashed line) develops curvature (red, dashed curve) in response to growth above. Bottom row: 2D top-down view of anterior surface. Outlines (black curves) show segments of the initial geometry of each time step. Von Mises stress is shown in arbitrary units. B. Radial thinning with volume-preserving circumferential and axial extension of the left stomach (transversely isotropic radial intercalation) generates the curvature seen in vivo. Initial geometry (\( t = 0 \)) deformed with incremental stretch ratios \( \lambda_r = 0.9 \), \( \lambda_\theta = \lambda_z \approx 1.05 \) over eighteen time steps, achieving total stretch ratios \( \lambda_r \approx 0.15 \) and \( \lambda_\theta = \lambda_z \approx 2.6 \). Top row: Short-term residual stress concentrates at edges of thinning patch. Bottom row: Growth pressure from differential growth posterior to it causes visible distortion of the lumen.
In this simulation (Fig 4.8B), the stomach tube bends progressively further to the right, generating “greater” and “lesser” curvatures, with the left side thinning and crossing the midline towards the right (resembling in vivo stomach shape; see Figure 1). The cross-sectional shape of the fixed upper surface also deforms asymmetrically; the bottom surface, originally flat, bends in response to longitudinal expansion on the left.

4.5 Discussion

4.5.1 Summary of findings

The rationale for this study was to confirm/validate models of LR asymmetric stomach morphogenesis suggested by in vivo observations of organ/tissue size and cellular architecture (12). Decomposing the overly broad notion of growth into separate concepts of volume change and cell rearrangements, carefully considering changes in length ratios in each direction, and accounting for both volume and force balance, reveals a much larger set of possible differential growth modes. Evaluating each of these modes identifies just one out of 16 modes that is consistent with in vivo observations, and predicts outcomes from 15 hypothetical experiments each of which substitutes a different growth mechanism.

We tested, in silico, multiple alternative ways in which LR differential "growth" could happen: Test 1, isotropic volumetric growth that is greater on the left side (Fig 4.4); Test 2, equal LR growth rates in two directions, but greater L growth in one of three directions, consistent with oriented cell division, and resulting in greater L volumetric growth (Fig 4.5); Test 3, volumetrically equal LR growth coupled to L cell intercalation in a variety of direction combinations (Fig 4.7). We found that mere LR differences in isotropic volumetric growth rate (Test 1, Fig 4.4) generate shapes which are not consistent with those observed in vivo. Indeed, when comparing left and right sides of the embryonic stomach, experimentalists detect no difference in number of cells or rate of proliferation (mitotic indices) or cell death (12). Likewise, anisotropic growth in the left wall (Test 2, Fig 4.5) was also unable to capture all aspects of in vivo stomach bending.

In contrast, simulations involving volume-conserving cell intercalations in the left wall were able to reproduce all aspects of in vivo bending. The only LR differential growth modes that qualitatively agreed with in vivo observations in the short-term were 1) equal LR volumetric growth coupled with radial intercalation lengthening the longitudinal axis (Fig 4.6B, Fig 4.7C) and 2) equal LR volumetric growth coupled with transversely isotropic radial intercalation (Fig 4.6D, Fig 4.7F). It has been shown that, in vivo, left wall tissues thin and expand relative to the right wall, and gradually exhibit fewer layers of nuclei (12). Thus, our results confirmed, independent from previous work, that a LR asymmetrical radial intercalation model is, in fact,
consistent with the predictions/principles of morphoelasticity.

Encouraged by these short-term observations, we further tested these two volume-preserving, radially thinning models over an extended period of growth, with residual stresses dissipating rapidly (Fig 4.8). Of these, only the model where radial thinning was balanced by isotropic expansion in the remaining longitudinal and circumferential directions (transversely isotropic) replicated normal stomach bending with proper morphology of the greater curvature.

The physical mechanistic model simulations clarify and refine the conceptual model, by showing that proper curvature of the stomach is captured only by concomitant expansion of left side tissues in both orthogonal directions, a non-obvious prediction supported by in vivo measurements of the developing stomach (12).

4.5.2 Biological implications

When comparing the volume-preserving, radially thinning, transversely isotropic modes with the other candidate modes of differential LR growth that fail to reproduce proper stomach morphology, it is worth considering the evolution of the relevant control mechanisms. Isotropic differential growth (Test 1, Figs 4.3C, 4.4) does not require complicated orthogonally coordinated signaling, only a LR difference in mitotic rates. In contrast, oriented cell divisions (Test 2, Figs 4.3D, 4.5) require a directional signal, such as cell shape, coupled with spindle orientation. In our formulation, they are also modeled as having an excess mitotic rate above the control right side. Finally, volume-preserving cell rearrangement (Figs 4.3E, 4.6-8) also requires a signal to guide intercalation in a specific direction. If we assume the signal for radial intercalation as given, and examine the modes of compensatory extension in the other directions (Figs 4.6-8), the transversely isotropic mode is the simplest (Fig 4.6), because it does not require an additional mechanism to distinguish longitudinal from circumferential extensions. We may expect that control systems evolve one new feature at a time, so perhaps it is more likely that transversely isotropic intercalation would be the underlying mechanism. That said, for curved epithelia which are thick relative to their lumen diameters, the geometry of cell packing may bias the directions of intercalation through purely physical means (22,23).

We noted in our extended-growth models that adjacent tissues of the esophagus and duodenum are deformed by the differential growth of the stomach (Fig 4.8). With our choices of boundary conditions, we observed the anterior end developing a small but noticeable LR asymmetry in its cross section, and the posterior end developing a curvature out of its transverse plane. We may speculate that the large shape changes of differential growth of the stomach may deform the adjacent esophageal and duodenal tissues enough to affect - or even mechanobiologically induce - the formation of relevant digestive features, such as the
respective sphincters.

### 4.5.3 Limitations and open questions

Although the transversely isotropic intercalation model agrees qualitatively with *in vivo* observations, a quantitative comparison would not be valid, because the model is based on simplifications in initial geometry, distribution of growth fields, and mechanics, including the potential mechanical role of adjacent tissues such as the liver and pancreas.

The four test scenarios conducted here use two distinct but related models. In one (Tests 1-3), the initial geometry is a straight elliptical cylinder with an open lumen, where \( G \) is applied to the entire L side. In the other (Test 4), \( G \) is applied to the central-left section, corresponding to L-sided expression of asymmetry genes such as *pitx2* and/or *sim2* (7,12), and includes the stomach’s connection to adjacent gut structures on its anterior and posterior surfaces. These growth domains are modeled as defined by sharp edges, which causes convergence issues at these edges, and results in unrealistic morphology during autonomous sustained growth. We modeled \( G \) as uniform on the L and R sides, but it may be that the resulting local growth rates are not uniform, due to nonuniform expression of other factors on one or both sides. Such non-uniformities may account for subtleties of the resulting *in vivo* form not seen in our models. We also note that oriented cell divisions could lead to a similar result, and future studies should continue to explore the interplay between physical phenomena (e.g. fluid dynamics, stress and strain, cellular behavior, etc.) and their effects on growth at the tissue and organ levels via mechanosensing or local gene expression.

Additionally, our experiments all assume that the R side is growing isotropically; future studies could assess to what extent this is true. A future model with a more realistic initial geometry, e.g., derived from 3D reconstructions of embryonic tissue sections, may ameliorate these issues. Future models could also incorporate different boundary conditions, different degrees of elongation in \( z \) and \( \theta \), or different degrees of thinning. The models presented here also neglect external structures such as the mesogastria, which connect to the stomach; future work should investigate how the associated mesenteries and nerves might remodel or move during the bending process. We also neglected individual tissue layers (e.g., endoderm and mesoderm), extracellular matrices and differences in external and internal (lumen) pressure, but these could be modeled using the framework presented here. Despite the laundry list of unaddressed details, our models’ simple geometry and simple mechanisms mean that our conclusions are robust, and applicable to many vertebrate species and their variations.

The models show residual stress accumulating near the internal boundaries where there is a jump in growth strains, in particular at the dorsal and ventral extremes where the L and R sides
meet. Residual stress could be revealed by dissection experiments in which the walls of the embryonic stomach are slit open; changes in opening angle in different directions correspond to magnitude and direction of residual stresses.

Our computational workflow represents a technical advance in continuum tissue growth modeling, and here demonstrates sustained finite deformations with stretch ratios up to 3 and down to 0.15. For parsimony, the models tested herein assumed zero initial residual stress, and dissipation of accumulated stress at each time step. Our modeling methodology could be adapted to account for accumulated stresses, stress signaling, and explicit models of stress dissipation, as well as coupling to models of differentiation, signaling, and transport.

Similar approaches could be used to investigate the validity of models of morphogenetic shape change in other tissues or organs - such as the heart, liver, lungs, and pancreas - which demonstrate LR asymmetry during development, many of which do so across species, downstream of similar genes and chemical pathways as the stomach (24–26). Failure to properly break symmetry during development leads to a variety of severe birth defects in many systems, thus this is an important area of future study (27–31).

4.5.4 Conclusions

A physics-based model framework, such as the one presented here, built on well-established principles, can efficiently evaluate the specifics of a hypothesis and its predictions, and can eliminate from further consideration mechanisms yielding predictions which contradict physical observations. Moreover, by testing a variety of alternative hypothesized mechanisms, many of which show results inconsistent with previous observations, predictions are immediately made as to the roles of mutations and treatments not yet observed or probed.
4.6 References

5.1 Summary

In the three preceding chapters, we have detailed three studies in two distinct systems, each with its own modeling framework.

In Chapter 2, a monodisperse liquid foam model of finite notochords described a novel relationship between surface tension ratio $\Gamma$ and geometric ratio $\lambda$, and compared this to previous results. We showed that $\Gamma$ is crucial to overall morphology of the notochord in this context, and of particular note was its impact on eccentricity, taper length and cusps between cells. There were clear differences in the results dependent upon configuration. However, surprisingly, many results were independent of the length of the notochord, suggesting that these relationships might transcend size of the organism.

In *vivo*, chordocytes have properties which vary along the length of the notochord. Bamboo configuration appears preferentially at the ends, where the notochord diameter tapers to a point (1). Not only does the notochord diameter vary along the length, but so do cell diameters and volumes, impacting $\lambda$. Thus, results could be applied to local portions of the notochord which display the packing pattern of interest. We treat material properties as continua over their respective surfaces, when in fact $\Gamma$ would also vary depending on local differences in cortical tension. An important limitation of this study was the inability to discern a relationship
between volume, pressure, and tension. Either volume or pressure could be fixed, but not both. Attempting a model with constant pressure rather than volume led to the biologically unrealistic problem of disappearing cells (2). Future work should incorporate relaxed packing assumptions, anisotropic, local material properties, and structures external to the notochord which we have neglected for parsimony. Little is known of the mechanosensing abilities of these cells, though it is clear from ablation experiments that mechanisms exist to restore pressure when it is disrupted (3).

In Chapter 3, a linear elastic model of finite notochords in bamboo and staircase configurations allowed us to study the relationship between varying internal pressure and stiffness ratios on $\Gamma$, $\lambda$, volume, and bulk modulus. To our knowledge, pressure vessels with such internal structures have not been previously studied in this way. Further, this framework allowed a parametric study on varying force in three-point bending tests on these pressurized structures. We determined that the resulting displacements provided insight into flexural rigidity, and that this flexural rigidity had concave up profiles with respect to pressure, dependent both on configuration, and the orientation of configuration. Since the staircase pattern is the only pattern known to induce eccentricity (4,5) (and vice versa), there are immediate implications for future work studying proper development or motility - that is, to understand how eccentricity and subsequent orientation of the staircase pattern originate, and to what extent they affect later structure and motility.

Similarly to the liquid foam model, the elastic membrane model assumed finite notochords with one consistent packing pattern, which is biologically unrealistic (1). Thus, results could be applied to local sections of the notochord in vivo which display the proper packing pattern. As in Chapter 2, the notochords are considered to be perfectly straight, but here we chose boundary conditions which effectively eliminate the tapered ends in order to compare bending results to beam theory. Finally, we assumed isotropic material properties, which could be unrealistic, given that fibers in the sheath of amphioxus notochords have directional orientation (6), which could induce anisotropic stiffness. Future work could relax these assumptions. Many of our assumptions were necessitated by the lack of experimental results with which to compare. As hydrostatic pressure is crucial for development in a variety of contexts, an ability to measure local variations in material properties would serve to elucidate issues with this model, and would certainly help to generate new theoretical and computational endeavors.

In Chapter 4, a neo-Hookean hyperelastic model of stomach morphogenesis was developed to test transverse isotropic tissue thinning of the left stomach. We further developed this computational regime to include iterative growth application by remeshing the deformed configurations and dissipating residual stress at each step. Again, many assumptions - such as incompressibility, and isotropic material properties - were necessary due to a lack of experi-
mental measurements in this organ, and at the developmental stage we scrutinized. Future work in measuring these properties would be beneficial, as there is much speculation as to which material model to use in order to capture the most realistic behavior in silico. Future models could analyze the differences between hyperelasticity, viscoelasticity, or superelasticity, among others. Future models should also explore the impact of different boundary conditions, and the effect of concomitant growth of the pancreas, liver, remodeling of the dorsal mesentery, vagus nerves, or other structures external to the stomach which we have neglected. Our initial geometry is simple, which allows the model to be robust with respect to modeling other types of organs. Different modeling frameworks which incorporate local gene expression patterns defining the growth domain, and which could dissipate residual stress in other ways, could help determine a more realistic domain of growth and consequent morphology.

5.2 Segmentation

Future work could be done to segment µCT-scans to develop a more realistic initial geometry to compare pre- and post-growth morphology to actual images. Segmentation is the process by which quantities of interest (bone, tissue, etc.) are identified and partitioned from their surroundings in an image - in this case, the embryonic stomach, from µCT data. In the figure below, we attempted to create a mesh from µCT-scans; during these attempts, we encountered a variety of issues. The first difficulty lies in identifying the stomach, which, apart from some striations in the tissue, is contiguous with and visually similar to the adjacent structures of the gut. This requires thorough investigation of slices in each of the coronal, sagittal, and transverse slice data sets. Once identified, initial segmentation was performed via thresholding (highlighting pixels above a certain intensity) and machine learning operations, which allow for segmentation of many slices at once. Invariably, these techniques introduced more issues. If some pieces of tissue are below the intensity threshold, either because they are in fact not contiguous with each other, or due to scanning resolution, or source scattering, then these would create ‘islands’ in the eventual mesh. Alternatively, shadowing or spaces between tissue might not be correctly highlighted, causing holes in the eventual mesh. In either case, a functional mesh could not be created. Instead, we performed segmentation by hand and attempted to smooth the external surfaces in post-processing. While this removed all ‘islands’ and holes, the resulting mesh was not smooth enough for our purposes. We also found at this stage of embryonic development that the stomach lumen is narrow, which may introduce stress singularities due to numerical issues at these sharp edges. Finally, the software exports a surface mesh, which must be made volumetric to implement growth in the context of Chapter 4. This is a separate issue not readily handled by all software packages. For all of these reasons
listed, we were unable to continue down this path, and hope that others will do so in the future.

Figure 5.1: An example of segmentation of embryonic stomach performed by the author on \( \mu \)CT-scans of an *X. laevis* embryo at stage NF35. Scans provided by the Nascone-Yoder lab. Segmentation performed in Dragonfly.

## 5.3 Final Remarks

The mathematical models presented herein shed new light on notochord biomechanics and advance perspectives on growth in early stomach development. These useful methodologies demonstrate a need for closer collaboration with experimentalists and engineers - models are only useful insofar as they corroborate real-world data, and without precise instruments and careful analysis, this data may be suspect. This thesis offers many perspectives and systems as proof that even relatively simple models, controlling for only a few parameters, can serve to test and even generate hypotheses, driving science forward.
5.4 References

APPENDIX
A.1 The Surface Evolver Datafile

The .txt datafile read by The Surface Evolver to generate the initial notochord geometry includes the following sections:

- Parameters (optional)
- Vertices
- Edges
- Faces
- Bodies
- Commands (optional)

The following is a brief walkthrough of the algorithm and example datafile for a notochord in bamboo configuration with $N = 2$, a schematic of which is shown in the figure below (Fig A.1). Values for vertices, edges, faces, and bodies were chosen to be stored in matrices for ease of iteration via loops, and to preserve the format used by the Surface Evolver Datafile.
Figure A.1: Initial geometry for a notochord in bamboo configuration with $N = 2$, with labels shown for vertices, edges, faces, and bodies. Note that Face VI is defined by traversing edges in the counterclockwise direction when describing Body 1, but is traversed in the clockwise direction when defining Body 2, in order to ensure the normal vector is outward with respect to the body to which it is being attributed.
A.1.1 Vertices

Vertices are numbered in order 1-12, each row containing the respective \( x \), \( y \), and \( z \) coordinates, separated by whitespace. Vertex 1 is at the origin, and subsequent vertices are traversed clockwise for all vertices at the same height. This process is repeated for all height values. In this example, there are three: two for the sheath-cell surfaces, and one cell-cell surface in the middle.

A.1.2 Edges

Edges are numbered 1-20. For a given height, edges begin at the \( z \)-axis and traverse vertices in a clockwise manner. Edges are oriented from their initial vertex towards their final vertex. Once all edges at a given height have been assigned, four more edges are created, connecting vertices from one height to the vertices directly above. Again, these edges begin at the \( z \)-axis and traverse counterclockwise. This process is repeated until the top level, at which no upward edges need be assigned. Properties like color or line tension can be assigned to individual edges using commands in the same row as the corresponding edge.

A.1.3 Faces

Faces are numbered 1-11. Each face initially corresponds to a quadrilateral, thus is defined by four edges. Care must be taken to traverse edges in the correct order, such that an edge's final vertex is the initial vertex of the subsequent edge. If this is not the case, an edge orientation can be flipped with a minus sign. The first face corresponds to the bottom of the notochord. Then, the four faces corresponding to the upward edges are defined, beginning with the face corresponding to the first edge at that height, and traversing clockwise. This process is repeated until the top level. Properties like color or surface tension can be assigned to individual faces, as in edges.

A.1.4 Bodies

Bodies are numbered 1 and 2 in this example, ordered from bottom to top. They are defined starting with their bottom face, followed by the sides in a clockwise manner, and finally by their top face. Again, care must be taken to define faces with the correct orientation. All faces surrounding a body must have an outward normal in the same direction. The direction of the outward normal can be found using the right-hand rule around its edges. If a face is defined in the wrong orientation for a certain body, its orientation can be flipped with a minus sign. In this example, the cell-cell boundary (Face 6) acts as the top of Body 1 and the bottom of Body
2. Properties like target volume or pressure can be assigned to individual bodies, in the same way as with faces and edges.

A.1.5 Parameters and Commands

While these sections are included in the datafile, they can be defined in The Surface Evolver after importing the geometry. Here, important parameters include the initial tension ratio and target cell volume. These are given to individual faces and bodies, respectively, by including these values in the same row as the edges and faces, respectively, which define them.

Commands are defined at the end of the datafile, following the ‘read’ command. This is useful to assign shortcuts to commands which will be used in every simulation - for example, setting properties like cell opacity, ‘U’ to initiate conjugate gradient method, or custom commands which evolve the surface and refine the mesh in a predictable way. Likely, these will not be sufficient to fully evolve the surface, but are useful to begin.

The figure below (Fig A.2) shows a vertex-edge-facet-body diagram generated by Surface Evolver for an example bamboo notochord of \( N = 2 \), the datafile for which is printed in the following section.
Figure A.2: Initial geometry for a notochord in bamboo configuration with $N = 2$, with labels shown for vertices, edges, and faces. Vertex values are circled, Edge values are boxed, and Face values are both boxed and signed, based on the direction of the vector normal to their respective surface.
A.1.6 Example Datafile for Bamboo Configuration, \( N = 2 \)

PARAMETER tensC =1.5
PARAMETER gamma =0.7
PARAMETER tensS =1.05
PARAMETER cell_opacity =0.75
PARAMETER cross_sectional_area =1.5394e-10
PARAMETER eccentricity =0
PARAMETER lambda =1.3

//The following are computed parameters.
//Do not change the formulas unless necessary.
PARAMETER tubeD = 2*sqrt(cross_sectional_area/pi)
PARAMETER celld = tubeD/lambda
PARAMETER cell_volume = (4*pi*(celld/2)^3)/3
PARAMETER initial_height = 2*cell_volume/cross_sectional_area

//initial positioning
PARAMETER radiusx = sqrt(cross_sectional_area/(pi*sqrt(1-eccentricity^2)))
PARAMETER radiusy = sqrt((1-eccentricity^2)*radiusx^2)
PARAMETER centerx = radiusx
PARAMETER centery = radiusy

vertices
1 0.000000 0.000000 0.000000
2 0.000014 0.000000 0.000000
3 0.000014 0.000014 0.000000
4 0.000000 0.000014 0.000000
5 0.000000 0.000000 0.000008
6 0.000014 0.000000 0.000008
7 0.000014 0.000014 0.000008
8 0.000000 0.000014 0.000008
9 0.000000 0.000000 0.000017
10 0.000014 0.000000 0.000017
11 0.000014 0.000014 0.000017
12 0.000000 0.000014 0.000017

edges
faces
1 1 2 3 4 color cyan tension tensS+tensC
2 1 6 -9 -5 color cyan tension tensS+tensC
3 2 7 -10 -6 color cyan tension tensS+tensC
4 3 8 -11 -7 color cyan tension tensS+tensC
5 4 5 -12 -8 color cyan tension tensS+tensC
6 9 10 11 12 color magenta tension 2*tensC
7 9 14 -17 -13 color cyan tension tensS+tensC
8 10 15 -18 -14 color cyan tension tensS+tensC
9 11 16 -19 -15 color cyan tension tensS+tensC
10 12 13 -20 -16 color cyan tension tensS+tensC
11 17 18 19 20 color cyan tension tensS+tensC

bodies

103
The following are two programs written in MATLAB which take parameter values (cell count, tension ratio, and so on) as inputs, and output a .txt file for an initial geometry of a notochord of an arbitrary number of total cells in bamboo or staircase configuration, respectively. These programs were used for the work detailed in Chapter 2.

### A.2 Bamboo Program

```matlab
% Generalized Bamboo
clear all
N = 50;  \% Number of cells

% parameters
tensC = 1.5;
gamma = 0.7;
tensS = gamma*tensC;
% press = 2;
cell_opacity = .75;
cross_sectional_area = 3.1415926*(7E-6)^2; \% \pi r^2 + assumes r=7um ;
```
eccentricity = 0;
lambda =1.3; %wild-type lambda 1.3
%the following are computed parameters
%dependent on the values above
tubeD = 2*sqrt(cross_sectional_area/pi);
celld = tubeD/lambda;
cell_volume = (4*pi*(celld/2)^3)/3;
initial_height = (cell_volume/cross_sectional_area)*(2);%*2
    -exclude *2 if checking bead unstable
%initial position computed parameters
radiusx = sqrt(cross_sectional_area/(pi*sqrt(1-eccentricity^2)));
radiusy = sqrt((1-eccentricity^2)*radiusx^2);
centerx = radiusx;
centery = radiusy;

%number of vertices
numVertex = 8+(4*(N-1));
vertMat = zeros(numVertex, 4); %one column for id, then x, y, z coords
vertMat(:,1) = 1:1:numVertex;

% Create vertices

for i=1:(N+1)
    %first vertex on level
    vertMat(4*(i-1)+1,2) = centerx-radiusx;
    vertMat(4*(i-1)+1,3) = centery-radiusy;
    vertMat(4*(i-1)+1,4) = (i-1)*initial_height;
    %second vertex on level
    vertMat(4*(i-1)+2,2) = centerx+radiusx;
    vertMat(4*(i-1)+2,3) = centery-radiusy;
    vertMat(4*(i-1)+2,4) = (i-1)*initial_height;
    %third vertex on level
    vertMat(4*(i-1)+3,2) = centerx+radiusx;
    vertMat(4*(i-1)+3,3) = centery+radiusy;

vertMat(4*(i-1)+3,4) = (i-1)*initial_height;
%fourth vertex on level
vertMat(4*(i-1)+4,2) = centerx-radiusx;
vertMat(4*(i-1)+4,3) = centery+radiusy;
vertMat(4*(i-1)+4,4) = (i-1)*initial_height;
end

%Create edges
totalEdges = 4+(8*N);
edgeMat = zeros(totalEdges,3);
edgeMat(:,1) = 1:1:totalEdges;
%bottom of current layer, then stilts upward
for i=1:N
    edgeMat((8*(i-1))+1,2) = 4*(i-1)+1;
    edgeMat((8*(i-1))+1,3) = 4*(i-1)+2;
    edgeMat((8*(i-1))+2,2) = 4*(i-1)+2;
    edgeMat((8*(i-1))+2,3) = 4*(i-1)+3;
    edgeMat((8*(i-1))+3,2) = 4*(i-1)+3;
    edgeMat((8*(i-1))+3,3) = 4*(i-1)+4;
    edgeMat((8*(i-1))+4,2) = 4*(i-1)+4;
    edgeMat((8*(i-1))+4,3) = 4*(i-1)+1;
    edgeMat((8*(i-1))+5,2) = 4*(i-1)+1;
    edgeMat((8*(i-1))+5,3) = 4*(i-1)+5;
    edgeMat((8*(i-1))+6,2) = 4*(i-1)+2;
    edgeMat((8*(i-1))+6,3) = 4*(i-1)+6;
    edgeMat((8*(i-1))+7,2) = 4*(i-1)+3;
    edgeMat((8*(i-1))+7,3) = 4*(i-1)+7;
    edgeMat((8*(i-1))+8,2) = 4*(i-1)+4;
    edgeMat((8*(i-1))+8,3) = 4*(i-1)+8;
end
%cap the top
edgeMat(totalEdges-3,2) = numVertex-3;
edgeMat(totalEdges-3,3) = numVertex-2;
edgeMat(totalEdges-2,2) = numVertex-2;
edgeMat(totalEdges-2,3) = numVertex-1;
edgeMat(totalEdges-1,2) = numVertex-1;
edgeMat(totalEdges-1,3) = numVertex;
edgeMat(totalEdges,2) = numVertex;
edgeMat(totalEdges,3) = numVertex-3;

%Create faces

totalFaces = 6+((N-1)*5); %bottom cube 6 faces, then 5 new
    for every new level (cell)
faceMat = zeros(totalFaces,5); %every face has four edges
faceMat(:,1) = 1:1:totalFaces;
for i=1:N
    faceMat(1+(5*(i-1)),2) = (8*(i-1))+1; %bottom face of
        level, always orient up
    faceMat(1+(5*(i-1)),3) = (8*(i-1))+2;
    faceMat(1+(5*(i-1)),4) = (8*(i-1))+3;
    faceMat(1+(5*(i-1)),5) = (8*(i-1))+4;
    faceMat(2+(5*(i-1)),2) = (8*(i-1))+1; %front face, always orient out
    faceMat(2+(5*(i-1)),3) = (8*(i-1))+6;
    faceMat(2+(5*(i-1)),4) = -((8*(i-1))+9);
    faceMat(2+(5*(i-1)),5) = -((8*(i-1))+5);
    faceMat(3+(5*(i-1)),2) = (8*(i-1))+2; %right face, always orient out
    faceMat(3+(5*(i-1)),3) = (8*(i-1))+7;
    faceMat(3+(5*(i-1)),4) = -((8*(i-1))+10);
    faceMat(3+(5*(i-1)),5) = -((8*(i-1))+6);
    faceMat(4+(5*(i-1)),2) = (8*(i-1))+3; %back face, always orient out
    faceMat(4+(5*(i-1)),3) = (8*(i-1))+8;
    faceMat(4+(5*(i-1)),4) = -((8*(i-1))+11);
    faceMat(4+(5*(i-1)),5) = -((8*(i-1))+7);
    faceMat(5+(5*(i-1)),2) = (8*(i-1))+4; %left face, always orient out
    faceMat(5+(5*(i-1)),3) = (8*(i-1))+5;
    faceMat(5+(5*(i-1)),4) = -((8*(i-1))+12);
faceMat(5+(5*(i-1)),5) = -((8*(i-1))+8);
end

% upper face, always orient up
faceMat(totalFaces,2) = totalEdges - 3;
faceMat(totalFaces,3) = totalEdges - 2;
faceMat(totalFaces,4) = totalEdges - 1;
faceMat(totalFaces,5) = totalEdges;

% Create bodies
totalBodies = N;
bodyMat = zeros(N,7); % each body has 6 faces
bodyMat(:,1) = 1:1:totalBodies;

% Face orientation (remember orientation of normal)
for i = 1:N
    bodyMat(i,2) = -(1+(5*(i-1))); % bottom face needs to orient down
    bodyMat(i,3) = 2+(5*(i-1));
    bodyMat(i,4) = 3+(5*(i-1));
    bodyMat(i,5) = 4+(5*(i-1));
    bodyMat(i,6) = 5+(5*(i-1));
    bodyMat(i,7) = 6+(5*(i-1)); % top already oriented up
end

% Now write the file to text

fileID = fopen('evan_bamboo_test.txt', 'wt');
%fprintf(fileID, 'GRAVITY_CONSTANT 0\n');
%fprintf(fileID, 'CONSIDER PUTTING FILE PATH HERE
');
%fprintf(fileID, '// C:\.....txt');
%fprintf(fileID, strcat('PRESSURE ', string(press), '\n'));
fprintf(fileID, strcat('PARAMETER tensC = ', string(tensC), '\n'));
fprintf(fileID, strcat('PARAMETER gamma = ', string(gamma), '\n'));
fprintf(fileID, strcat('PARAMETER tensS = ',string(gamma*tensC),'
'));
fprintf(fileID, strcat('PARAMETER cell_opacity = ',string(cell_opacity),'
'));
fprintf(fileID, strcat('PARAMETER cross_sectional_area = ',string(cross_sectional_area),'
'));
fprintf(fileID, strcat('PARAMETER eccentricity = ',string(eccentricity),'
'));
fprintf(fileID, strcat('PARAMETER lambda = ',string(lambda),'
'));
fprintf(fileID, '// The following are computed parameters.
');
fprintf(fileID, '// Do not change the formulas unless necessary.
');
fprintf(fileID, 'PARAMETER tubeD = 2*sqrt(cross_sectional_area/pi)
');
fprintf(fileID, 'PARAMETER celld = tubeD/lambda
');
fprintf(fileID, 'PARAMETER cell_volume = (4*pi*(celld/2)^3)/3
');
fprintf(fileID, 'PARAMETER initial_height = 2*cell_volume/cross_sectional_area
');
fprintf(fileID, '// initial positioning
');
fprintf(fileID, 'PARAMETER radiusx = sqrt(cross_sectional_area/(pi*sqrt(1-eccentricity^2)))
');
fprintf(fileID, 'PARAMETER radiusy = sqrt((1-eccentricity^2)*radiusx^2)
');
fprintf(fileID, 'PARAMETER centerx = radiusx
');
fprintf(fileID, 'PARAMETER centery = radiusy
');
fprintf(fileID, 'constraint 1 // constrains bottom to z=0
');
fprintf(fileID, 'constraint 2 // constrains top to z=initial_height
');
fprintf(fileID, 'formula: x3 = 0
');
fprintf(fileID, 'formula: x3 =',string(N),'*initial_height
');
fprintf(fileID, 'vertices
');
for i=1:numVertex
    if i<=4
        fprintf(fileID,'%d %f %f %f
', [vertMat(i,1); vertMat(i,2); vertMat(i,3); vertMat(i,4)]);
    end
    if i>=numVertex-3
        fprintf(fileID,'%d %f %f %f
', [vertMat(i,1); vertMat(i,2); vertMat(i,3); vertMat(i,4)]);
    end
    if (i>4 && i<numVertex-3)
        fprintf(fileID,'%d %f %f %f
', [vertMat(i,1); vertMat(i,2); vertMat(i,3); vertMat(i,4)]);
    end
end
fprintf(fileID,'\nedges\n');
for i=1:totalEdges
    if i<=4
        fprintf(fileID,'%d %d %d
', [edgeMat(i,1); edgeMat(i,2); edgeMat(i,3)]);
    end
    if (i>4 && i<totalEdges-3)
        fprintf(fileID,'%d %d %d
', [edgeMat(i,1); edgeMat(i,2); edgeMat(i,3)]);
    end
    if i>=totalEdges-3
        fprintf(fileID,'%d %d %d
', [edgeMat(i,1); edgeMat(i,2); edgeMat(i,3)]);
    end
end

% get flat faces - these will have tension 2*tensC
% otherwise, these are upright faces, with tension tensS+ tensC
flatFaces = 1:1:(N+1);
for i=1:(N+1)
    flatFaces(1,i) = (5*(i-1))+1;
fprintf(fileID,'\nfaces\n');

for i=1:totalFaces
    if i==1
        fprintf(fileID,'%d %d %d %d %d color cyan tension tensS+tensC\n', [faceMat(i,1); faceMat(i,2); faceMat(i,3); faceMat(i,4); faceMat(i,5)]);
    elseif ismember(i, flatFaces) && i~=1 && i~=totalFaces
        fprintf(fileID,'%d %d %d %d %d color magenta tension 2*tensC\n', [faceMat(i,1); faceMat(i,2); faceMat(i,3); faceMat(i,4); faceMat(i,5)]);
    elseif i==totalFaces
        fprintf(fileID,'%d %d %d %d %d color cyan tension tensS+tensC\n', [faceMat(i,1); faceMat(i,2); faceMat(i,3); faceMat(i,4); faceMat(i,5)]);
    else
        fprintf(fileID,'%d %d %d %d %d color cyan tension tensS+tensC\n', [faceMat(i,1); faceMat(i,2); faceMat(i,3); faceMat(i,4); faceMat(i,5)]);
    end
end

fprintf(fileID,'\nbodies\n');

for i=1:totalBodies
    fprintf(fileID,'%d %d %d %d %d %d %d volume cell_volume \n', [bodyMat(i,1); bodyMat(i,2); bodyMat(i,3); bodyMat(i,4); bodyMat(i,5); bodyMat(i,6); bodyMat(i,7)]);
end

fprintf(fileID,'\n\nread\n');

fprintf(fileID,'U\n'); % for conjugate gradient method
fprintf(fileID,'delete facets where area=0\n');
fprintf(fileID,'set facet.opacity cell_opacity\n');
fprintf(fileID,'unfix radiusx\n');
fprintf(fileID, 'unfix radiusy\n');
fprintf(fileID, 'inner := { show facets ff where sum(ff.
  body,1) == 2 }\n');
fprintf(fileID, 'gogo := { g 3; r; g 5; r; V 25; g 25; V
  25; g 25; V 25; g 25; V 25; g 25; V 25; g 25; V 25; }
');
fprintf(fileID, 'iter := { g 25; V 10; }\n');
fprintf(fileID, 'iter2 := { u; iter; }\n');
fprintf(fileID, 'start := { gogo; iter 25; r; iter2 10; }\n');

fclose(fileID);

type evan_bamboo_test.txt
A.3 Staircase Program

%Generalized Staircase
clear all
N = 48;
% ADD TWO to this for total number of cells (half-cells on each end)
% Number of cells (should be even if you want red-blue pairs.)
% Should also be N>0 otherwise edge case will break (there are no red or
% blue cells in that case, only half-cells)

%parameters
tensC = 1.5;
gamma = 0.7;
tensS = gamma*tensC;
cell_opacity = .5;
cell_radius = (7E-6); % microns
cross_sectional_area = 3.1415926*(cell_radius)^2; %pi*r^2
eccentricity = 0;
lambda = 1.3; % wild-type lambda 1.3
%the following are computed parameters
%dependent on the values above
%These formulas should not be changed
tubeD = 2*sqrt(cross_sectional_area/pi);
celld = tubeD/lambda;
%celld = cell_radius*2;
cell_volume = (4*pi*(celld/2)^3)/3;
initial_height = (cell_volume/cross_sectional_area)*2.5;%
        --exclude *2 if checking bead instable
%initial position computed parameters
radiusx = sqrt(cross_sectional_area/(pi*sqrt(1-eccentricity^2)));
radiusy = sqrt((1-eccentricity^2)*radiusx^2);
centerx = radiusx;
centery = radiusy;

% number of vertices
numVertex = 8+(3*N);
vertMat = zeros(numVertex, 4); % one column for id, then x, y, z coords
vertMat(:,1) = 1:1:numVertex;

% Create vertices
% fill in first four vertices then last four
vertMat(1,2) = centerx - radiusx;
vertMat(1,3) = centery - radiusy;
vertMat(1,4) = 0;
vertMat(2,2) = centerx + radiusx;
vertMat(2,3) = centery - radiusy;
vertMat(2,4) = 0;
vertMat(3,2) = centerx + radiusx;
vertMat(3,3) = centery + radiusy;
vertMat(3,4) = 0;
vertMat(4,2) = centerx - radiusx;
vertMat(4,3) = centery + radiusy;
vertMat(4,4) = 0;
vertMat(numVertex -3,2) = centerx - radiusx;
vertMat(numVertex -3,3) = centery - radiusy;
vertMat(numVertex -3,4) = (N+1)*initial_height/2;
vertMat(numVertex -2,2) = centerx + radiusx;
vertMat(numVertex -2,3) = centery - radiusy;
vertMat(numVertex -2,4) = (N+1)*initial_height/2;
vertMat(numVertex -1,2) = centerx + radiusx;
vertMat(numVertex -1,3) = centery + radiusy;
vertMat(numVertex -1,4) = (N+1)*initial_height/2;
vertMat(numVertex ,2) = centerx - radiusx;
vertMat(numVertex ,3) = centery + radiusy;
vertMat(numVertex ,4) = (N+1)*initial_height/2;
% now fill in middle vertices
for i = 1:N
    if rem(i,2) ~= 0 % if an odd level, odd numerator for height and orientation
        vertMat(2+3*i,2) = centerx - radiusx;
        vertMat(2+3*i,3) = centery - radiusy;
        vertMat(2+3*i,4) = i*initial_height/2;
        vertMat(3+3*i,2) = centerx + radiusx;
        vertMat(3+3*i,3) = centery + radiusy;
        vertMat(3+3*i,4) = i*initial_height/2;
        vertMat(4+3*i,2) = centerx - radiusx;
        vertMat(4+3*i,3) = centery + radiusy;
        vertMat(4+3*i,4) = i*initial_height/2;
    end
    if rem(i,2) == 0 % if an even level, even numerator for height and orientation
        vertMat(2+3*i,2) = centerx - radiusx;
        vertMat(2+3*i,3) = centery - radiusy;
        vertMat(2+3*i,4) = i*initial_height/2;
        vertMat(3+3*i,2) = centerx + radiusx;
        vertMat(3+3*i,3) = centery - radiusy;
        vertMat(3+3*i,4) = i*initial_height/2;
        vertMat(4+3*i,2) = centerx + radiusx;
        vertMat(4+3*i,3) = centery + radiusy;
        vertMat(4+3*i,4) = i*initial_height/2;
    end
end

% Create edges
totalEdges = 14+(N*6);
edgeMat = zeros(totalEdges,3);
edgeMat(:,1) = 1:1:totalEdges;

% bottom layer of edges + stilts upward
edgeMat(1,2) = 1;
edgeMat(1,3) = 2;
edgeMat(2,2) = 2;
edgeMat(2,3) = 3;
edgeMat(3,2) = 3;
edgeMat(3,3) = 4;
edgeMat(4,2) = 4;
edgeMat(4,3) = 1;
edgeMat(5,2) = 1;
edgeMat(5,3) = 3;
edgeMat(6,2) = 1;
edgeMat(6,3) = 5;
edgeMat(7,2) = 2;
edgeMat(7,3) = 9;
edgeMat(8,2) = 3;
edgeMat(8,3) = 6;
edgeMat(9,2) = 4;
edgeMat(9,3) = 7;

% now top layer of edges
edgeMat(totalEdges-4,2) = numVertex-3;
edgeMat(totalEdges-4,3) = numVertex-2;
edgeMat(totalEdges-3,2) = numVertex-2;
edgeMat(totalEdges-3,3) = numVertex-1;
edgeMat(totalEdges-2,2) = numVertex-1;
edgeMat(totalEdges-2,3) = numVertex;
edgeMat(totalEdges-1,2) = numVertex;
edgeMat(totalEdges-1,3) = numVertex-3;
edgeMat(totalEdges,2) = numVertex-3;
edgeMat(totalEdges,3) = numVertex-1;

% now loop through the layers
% handle odd layers differently than even based on orientation of diagonal
for i=1:(N)
    % create edges for flat surface
    edgeMat(2*(5+(3*(i-1))),2) = 5+(3*(i-1));
    edgeMat(2*(5+(3*(i-1))),3) = 5+(3*(i-1))+1;
    edgeMat(2*(5+(3*(i-1)))+1,2) = 5+(3*(i-1))+1;
    edgeMat(2*(5+(3*(i-1)))+1,3) = 5+(3*(i-1))+2;
edgeMat(2*(5+(3*(i-1)))+2,2) = 5+(3*(i-1))+2;
edgeMat(2*(5+(3*(i-1)))+2,3) = 5+(3*(i-1));
end

% now use remainder trick to create upward stilts in correct spots
for i=1:(N-1)
    if rem(i,2)~=0 % this is an odd row
        edgeMat(7+(6*i),2) = 5+(3*(i-1));
        edgeMat(7+(6*i),3) = 5+(3*(i-1))+3;
        edgeMat(7+(6*i)+1,2) = 5+(3*(i-1))+1;
        edgeMat(7+(6*i)+1,3) = (5+(3*(i-1))+1)+4; % for even, it would be +2
        edgeMat(7+(6*i)+2,2) = 5+(3*(i-1))+2;
        edgeMat(7+(6*i)+2,3) = (5+(3*(i-1))+2)+6;
    end
    if rem(i,2)==0
        edgeMat(7+(6*i),2) = 5+(3*(i-1));
        edgeMat(7+(6*i),3) = 5+(3*(i-1))+3;
        edgeMat(7+(6*i)+1,2) = 5+(3*(i-1))+1;
        edgeMat(7+(6*i)+1,3) = (5+(3*(i-1))+1)+6; % for even, it would be +2
        edgeMat(7+(6*i)+2,2) = 5+(3*(i-1))+2;
        edgeMat(7+(6*i)+2,3) = (5+(3*(i-1))+2)+2;
    end
end
% this is for the edge case, where you're on the last level before the top
% odd case
if rem(N,2)~=0
    edgeMat(totalEdges-7,2) = numVertex-6;
    edgeMat(totalEdges-7,3) = numVertex-3;
    edgeMat(totalEdges-6,2) = numVertex-5;
    edgeMat(totalEdges-6,3) = numVertex-1;
    edgeMat(totalEdges-5,2) = numVertex-4;
    edgeMat(totalEdges-5,3) = numVertex;
end
if rem(N,2)==0 %even case
    edgeMat(totalEdges-11,2) = numVertex-7;
    edgeMat(totalEdges-11,3) = numVertex;
    edgeMat(totalEdges-7,2) = numVertex-6;
    edgeMat(totalEdges-7,3) = numVertex-3;
    edgeMat(totalEdges-6,2) = numVertex-5;
    edgeMat(totalEdges-6,3) = numVertex-2;
    edgeMat(totalEdges-5,2) = numVertex-4;
    edgeMat(totalEdges-5,3) = numVertex-1;
end

%Create faces
totalFaces = 4+N+(N+1)+(2*N)+4; %bottom 2 plus top 2 plus shared faces (N+1) plus cell walls (2*N) plus half-cell walls (4)
faceMat = zeros(totalFaces,6);
faceMat(:,1) = 1:1:totalFaces;
%bottom of half-cell
faceMat(1,2) = 5;
faceMat(1,3) = 3;
faceMat(1,4) = 4;
%sides of half-cell
faceMat(2,2) = 4;
faceMat(2,3) = 6;
faceMat(2,4) = -12;
faceMat(2,5) = -9;
faceMat(3,2) = 3;
faceMat(3,3) = 9;
faceMat(3,4) = -11;
faceMat(3,5) = -8;
%bottom of first red cell or bottom of second half-cell if N=0. Always
%here, so hard-coded
faceMat(4,2) = 1;
faceMat(4,3) = 2;
faceMat(4,4) = -5;
short sides of top half-cell
%depends on whether top half-cell is on right or left
if rem(N,2)==0 %even case
    faceMat(totalFaces-3,2) = totalEdges-10;
    faceMat(totalFaces-3,3) = totalEdges-6;
    faceMat(totalFaces-3,4) = -(totalEdges-4);
    faceMat(totalFaces-3,5) = -(totalEdges-7);
    faceMat(totalFaces-2,2) = totalEdges-9;
    faceMat(totalFaces-2,3) = totalEdges-5;
    faceMat(totalFaces-2,4) = -(totalEdges-3);
    faceMat(totalFaces-2,5) = -(totalEdges-6);
end
if rem(N,2)~=0 %odd case
    faceMat(totalFaces-3,2) = totalEdges-8;
    faceMat(totalFaces-3,3) = totalEdges-7;
    faceMat(totalFaces-3,4) = -(totalEdges-1);
    faceMat(totalFaces-3,5) = -(totalEdges-5);
    faceMat(totalFaces-2,2) = totalEdges-9;
    faceMat(totalFaces-2,3) = totalEdges-5;
    faceMat(totalFaces-2,4) = -(totalEdges-2);
    faceMat(totalFaces-2,5) = -(totalEdges-6);
end
%top two triangular faces
faceMat(totalFaces-1, 2) = totalEdges;
faceMat(totalFaces-1, 3) = totalEdges-2;
faceMat(totalFaces-1, 4) = totalEdges-1;
faceMat(totalFaces, 2) = totalEdges-4;
faceMat(totalFaces, 3) = totalEdges-3;
faceMat(totalFaces, 4) = -totalEdges;

%hard-code the middle and sides of the first red wall
%it's the only one that doesn't fit the pattern
if N>=1
    faceMat(5,2) = 5;
    faceMat(5,3) = 8;
    faceMat(5,4) = -10;
faceMat(5,5) = -6; %this ends shared middle
faceMat(6,2) = 1;
faceMat(6,3) = 7;
faceMat(6,4) = -16;
faceMat(6,5) = -13;
faceMat(6,6) = -6; %this ends one side
faceMat(7,2) = 2;
faceMat(7,3) = 8;
faceMat(7,4) = 14;
faceMat(7,5) = -17;
faceMat(7,6) = -7; %this ends other side
%now the last horizontal piece and middle interface at the top
%tried not to hard-code but it breaks the pattern
faceMat(totalFaces-5,2) = totalEdges-10;
faceMat(totalFaces-5,3) = totalEdges-9;
faceMat(totalFaces-5,4) = totalEdges-8;
if rem(N,2) == 0 %top middle interface, even case
    faceMat(totalFaces-4,2) = -(totalEdges-8);
    faceMat(totalFaces-4,3) = totalEdges-5;
    faceMat(totalFaces-4,4) = -totalEdges;
    faceMat(totalFaces-4,5) = -(totalEdges-7);
%second-to-last middle interface to the top
faceMat(totalFaces-8,2) = totalEdges-16;
faceMat(totalFaces-8,3) = totalEdges-12;
faceMat(totalFaces-8,4) = totalEdges-8;
faceMat(totalFaces-8,5) = -(totalEdges-13);
%now the sides
faceMat(totalFaces-7,2) = totalEdges-14;
faceMat(totalFaces-7,3) = totalEdges-13;
faceMat(totalFaces-7,4) = totalEdges-7;
faceMat(totalFaces-7,5) = -(totalEdges-1);
faceMat(totalFaces-7,6) = -(totalEdges-11);
faceMat(totalFaces-6,2) = totalEdges-15;
faceMat(totalFaces-6,3) = totalEdges-11;
faceMat(totalFaces-6,4) = -(totalEdges-2);
faceMat(totalFaces-6,5) = -(totalEdges-5);
faceMat(totalFaces-6,6) = -(totalEdges-12);
end
if rem(N,2) ~= 0 %top middle interface, odd case
  faceMat(totalFaces-4,2) = totalEdges-10;
  faceMat(totalFaces-4,3) = totalEdges-6;
  faceMat(totalFaces-4,4) = -totalEdges;
  faceMat(totalFaces-4,5) = -(totalEdges-7);
end %second-to-last middle interface to the top
faceMat(totalFaces-8,2) = -(totalEdges-16);
faceMat(totalFaces-8,3) = totalEdges-11;
faceMat(totalFaces-8,4) = -(totalEdges-10);
faceMat(totalFaces-8,5) = -(totalEdges-13);
end %now the sides
faceMat(totalFaces-7,2) = totalEdges-16;
faceMat(totalFaces-7,3) = totalEdges-12;
faceMat(totalFaces-7,4) = -(totalEdges-4);
faceMat(totalFaces-7,5) = -(totalEdges-7);
faceMat(totalFaces-7,6) = -(totalEdges-14);
faceMat(totalFaces-6,2) = totalEdges-15;
faceMat(totalFaces-6,3) = totalEdges-11;
faceMat(totalFaces-6,4) = totalEdges-6;
faceMat(totalFaces-6,5) = -(totalEdges-3);
faceMat(totalFaces-6,6) = -(totalEdges-12);
end %Work way up staircase - bottom then shared then walls
if N>=1
  for i=1:(N-1) %iterate over levels
    for j=1:numVertex %search vertices for first vertex at that height
      if ((vertMat(j,4) == i*initial_height/2) && (vertMat(j,2) == centerx-radiusx) && (vertMat(j,3) == centerx-radiusx))

% now you have the first vertex on that surface!
% the first edge is the edge connecting the next vertex!
for k = 1:totalEdges
    if (edgeMat(k,2) == j && edgeMat(k,3) == (j+1))
        % bottom, then sides, then shared middle
        % bottom always oriented up, sides always out,
        % middle toward +x
        faceMat(8+(4*(i-1)),2) = k;
        faceMat(8+(4*(i-1)),3) = k+1;
        faceMat(8+(4*(i-1)),4) = k+2;
    if i < (N-1) % the middle and sides algorithm only works until you get to a cell
        % which hits the bottom or the top - that's why
        % bottom is hard-coded as well!
        % now the shared middle - split into even and odd
        if rem(i,2)==0 % even case
            faceMat(9+(4*(i-1)),2) = -(k+2);
            faceMat(9+(4*(i-1)),3) = k+5;
            faceMat(9+(4*(i-1)),4) = -(k+6);
            faceMat(9+(4*(i-1)),5) = -(k+3);
        % now the sides
            faceMat(10+(4*(i-1)),2) = k;
            faceMat(10+(4*(i-1)),3) = k+4;
faceMat(10+(4*(i-1)),4) = -(k+12);
faceMat(10+(4*(i-1)),5) = -(k+9);
faceMat(10+(4*(i-1)),6) = -(k+3);
faceMat(11+(4*(i-1)),2) = k+1;
faceMat(11+(4*(i-1)),3) = k+5;
faceMat(11+(4*(i-1)),4) = k+10;
faceMat(11+(4*(i-1)),5) = -(k+13);
faceMat(11+(4*(i-1)),6) = -(k+4);

end

if rem(i,2)~=0 %odd case
faceMat(9+(4*(i-1)),2) = k;
faceMat(9+(4*(i-1)),3) = k+4;
faceMat(9+(4*(i-1)),4) = k+8;
faceMat(9+(4*(i-1)),5) = -(k+3);

%now the sides
faceMat(10+(4*(i-1)),2) = k+2;
faceMat(10+(4*(i-1)),3) = k+3;
faceMat(10+(4*(i-1)),4) = k+9;
faceMat(10+(4*(i-1)),5) = -(k+14);
faceMat(10+(4*(i-1)),6) = -(k+5);
\[
\text{faceMat}(11+(4\times(i-1)),2) = k +1;
\text{faceMat}(11+(4\times(i-1)),3) = k +5;
\text{faceMat}(11+(4\times(i-1)),4) = -(k+13);
\text{faceMat}(11+(4\times(i-1)),5) = -(k+11);
\text{faceMat}(11+(4\times(i-1)),6) = -(k+4);
\]

end
end
end
end
end
end

% Bodies
totalBodies = N+2;
bodyMat = zeros(N+2,7);
bodyMat(:,1) = 1:1:totalBodies;

% Orient faces correctly (check orientation of normals)

% body 1 should always be the bottom half-cell
% bottom half-cell always on left!
bodyMat(1,2) = -1;
bodyMat(1,3) = 2;
bodyMat(1,4) = 3;
bodyMat(1,5) = 5;
bodyMat(1,6) = 8;
% the last body should always be the top half-cell
% if N even, half-cell on right
% if N odd, half-cell on left!
%hard-code the second-to-last body (which is the last full cell)
%because it changes which faces it has based on odd vs even
if rem(N,2)~=0 %odd case
    bodyMat(totalBodies,2) = totalFaces -1;
    bodyMat(totalBodies,3) = totalFaces -2;
    bodyMat(totalBodies,4) = totalFaces -3;
    bodyMat(totalBodies,5) = totalFaces -4;
    bodyMat(totalBodies,6) = -(totalFaces -5);
    bodyMat(totalBodies-1,2) = -(4*N);
    bodyMat(totalBodies-1,3) = -(4*N+1);
    bodyMat(totalBodies-1,4) = 4*N+2;
    bodyMat(totalBodies-1,5) = 4*N+3;
    bodyMat(totalBodies-1,6) = -(4*N+5);
    bodyMat(totalBodies-1,7) = totalFaces;
end
if rem(N,2)==0 %even case
    bodyMat(totalBodies,2) = totalFaces;
    bodyMat(totalBodies,3) = totalFaces -2;
    bodyMat(totalBodies,4) = totalFaces -3;
    bodyMat(totalBodies,5) = -(totalFaces -4);
    bodyMat(totalBodies,6) = -(totalFaces -5);
    bodyMat(totalBodies-1,2) = -(4*N);
    bodyMat(totalBodies-1,3) = 4*N+1;
    bodyMat(totalBodies-1,4) = 4*N+2;
    bodyMat(totalBodies-1,5) = 4*N+3;
    bodyMat(totalBodies-1,6) = totalFaces -4;
    bodyMat(totalBodies-1,7) = totalFaces -1;
end

%now the red cells and blue cells
%might need to hard code the cell touching the bottom
%might need to hard code the cell touching the top
for i=1:(N-1)
    bodyMat(i+1,2) = -(i*4);
    if rem(i,2)~=0
bodyMat(i+1,3) = -((i*4)+1);
bodyMat(i+1,6) = -((i*4)+5);
end
if rem(i,2)==0
    bodyMat(i+1,3) = (i*4)+1;
    bodyMat(i+1,6) = (i*4)+5;
end
bodyMat(i+1,4) = (i*4)+2; % side already oriented out
bodyMat(i+1,5) = (i*4)+3; % side already oriented out
bodyMat(i+1,7) = (i*4)+8;
end

% rotate vertices 45 degrees
centerOfRotX = radiusx/2;
centerOfRotY = radiusy/2;
for i = 1:numVertex
    newX = (vertMat(i,2)-centerOfRotX); % translate center of rotation to origin
    newY = (vertMat(i,3)-centerOfRotY); % same as above
    newX2 = (newX-newY)/sqrt(2);
    newY2 = (newX+newY)/sqrt(2);
    newX3 = newX2+centerOfRotX;
    newY3 = newY2+centerOfRotY;
    vertMat(i,2) = newX3;
    vertMat(i,3) = newY3;
end

% Now write the file to text

fileID = fopen('evan_staircase_test.txt','wt');
fprintf(fileID, 'GRAVITY_CONSTANT 0
');
% CONSIDER PUTTING FILE PATH HERE
fprintf(fileID, strcat('PARAMETER tensC = ',string(tensC),' 
'));
fprintf(fileID, strcat('PARAMETER gamma = ',string(gamma),'
'));

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\n
\n');
fprintf(fileID, strcat('PARAMETER tensS = ',string(gamma*
tensC),'
'));
fprintf(fileID, strcat('PARAMETER cell_opacity = ',string(
cell_opacity),'
'));
fprintf(fileID, strcat('PARAMETER cross_sectional_area = ',
string(cross_sectional_area),'
'));
fprintf(fileID, strcat('PARAMETER eccentricity = ',string(
eccentricity),'
'));
fprintf(fileID, strcat('PARAMETER lambda = ',string(lambda) ,'
'));
fprintf(fileID, '//The following are computed parameters.
');
fprintf(fileID, '//Do not change the formulas unless
necessary.
');
fprintf(fileID, 'PARAMETER tubeD = 2* sqrt(
cross_sectional_area/pi)
');
fprintf(fileID, 'PARAMETER celld = tubeD/lambda
');
fprintf(fileID, 'PARAMETER cell_volume = (4*pi*(celld/2)^3)/3
');
fprintf(fileID, 'PARAMETER initial_height = 2*cell_volume/
cross_sectional_area
');
fprintf(fileID, '//initial positioning
');
fprintf(fileID, 'PARAMETER radiusx = sqrt(
cross_sectional_area/(pi*sqrt(1-eccentricity^2)))
');
fprintf(fileID, 'PARAMETER radiusy = sqrt((1-eccentricity 
^2)*radiusx^2)
');
fprintf(fileID, 'PARAMETER centerx = radiusx
');
fprintf(fileID, 'PARAMETER centery = radiusy
');
fprintf(fileID, 'constraint 1 //constrains bottom to z=0
');
fprintf(fileID, 'formula: x3 = 0
');
fprintf(fileID, 'constraint 2 //constrains top to z=
initial_height
');
fprintf(fileID, 'formula: x3 = (N+1)*initial_height/2
');
fprintf(fileID, 'vertices
');
for i=1:numVertex
    if i<=4
        fprintf(fileID, '%d %f %f %f 
', vertMat(i,1); vertMat(i,2); vertMat(i,3); vertMat(i,4));
    end
    if i>=numVertex-3
        fprintf(fileID, '%d %f %f %f 
', vertMat(i,1); vertMat(i,2); vertMat(i,3); vertMat(i,4));
    end
    if (i>4 && i<numVertex-3)
        fprintf(fileID, '%d %f %f %f 
', vertMat(i,1); vertMat(i,2); vertMat(i,3); vertMat(i,4));
    end
end
fprintf(fileID, '
edges
');
for i=1:totalEdges
    if i<=5
        fprintf(fileID, '%d %d %d 
', edgeMat(i,1); edgeMat(i,2); edgeMat(i,3));
    end
    if (i>5 && i<totalEdges-4)
        fprintf(fileID, '%d %d %d 
', edgeMat(i,1); edgeMat(i,2); edgeMat(i,3));
    end
    if i>=totalEdges-4
        fprintf(fileID, '%d %d %d 
', edgeMat(i,1); edgeMat(i,2); edgeMat(i,3));
    end
end

% need middle faces, so can change their tension correctly in face section
middleMat = zeros(1,N+1);
for i=0:N
    middleMat(1,i+1)=(4*i)+5;
flatMat = zeros(1,N);
for i =0:N-1
    flatMat(1,i+1)=(4*i)+8;
end

fprintf (fileID , \nfaces 
);

for i=1:totalFaces
    if (i==1 || i==4)
        fprintf (fileID , '%d %d %d %d tension tensS+tensC 
         color cyan\n',[faceMat(i,1);faceMat(i,2);faceMat (i,3);faceMat(i,4)]);
    elseif (i==totalFaces -1 || i==totalFaces)
        fprintf (fileID , '%d %d %d %d tension tensS+tensC 
         color cyan\n',[faceMat(i,1);faceMat(i,2);faceMat (i,3);faceMat(i,4)]);
    elseif ismember (i,middleMat)
        fprintf (fileID , '%d %d %d %d %d tension 2*tensC 
         color magenta\n',[faceMat(i,1);faceMat(i,2); faceMat(i,3);faceMat(i,4);faceMat(i,5)]);
    elseif (i==2 || i==3 || i==totalFaces -2 || i==
        totalFaces -3)
        fprintf (fileID , '%d %d %d %d %d %d tension tensS+tensC 
         color cyan\n',[faceMat(i,1);faceMat(i,2);faceMat (i,3);faceMat(i,4);faceMat(i,5)]);
    elseif ismember (i,flatMat)
        fprintf (fileID , '%d %d %d %d %d tension 2*tensC color 
         magenta\n',[faceMat(i,1);faceMat(i,2);faceMat(i ,3);faceMat(i,4)]);
    else
        fprintf (fileID , '%d %d %d %d %d %d %d tension tensS+ 
         tensC color cyan\n',[faceMat(i,1);faceMat(i,2); faceMat(i,3);faceMat(i,4);faceMat(i,5);faceMat(i ,6)]);
    end

fprintf (fileID, '\nbodies\n');
for i=1:totalBodies
   if bodyMat(i,7)==0
      fprintf (fileID, '%d %d %d %d %d %d volume cell_volume
', [bodyMat(i,1);bodyMat(i,2);bodyMat(i,3);bodyMat(i,4);bodyMat(i,5);bodyMat(i,6)]);
   end
   if bodyMat(i,7)~=0
      fprintf (fileID, '%d %d %d %d %d %d %d volume cell_volume
', [bodyMat(i,1);bodyMat(i,2);bodyMat(i,3);bodyMat(i,4);bodyMat(i,5);bodyMat(i,6);bodyMat(i,7)]);
   end
end
fprintf (fileID, '\n\nread\n');
fprintf (fileID, 'U\n'); % for conjugate gradient method
fprintf (fileID, 'delete facets where area=0\n');
fprintf (fileID, 'set facet.opacity cell_opacity\n');
fprintf (fileID, 'unfix radiusx\n');
fprintf (fileID, 'unfix radiusy\n');
fprintf (fileID, 'inner := { show facets ff where sum(ff.
   body,1) == 2 }\n');
fprintf (fileID, 'covershow:={ show facets ff where (sum(ff.
   body,1)==2 or ff.frontbody==1 or ff.backbody==1 or ff.
   frontbody==3 or ff.backbody==3 or ff.frontbody==5 or ff.
   backbody==5 or ff.frontbody==7 or ff.backbody==7 or ff.
   frontbody==9 or ff.backbody==9 or ff.frontbody==11 or ff.
   backbody==11 or ff.backbody==13 or ff.frontbody==13 or ff.
   backbody==15 or ff.frontbody==15 or ff.frontbody==17
   or ff.backbody==17 or ff.frontbody==19 or ff.backbody
   ==19)}\n');
fprintf (fileID, 'gogo := { g 3; r; g 5; r; V 25; g 25; V

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fprintf(fileID, 'iter := { g 25; V 10; }
');
fprintf(fileID, 'iter2 := { u; iter; }
');
fprintf(fileID, 'start := { gogo; iter 25; r; iter2 10; }
');

fclose(fileID);

type evan_staircase_test.txt