THE MECHANICS OF EARLY EMBRYO DEVELOPMENT: INSIGHTS FROM FINITE ELEMENT MODELING

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Abstract

A finite element-based simulation of neurulation, a critical developmental event common to all vertebrates, is presented for an amphibian embryo. During this process, a sheet of tissue rolls up to form a tube, the precursor of the spinal cord and brain. Material property data for the simulation are based on the cellular fabric of the tissues and on tensile test data, and geometric data are obtained from three-dimensional reconstructions. A spatio-temporal correlation system is used to organize and correlate the data and to construct the finite element model. The simulations predict morphogenetic movements similar to those which occur in real embryos.

Keywords: mechanics of morphogenesis, finite element analysis; constitutive models; fabric evolution

Introduction

During early embryo development, structured sheets of cells undergo dramatic self-driven changes of shape in order to form organs and other crucial structures (Fig. 1) [Alberts *et al.* 1989; Gilbert, 2003]. When anomalies occur in these motions, serious and debilitating malformation birth defects, such as spina bifida, can result. In order to develop effective new strategies for preventing malformation defects it is necessary to understand the mechanics that underlie these motions.

To investigate the mechanics of "morphogenetic movements" is challenging because large strains occur in the tissues and their small scale makes it difficult to obtain accurate geometric and mechanical property data. For example, during formation of the neural tube (Fig. 1), the precursor of the spinal cord and brain, certain tissues experience strains as high as several hundred percent. Direct measurement of the tensile properties of the tissues involved in this process is difficult because they are less than half a millimeter in size and are extremely fragile.

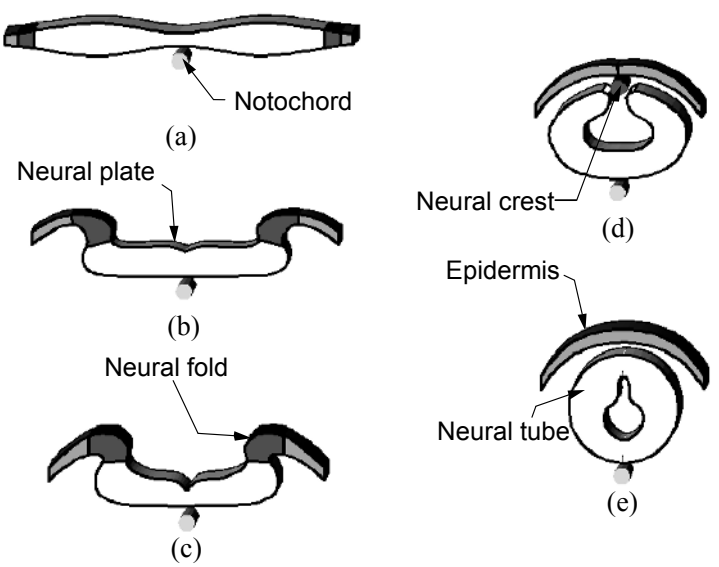

Figure 1. The process of neurulation

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Traditionally, embryological research has focused on identifying the molecular and genetic aspects of development [Huang and Ingber, 1999; Lecuit, 2003], and relatively little effort has been devoted to understanding the relevant mechanics. Recent work [Swartz, 2001; Stephanou, 2002; Chen, 2004; Robinson, 2004; Brodland, 2005; Moore et al, 2005] has shown, however, that mechanical processes must function in concert with chemical signal regulation and genetics to produce morphogenetic movements. In the present study, axolotls (*Ambystoma mexicanum*) embryos are used. Like a number of other amphibian embryos, they share important geometric similarities with human embryos and, for this reason, are often used as an animal model for neurulation.

This paper presents a whole-embryo finite element model of neurulation; the first of its kind. To capture the mechanical interactions that occur across cellular, tissue and whole-embryo scales, an advanced, multi-scale finite element approach is used. Cell-based simulations are used to construct a system of constitutive equations for embryonic tissues, and experimental data are used to determine the parameters in these equations. Images of live embryos and serial sections of fixed embryos provide the geometric data needed to complete the whole-embryo model.

Constitutive Model

In embryonic tissues, cells readily rearrange themselves, and, as a result, the stress-strain characteristics of embryonic tissues are quite different from those of mature tissues. Analytical studies and computer simulations show that the constitutive relationship is greatly affected by cell-level activities such as reshaping, rearrangement, and mitosis [Brodland *et al.*, 2000; Chen and Brodland, 2000; Brodland et al., 2005], a finding that is supported by experimental results [Brodland and Wiebe, 2004].

The model on which the analysis and simulations are based involves two primary assumptions:

1. Interfacial tension, γ, is assumed to be a primary driving force of cell-cell interactions. This tension is generated by circumferential microfilament bundles (CMBs), membrane-associated proteins, and cell membranes, and cell-cell adhesion generated by cell adhesion molecules (CAMs) and other mechanisms reduce the net contraction [Chen and Brodland, 2000].
2. Cell cytoplasm is assumed to be incompressible and characterized by an effective viscosity, μ.

To model cells with these characteristics using the finite element method, each n-sided cell is divided into n triangular elements (Fig. 2b) and rod-like elements are employed to model the interfacial tension. To account for the volume change that would occur in individual triangular elements due to motion of cytoplasm from one triangular element to another Poisson's ratio for the cytoplasm is set to zero. To keep the total volume in each cell constant, a volume constraint is applied to each cell.

Standard finite element approaches are used to determine element stiffness matrices and equivalent nodal loads and to assemble these results into a system of simultaneous equations [Chen and Brodland 2000],

$$\mathbf{C}\dot{\mathbf{u}} = \mathbf{f}\,, \tag{1}$$

where $\mathbf{C}$ is the damping matrix of the system and is derived from the triangular elements, $\mathbf{f}$ is the vector of driving forces produced by the rod-like elements carrying γ, and $\dot{\mathbf{u}}$ is the vector of nodal velocit**ies**. Using a forward difference scheme, this nonlinear equation can be rewritten as

$$\mathbf{C}\dot{\mathbf{u}} \approx \mathbf{C}\frac{\Delta\mathbf{u}}{\Delta t} = \frac{1}{\Delta t}\mathbf{C}\Delta\mathbf{u} = \mathbf{f}\,, \tag{2}$$

and

$$\frac{1}{\Delta t}\mathbf{C}(\mathbf{u}_{q+1} - \mathbf{u}_q) = \mathbf{f}_q\,. \tag{3}$$

Solution of these equations gives the time course of the resulting cell-cell interactions.

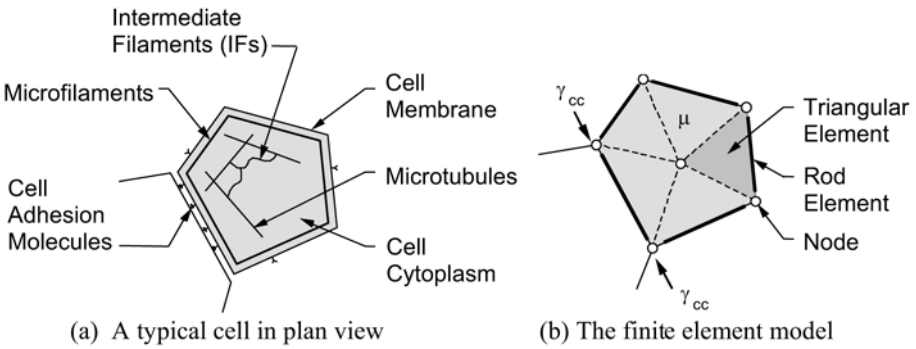


Figure 2. Schematic of a cell and its corresponding finite element model. [Chen and Brodland, 2000]

Insights from finite element models of this kind have lead to the development of constitutive equations for "patches" of such cells (Fig. 3a). The cells in a patch are characterized by a composite cell (Fig. 3b) that captures average aspect ratio, κ, long-axis mean angle, α, and cell density, β [Brodland and Wiebe, 2004].

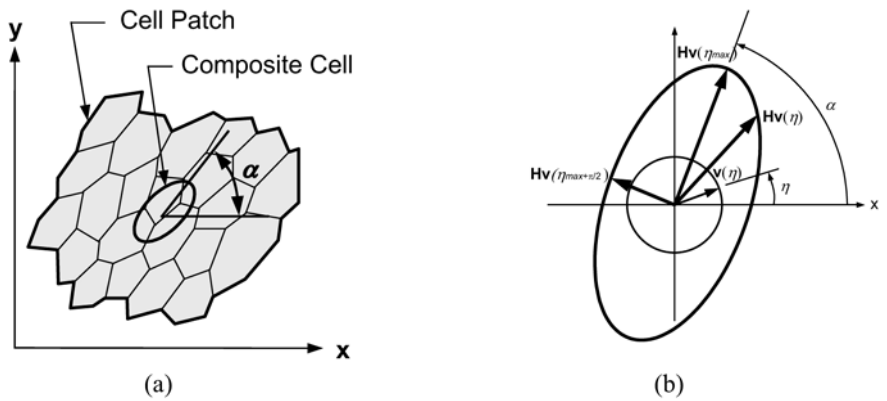


Figure 3. A cell aggregate and its composite cell. [Brodland, 2004]

Brodland and Wiebe (2004) have demonstrated that the principal stresses in the sheet are generated by interfacial tensions according to Equations 4 and 5:

$$\sigma_1 = \frac{\rho\gamma\kappa}{2\pi}\int_0^{2\pi}\frac{\cos^2\theta}{\sqrt{\kappa\cos^2\theta+\frac{\sin^2\theta}{\kappa}}}d\theta \tag{4}$$

$$\sigma_2 = \frac{\rho\gamma}{2\pi\kappa}\int_0^{2\pi}\frac{\cos^2\theta}{\sqrt{\kappa\sin^2\theta+\frac{\cos^2\theta}{\kappa}}}d\theta \tag{5}$$

where stress σ_1 is in the α direction and stress σ_2 is in the direction normal to σ_1. These stresses result in an internal pressure,

$$p = \frac{\sigma_1+\sigma_2}{2}, \tag{6}$$

that acts together with the tension stresses.

Ultimately, the stress-strain characteristics of the tissue depend on the five parameters, γ, μ, κ, α and β [Brodland et al., 2005]. These equations can be incorporated into a tissue-level finite element model used to model an entire embryo (Fig. 4). Values for all parameters can be obtained from suitable images of real embryos and from mechanical property tests on tissue specimens. Tests have shown that these parameters vary with tissue type, location, orientation, and developmental stage [Brodland and Wiebe, 2004; Wiebe and Brodland, 2005].

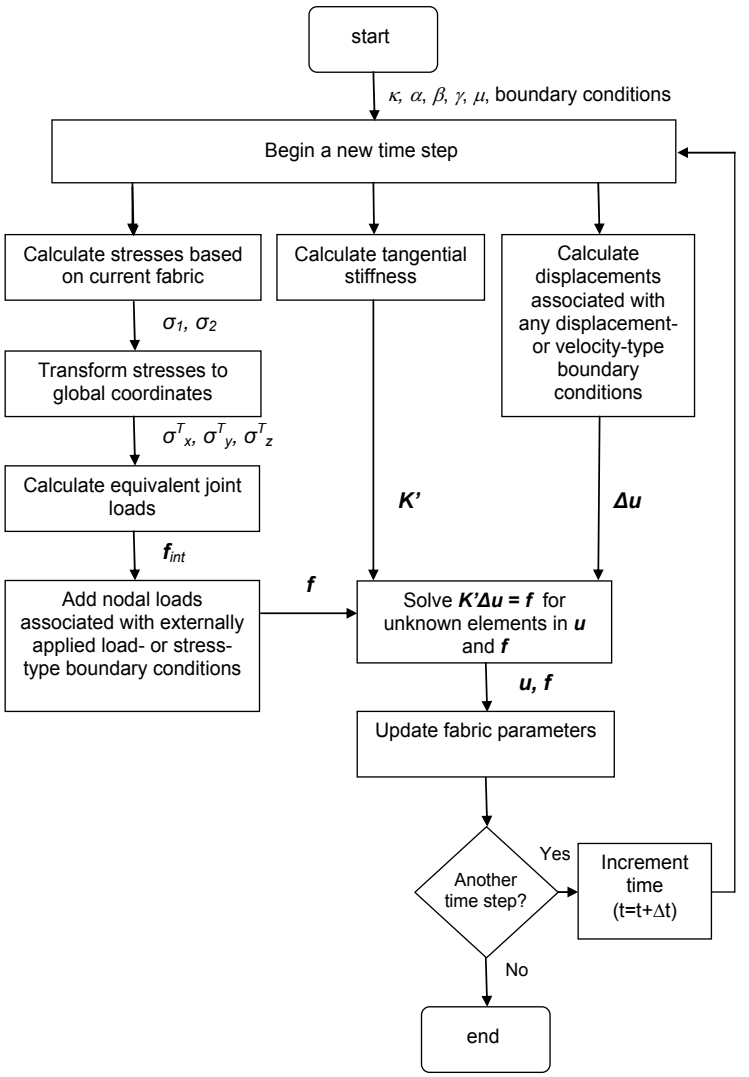


Figure 4. Finite element implementation of the constitutive equations.

Geometric Data

The geometry of an embryo is complex and varies with time. A complete geometric description, therefore, requires both thickness and shape data for each tissue layer and the fabric and material properties of each tissue, plus how these characteristics develop with time. The four primary steps involved in the collection of this "spatial-temporal data" are 1. determining embryo shape, 2. defining tissue fabric parameters, 3. determining embryo layer thickness, and 4. measuring material properties.

1. Determining embryo shape

To identify the specific three-dimensional (3D) shape of a live embryo is technically challenging, and our lab has developed an optical method for extracting live embryo 3D geometric information using robotic microscope images taken at several viewing angles (Fig. 5a). By establishing point correspondences among these views and employing suitable reconstruction algorithms [Brodland and Veldhuis 1998, Bootsma, 2005], we can calculate the spatial positions of a collection of surface points. These points can then be meshed and used to obtain a surface representation of the embryo (Fig. 5b). In-plane tissue motions and strain rates are determined using image processing methods that track the motion of groups of cells [Brodland and Veldhuis 1998; Bootsma, 2005].

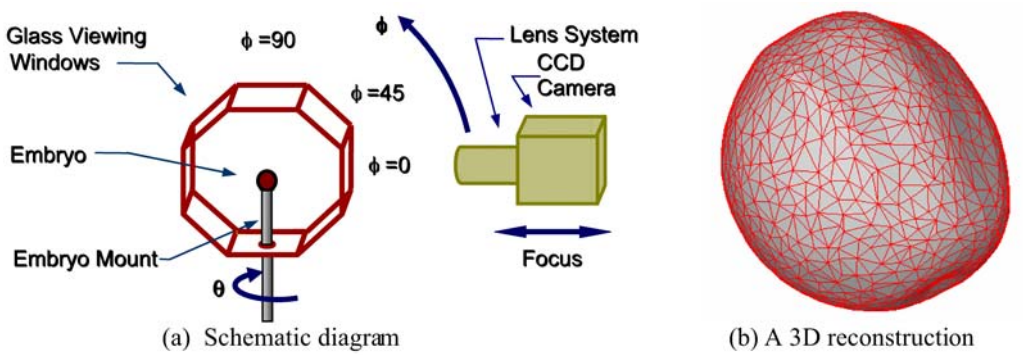


Figure 5. The Robotic microscope and a reconstruction made using it.

2. Defining tissue fabric parameters

Tissue fabric parameters are extracted from the time-lapse images using specially designed image processing software. The software detects the boundaries of the cells and uses Fourier analysis to determine κ, α , and β values. An interpolation scheme is used to map fabric parameters onto the surface mesh.

3. Determining embryo layer thickness

At the beginning of neurulation, the embryo consists of three layers – the ectoderm, the mesoderm, and the endoderm. The thicknesses of each of these layers can be obtained from serial sections (Fig. 6) and merged with the surface reconstruction data. In practice, layer data are obtained from several embryos and averaged to improve data quality.

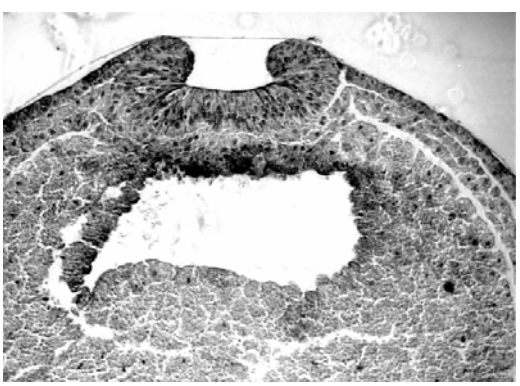


Figure 6. A transverse serial section

4. Measuring material properties

The tissues in an embryo are extremely fragile and, as a result, measurement of material properties is difficult. To obtain stress-strain data, a small specimen (300 by 500 μm) of tissue is attached to very thin wires. The specimen is stretched by drawing the wires apart under computer control. Applied force is calculated from measurements of wire bending, and real-time feedback is used to prevent strain reduction that would otherwise arise from the wire deflections. Companion, cell-level finite simulations are used to back calculate the γ and μ values [Wiebe and Brodland, 2005].

Feature Grid for Correlating Data

The spatial-temporal data collected in the above steps are obtained from a variety of sources, and each has a different natural coordinate system. To combine these data we devised a surface coordinate system, or feature grid. The grid and its attendant interpolation software serve as a vehicle through which element-specific data can be obtained for the whole-embryo finite element model.

Biologists generally divide the surface of a neurulation-stage embryo into three main regions: the neural plate region, the neural fold region, and the non-neural ectoderm region (Fig. 7). Potential feature grids devised for the neural plate are shown in Figure 8. The "parabolic" grid (Fig. 8a) was chosen for the neural plate region, and grid systems compatible with it were chosen for the other two regions.

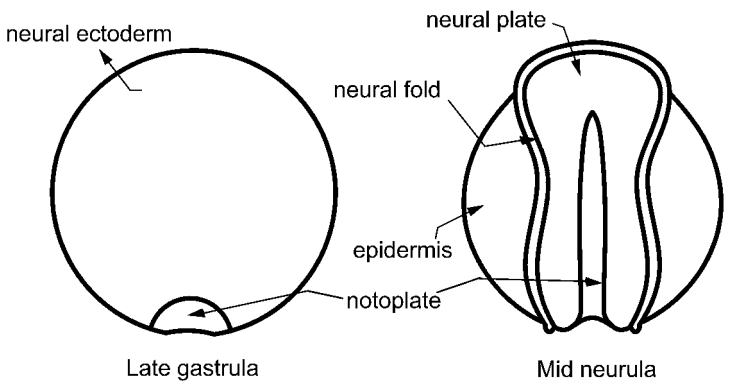
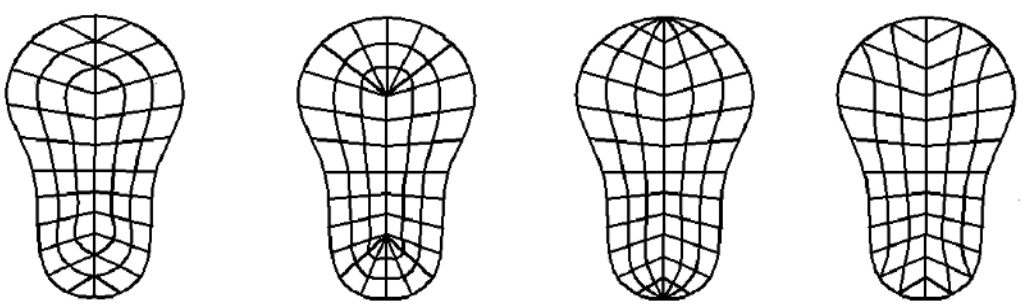
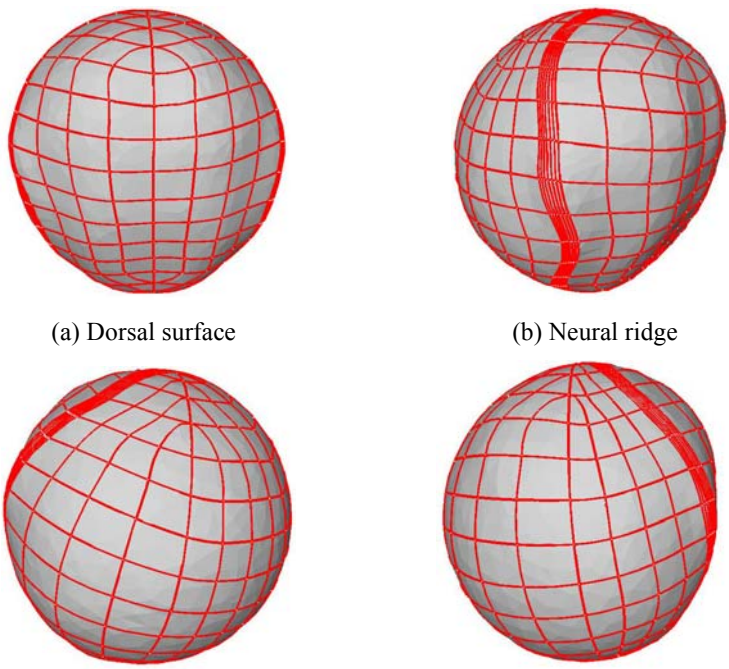


Figure 7. Embryonic region identification



(a) Parabolic (b) Arched (c) Rectangular (d) Gothic

Figure 8. Parametric coordinate systems



(a) Dorsal surface (b) Neural ridge

(c) Ventral region I (d) Ventral region II

Figure 9. Parametric coordinates shown from a variety of viewing angles

In the feature grid system, each surface point has unique u and v coordinate values, and the grid is mapped onto the 3D embryo surface reconstruction (Fig. 9) using a mathematical mapping (u, v) → (x, y, z). Mappings to other data sets are accomplished in a similar way. Details of the process [Bootsma, 2003] are beyond the scope of the present paper. All data are stored in both their raw form and in a feature grid form (Figure. 10).

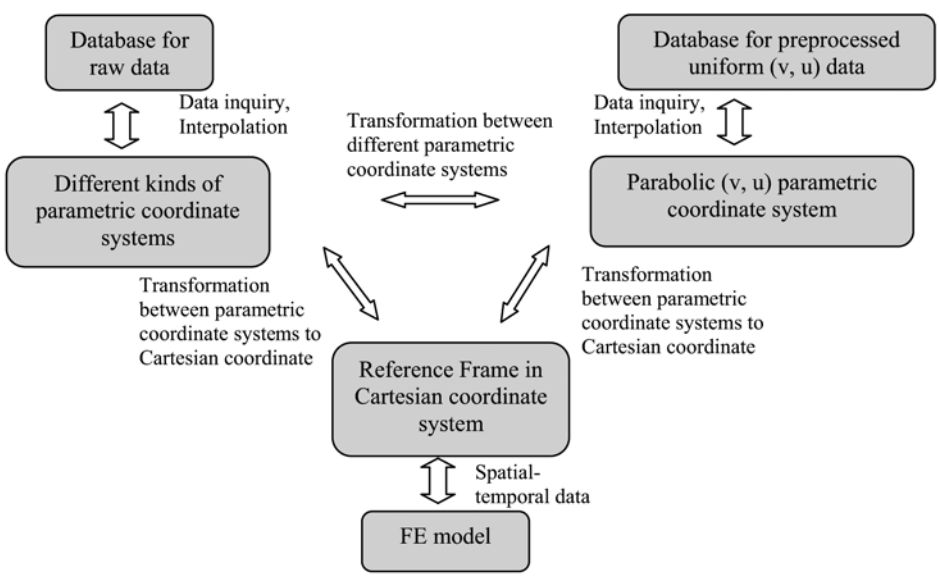
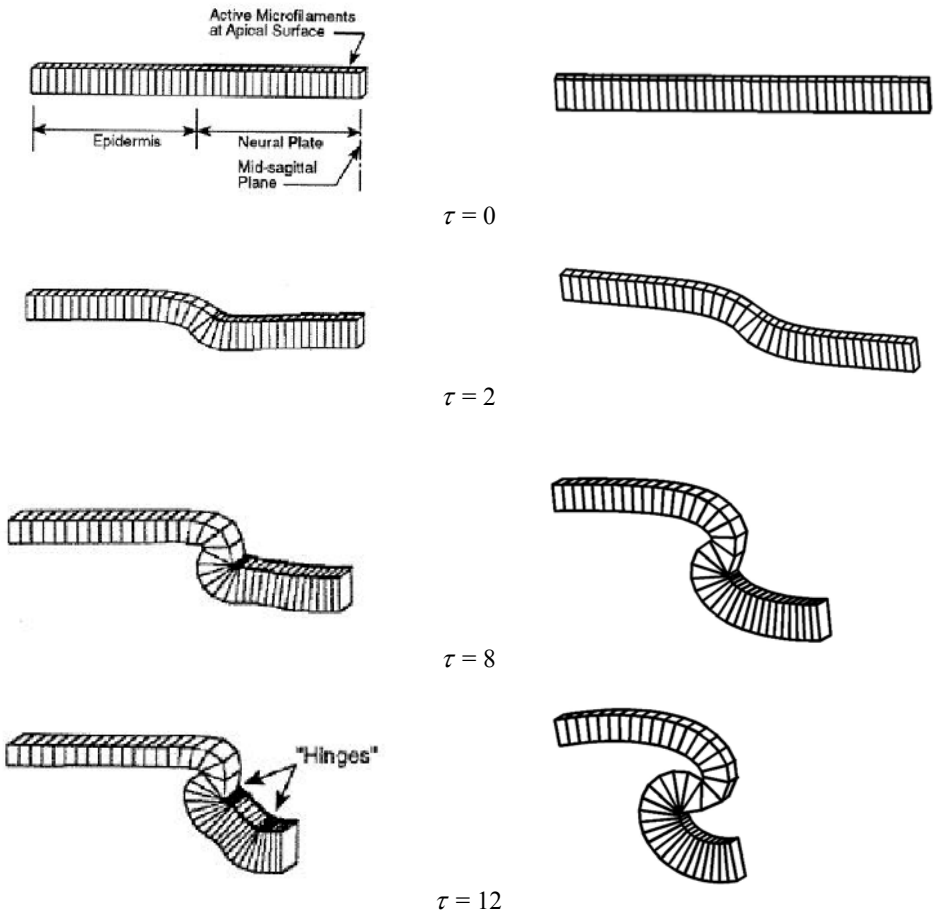


Figure 10. Schematic chart for inquiry system

The finite element mesh generator uses the feature grid as a basis for data inquiry and interpolation, and uses it to determine on an element-by-element basis, tissue geometry and thickness, cellular fabric and mechanical properties.

2D Simulations

To assess the accuracy of the collected data, the interpolation software and the finite element engine, a series of 2D simulations were conducted. Simulations of transverse aspects of neural tube development (Fig. 11) were found to be generally consistent with those obtained by Clausi and Brodland (1994). Which study's simulation best represents actual embryos is not yet known. The differences between the simulations are apparently due to differences in how the contraction forces are calculated: Clausi and Brodland used non-linear truss elements, whereas the current study uses cellular fabric.



(a) Simulations conducted by Clausi and Brodland

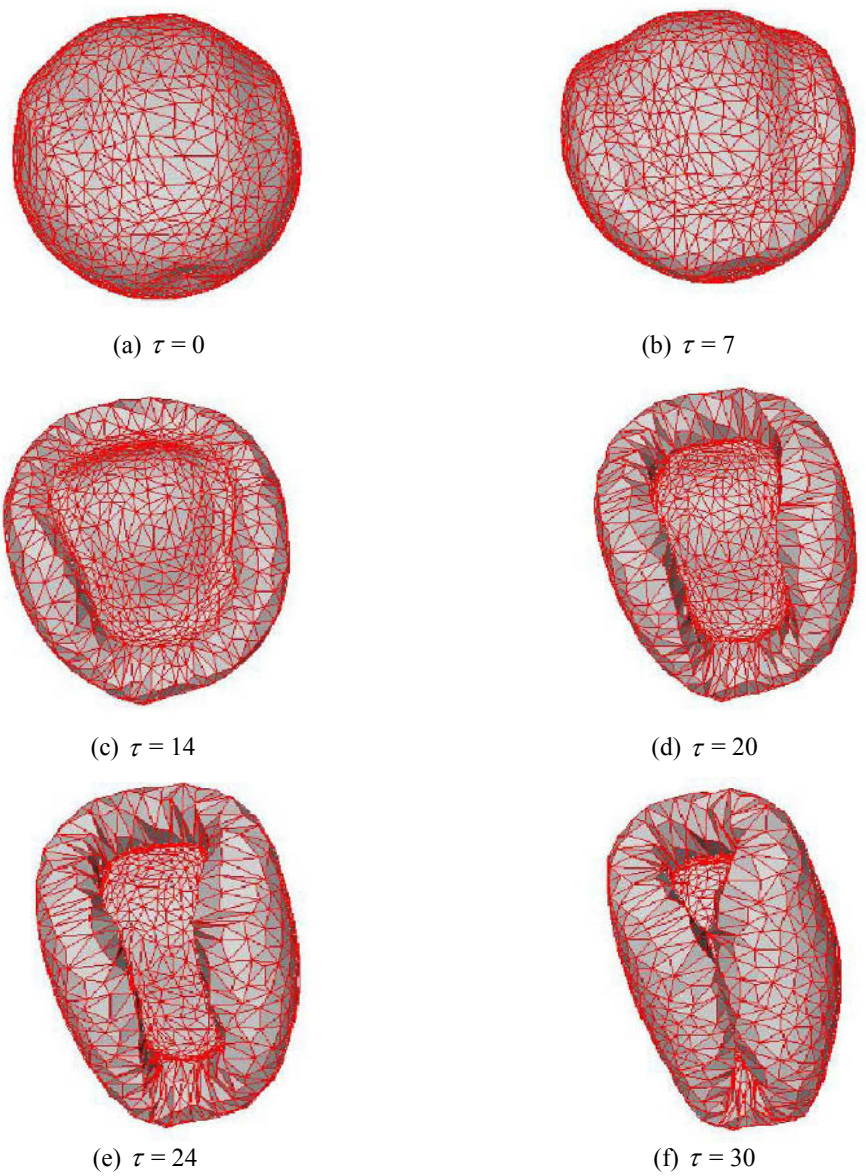
(b) New simulations

Figure 11. Comparison of simulations of a transverse section of the embryo

3D Simulations

The initial configuration of a complete, three-dimensional finite element model, based on available geometric, fabric, and mechanical-property data, is shown in Fig. 12. Each finite element represents a triangular patch of approximately 150 cells. The finite element engine was used to determine how the tissues in the model embryo would interact with each other over time. The engine shows that the neural tube of the model embryo does indeed roll up (Fig. 12b-f) and that it does so in a manner that is similar to neural tube closure in real embryos.

Sensitivity analyses showed that morphogenetic movements are highly sensitive to details of the initial configuration and tissue mechanical properties, a finding consistent with previous 2D simulations [Clausi and Brodland, 1994].



(a) $\tau = 0$ (b) $\tau = 7$

(c) $\tau = 14$ (d) $\tau = 20$

(e) $\tau = 24$ (f) $\tau = 30$

Figure 12. Whole-embryo simulations of neural tube rolling

Discussion and Conclusions

The finite element simulation presented here is the first published full-embryo model of neurulation. In time, it will be refined, as were its 2D predecessors, so that differences between the model and real embryos can be eliminated. The process of resolving these differences is an important scientific task because through it, deficits in understanding can be identified and relevant new experiments conceived.

The model demonstrates that realistic tissue motions are possible when a suitable cell-based constitutive equation is used. A key feature of the current constitutive model is that cells are able to flow past each other in-plane, a characteristic known to be important in real embryos. In previous (unpublished) attempts to model neurulation in 3 dimensions, cells were not free to rearrange in plane. This deficiency caused the tissues to be excessively stiff, especially with respect to in-plane shear, and it impeded the complex 3D deformations that must occur near the ends of the neural plate region. The present constitutive equation also made it possible for single finite elements to model multiple cells, making whole-embryo models computationally practical.

Recent experiments have shown that, during development, the fabric of the embryonic epithelia varies substantially with tissue type, location, and development stage. The constitutive model used here is able to successfully predict fabric evolution during *in vitro* tests, but additional statistical analysis software must be written before the accuracy of those predictions can be assessed in the context of neurulation. Such comparisons, however, are important to full model validation.

The constitutive model is structured so that, as the molecular pathways involved in tissue regulation are identified, their effects can be incorporated into the model. The model can hence serve as a bridge between gene expression and the morphogenetic movements of critical developmental events. This important integration of biology and mechanics is possible because the finite element method provides an open computational framework.

Simulations conducted to date show that tissue motions are highly sensitive to the geometry of the initial configuration and to tissue mechanical properties. This finding suggests that spina bifida and other neural tube defects might arise through a variety of subtle mechanical means. It also suggests that modest interventions might be sufficient to prevent neural tube defects. Identifying appropriate intervention methods is a critical goal for additional research, and the finite element model presented here holds promise as means to carry out preliminary evaluations of proposed interventions.

Acknowledgements

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