The chick forelimb is a model system in the study of the biological processes involved in tissue growth and pattern formation. The manipulation of these mechanisms in numerous experimental studies has resulted in the elucidation of many qualitative, as well as quantitative aspects involved. We use this information to formulate a spatio-temporal model for growth based on the coupling of the intracellular and extracellular morphogenetic mechanisms with the mechanics of the ecto- dermal and mesodermal tissues. The resulting model is comprised of system of non-linear partial differential equations which are solved on an approximation of the geometry of the stage 16 (HH16) limb bud. The resulting model provides a feasible computational tool for studying the complex interactions of growth and patterning in the vertebrate limb.

**ABSTRACT**

To study growth and spatial patterning in the developing chick limb, we develop a model that includes both the morphogenetic and the tissue mechanics. We first simulate these components separately and then combine them together and examine their interactions and the resulting effects on patterning.

**COMPUTATIONAL MODEL**

Motivation

Limb deformities caused by birth defects affect 1 in 300,000 human children. Since the general structure and signal transition network of the limb are evolutionarily well-conserved between the vertebrates, studying limb development in any vertebrate will advance our understanding in all. For example, many vertebrates, such as salamanders, can regenerate excised limbs, and there is recent evidence which suggests that mammals have this capability as well. Thus, the chick is a model system for studying the pattern formation that results from the hierarchical interactions between genes, their protein products, and tissue-level growth and cell division.

**BACKGROUND**

Limb Development

We consider the limb bud of the chick from Hamburger-Hamilton stage 16 (HH16) through HH25, a period of approximately 72 hours.

**RESULTS**

Analysis of Reaction and Diffusion

First, we compare the results of the reaction-diffusion system alone, using two distinct computational schemes. An ADI code was implemented to solve the kinetic system for 1 < t < 75 in a 3Dx3D grid. Additionally, we implemented the reaction-diffusion model in Comsol Multiphysics. The parameters, equations, initial conditions, boundary conditions, and other expressions were configured to be equal between both codes, and the same non-dimensionalization was used. The results shown below are the same as those shown above.

Next we created a more accurate geometric representation in Comsol of an HH20 limb bud [6] in which to study the interaction of reaction and diffusion. This approach allows us to more easily perform implant, excision, and other simulated experimental procedures, due to the malleable interface. The results shown below are the same as described in the wild-type network above, as were simulated in the ADI code.

**FUTURE WORK**

- Due to the spatial heterogeneity of growth in the limb, convergence problems arise in regions of the domain, particularly where the boundary is nearly concave. By including a proper finite element mesh, we may be able to distribute the displacements, as well as increase the biological accuracy of the model. Mesh refinement algorithms are also being investigated.
- Further comparisons of the kinetic and mechanical solutions with both experimental solutions will be used to validate the numerical procedures and the computational algorithm. This will ensure that predictions made by the model are valid.
- Simulation of experimental procedures, including excision, implant, and genetic modification will allow us to test the accuracy of model predictions against experimental results and to test new experimental hypotheses.
- New genes have been implicated in digit formation, but their upstream regulation and downstream effects are the subject of much research. By allowing us to control these genes, the model can be used to address questions about how, when, and why these genes are activated.
- Control of growth in the model is dominated by the concentration of the FGF family of morphogens, but does not incorporate the flow of mass into the limb, nor is the possibility of chemotactic movement of the cells included.
- After stage 25, many other processes occur in the limb, including vasculogenesis, chordogenesis, and apoptosis. The formulation and integration of these processes into a "whole limb" model may be used for investigating the effects these processes have on each other.

**ACKNOWLEDGEMENTS**

- The Minnesota Supercomputing Institute and the Digital Technology Center at the University of Minnesota.
- NIH grant GM29-123 and NSF grant DMS-051-7884.

**REFERENCES**