A Biologically Derived Approach to Tissue Modeling

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Abstract. Our approach to tissue modeling incorporates biologically derived primitives into a computational engine (CellSim®) coupled with a genetic search algorithm. By expanding an evolved synthetic genome CellSim® is capable of developing a virtual tissue with higher order properties. Using primitives based on cell signaling, gene networks, cell division, growth, and death, we have encoded a 64-cell cube-shaped tissue with emergent capacity to repair itself when up to 60% of its cells are destroyed. Other tissue shapes such as sheets of cells also repair themselves. Capacity for self-repair is an emergent property derived from, but not specified by, the rule sets used to generate these virtual tissues.

1. Introduction

Most models of biological tissues are based on principles of systems engineering [1]. For example, tissue structure and elasticity can be modeled as dampened springs, electrically excitable tissues can be modeled as core conductors, and tissues such as blood can be modeled according to principles of fluid mechanics. As different as these models are, they share a number of general features: they are constructed from the perspective of an external observer and designer of the system; they are grounded in laws (Hook, Kirchoff, Ohm, Bernoulli, etc.) that describe predictable behavior of the physical world in a manner that can be verified empirically, by measurement; they incorporate feedback controls to optimize system performance by tuning of adjustable elements; their complexity requires some kind of computational approach.

Although models based on a systems engineering approach contain a number of features that mimic the way that natural living systems are built and how they function, such models differ from natural systems in important ways (Table 1). Notably, living organisms have been designed by evolutionary processes characterized by descent with modification from ancestral forms, not by a single-minded, purposeful, intelligent architect. This means that natural designs are constrained by evolutionary legacies that may be sub-

Table 1. General features of natural and human-designed systems

	Natural Systems	**Human-engineered Systems**
Design	Selection by evolutionary process	Optimization by architect
Construction	Self-constructs by development; continuous turnover of components	Built by a separate process and apparatus prior to operation
Control	Feedback, homeostasis, self-repair, regeneration	Automated feedback
Tuning/ Operation	Contingent, adaptable and plastic; monitors complex, unpredictable environment	Task-specific; monitors only a few parameters

optimal but unavoidable consequences, for example, of the process of development in a given species. Even so, living systems incorporate a number of remarkable features that human-designed systems lack, such as self-construction via development, self-repair, plasticity, and adaptability –the ability to monitor and respond to complex, unpredictable environments*.

In an attempt to devise tissue models that conform more closely to the living systems they emulate, we have begun to incorporate biologically-derived primitives into a computational framework [3,4]. Relevant features include: 1) a developmental engine (CellSim®) that expands a synthetic genome to develop a virtual tissue composed of cells; 2) an evolutionary selection process based on genetic algorithms; 3) rule-based architecture using cellular processes such as growth, division, and signaling; and 4) higher order (emergent) properties such as self-repair. In summary, such an automated modeling process minimizes human intervention, reduces human error, and yields robust, versatile rule-based systems of encoding.

2. Computational Platform

2.1 Biological Primitives

Our computational platform is designed to incorporate principles of biology, particularly those primitive features of living systems that are fundamental to their construction and operation and that distinguish them from non-living. Living organisms share five basic features (Table 2): 1) compartmental organization (a form of anatomical modularity); 2) self-replication and repair; 3) adaptation (sensitivity to environment and ability to evolve); 4) selective communication among components; 5) requirement for energy (dissipative, non-equilibrium).

Table 2. Biological Primitives and Their Representation in CellSim®

Biological Primitive	**Representation in CellSim®**
Compartments	Cells, each with genome containing gene-like elements
Self-replication & Repair	Cell division and growth; replacement of dead cells
Adaptation	GA search engine with genetic operators and defined fitness function
Selective Communication	Signals to/from environment and neighboring cells; gene regulatory networks
Energy Requirement	Growth substance ≥ threshold value

Each of the above features is intertwined with at least one of the others. For example, despite their sometimes static appearance, living organisms are in a continual state of repair and renewal. This requires energy to build, replicate, disassemble, monitor and repair components. In addition, regulatory capacities such as homeostasis derive from the processes of cell signaling, compartmental organization, and selective feedback communication among components.

The most fundamental compartment of living systems is the cell, the smallest unit capable of self-replication. Each cell contains a genome, and it has a boundary that both delimits the cell and its contents and mediates exchange and communication between the cell and its surroundings. Accordingly, we have chosen to focus our simulations on a cellular level of granularity: processes such as division, differentiation, growth, and death are encoded in gene-like data structures, without reference to their complex biochemical basis *in vivo*. However, signaling and control of gene expression are treated computationally as molecular processes.

2.2 Basic Operation of CellSim®

The computational engine CellSim® models tissue phenotype (appearance, traits, properties) as the result of a developmental process, starting from a single cell and its genome. Properties such as tissue morphology and self-repair arise from the interaction of gene-like elements as the multicellular virtual tissue develops.

CellSim® defines and controls all of the environmental parameters necessary for development, including placement of nutrients, defining space for cells to grow, sequencing of actions, and rules that govern the physics of the environment. To make CellSim® more flexible, all of the environmental parameters (e.g., rules governing the calculation of molecular affinity and the placement and concentration of nutrients or other molecules) are configurable at run-time. If no value is specified for a parameter, default settings apply.

After the parameters of the CellSim® environment are configured, development is initiated by placing a single cell into that environment. The cell's genome then interacts with any molecules in the environment as well as any molecules that are produced internally by the cell. Depending upon these interactions, each gene within the cell may be turned on (or off). When a gene is turned on, the transcription apparatus of the cell produces the molecules defined by the gene's structural region. These newly produced molecules may in turn interact with the cell's genome, affecting rates of transcription at the next time step. Development is thus governed by inputs from the external environment, and also by internal feedback mechanisms.

In addition to transcription, two primary actions– cell death (apoptosis) and cell growth/ division– are available to each cell in the current version of CellSim®. The genome of a cell may include genes that encode death molecules (and/or growth molecules) and as the genes that encode either growth or death molecules are transcribed, the concentration of these molecules in the cell's cytoplasm increases. Growth or death is then a function of the concentration of these two types of molecules. When a cell dies, it is removed from the CellSim® environment. Alternately, if a cell grows and divides, a new cell is placed in a location adjacent to the existing (mother) cell. If all adjacent positions are already occupied, that cell may not divide even if the concentration of growth substance exceeds the threshold for growth.

2.3 Cell Signaling

In addition to environmental factors and internally produced molecules, a cell may also receive information from neighboring cells. The simplest neighborhood of a cell consists of those cells that are spatially adjacent to (touching) the cell of interest. However, CellSim® allows a cell's neighborhood to be configured as any arbitrary group of cells. For example, a neighborhood (the cells to/ from which it will send/ receive signals) could include cells that are not adjacent, as occurs *in vivo* with cells that are able to signal non-local cells via hormones.

Cellular signaling is based on a handshake approach that requires both the sender and the receiver to create specific molecules in order for a signal to be transmitted. To send a signal, a cell must create molecules of type 'signal'. At each time step, each cell determines which cells are in its neighborhood and presents the signal(s) it has produced to its neighbors. For a cell to receive a signal that is being presented to it, the cell must build receiver molecules that are tuned to the signal. This completes the handshake portion of the cell signaling process – i.e. in order for a signal to be passed between two cells, the sender cell's signal must be compatible with the receiver molecules built by the receiver cell. Finally, when a receiver senses a signal for which it is designed it generates an internal

signal that is defined by the receiver molecule (which is ultimately defined and produced by the cell's genome), but is independent of the particular signal a receiver molecule is designed to detect. This third component has been decoupled from the receiver and signal to allow different cells to produce entirely different internal signals from the same external stimulus. The strength of the internal signal is a configurable function of the concentration of signal molecules produced by the sender and the concentration of receiver molecules that the receiver has produced.

2.4 GA-Based Search

To automate the process of tissue modeling, genetic algorithms (GAs) are used to search for a genome with proper encoding to render the desired (target) tissue shape and function [3,4]. Typically a seed population of cells, each with a different genome, develop to yield a population of individuals, each a multicellular tissue with different properties. An individual is defined by both its genome and the CellSim® configuration that develops it; during evolution this permits modification of the genome (using genetic operators) or alteration of the context for development, or both.

Three basic steps are required to process each individual in the population. First, a CellSim® environment is instantiated using the configuration specified by the individual, and a single cell with a defined genome is placed in that environment. Then the CellSim® engine is allowed to run until a stable configuration is reached (or a maximum number of time steps is reached). If the configuration stabilizes, the fitness of the resulting individual is evaluated.

Currently, we have chosen to focus on the relatively simple problem of producing a multicellular tissue with a particular shape. Accordingly, the fitness of an individual is a function of how closely the stable configuration of cells matches the target shape. As we begin to produce more complex tissues, other target properties such as elasticity, connectivity, reactivity, contraction, and cellular state of differentiation will be incorporated into more complex fitness functions. After each individual in a population has been evaluated and scored according to a fitness function, the GA selects a subpopulation, usually those individuals with the highest fitness, as the starting set for the next generation. Genetic operators (mutation, duplication, deletion, or cross-over) increase the genetic variation of the seed population for another round of development by CellSim®, and the cycle repeats.

3. Simple Virtual Tissues

3.1 Initial Conditions

To test the capabilities of our computational platform and search algorithms we chose a cube as one of the first target tissue shapes. A cube is a relatively simple shape, but it is not necessarily easy to encode using CellSim®, because CellSim® naturally lends itself to developing shapes with curved surfaces and smooth edges (e.g., ellipses or spheroids), but shapes with large, flat surfaces and sharp edges pose a greater challenge.

The target shape for the GA search was a 4x4x4 cell cube, and the developmental engine was initialized with a population of 50 individuals, each defined by its genome and by its CellSim® configuration. Each individual's starting genome was a single gene with two control regions, one promoter and one repressor, linked to a single structural region coding for cell growth factor. In addition, the GA search algorithm was configured with 13

different types of mutation operators, most of which alter the control and structural regions of the cell's genome, modifying parameters that dictate the type of molecule a particular control region interacts with, the effect of this interaction on transcription, and the type(s) of molecule(s) encoded by the structural region. The other mutation operators modify the CellSim® configuration, in particular the placement and concentration of molecules in the environment. The mutation rate varied between 10 and 20 percent, depending upon the type of mutation operator.

3.2 Evolving a Cube-Shaped Tissue

The best individual in the starting population had a fitness of 0.37 (fitness = 1.0 indicates a perfect match). Fitness remained constant until the 19th generation, when an individual of fitness 0.64 was produced. At this point, the best individual's genome and the CellSim® starting configuration had been modified extensively by mutation, with relatively large changes in the parameters governing interaction of the gene control regions, and addition of 4 point sources of growth molecules to the CellSim® environment. Over the next 31 generations the GA produced incremental improvements to the average fitness of the population, until at the 50th generation the GA converged to a perfect solution, and the search terminated (Fig. 1A).

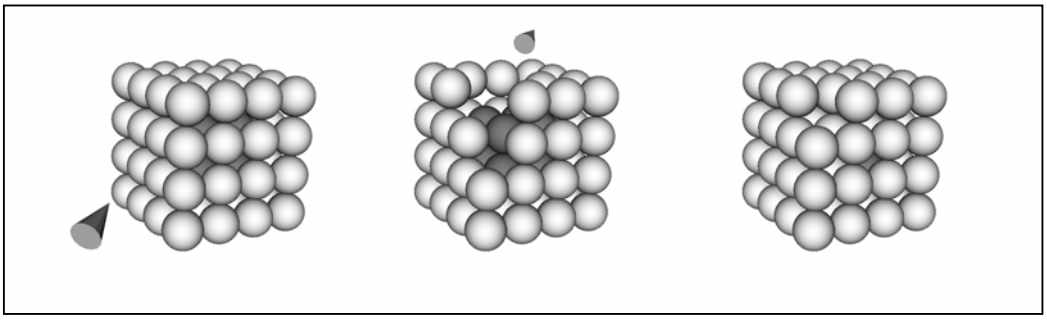

Figure 1. Cube embryo; fitness 1.0 individual produced by a GA search. Self-repair of the cube after being damaged by a projectile (conical shape, 1st panel, lower left). The projectile approaches the cube from the lower left, passing upward, through, and exiting near the midpoint of the upper surface, leaving a visible hole (middle image). Dark inner cells are ready to divide. A partially repaired embryo (right image), still missing a cell along the left front edge.

3.3 Self-Repair

The GA-based search is able to find an individual that matches the target shape but furthermore, this individual is capable of sustaining a relatively large amount of damage to its structure during development without compromising its ability to produce a stable cube. Indeed, even after the individual produces a stable cube shape, if the cube sustains damage (loss of up to 60% of its structure) the individual detects the damage and repairs it. Although the undisturbed cube appears static, injury reveals that the capacity for self repair remains latent.

Using other starting genomes and CellSim® configurations we have been able to evolve tissues with different shapes, such as single- or multi-layered sheets of cells. While not all of the tissue shapes that have been produced in our experiments can repair damage during all phases of the development process, all of the shapes produced to date do have some ability to repair damage, depending upon when the damage is introduced. For example, using a more complicated development process that involves cell signaling to

cause death of cells that grow in undesired positions and to terminate cell growth at the appropriate time, we have evolved an individual that produces a stable 5x5 single-layer sheet of cells. In this case, incoming signals from neighboring cells cause other cells to adopt a state that stabilizes the sheet, but also inhibits the ability to repair damage to the sheet. Consequently, this individual is not as resilient to damage as the cube. Nevertheless, while the sheet individual is in the growth/development phase, it can sustain moderate damage yet it still produces the desired target shape.

4. Discussion and Conclusions

This paper presents an automated, bottom-up approach to tissue modeling, the goal of which is to derive higher-level properties of biological systems –self-directed development, self-repair, feedback, resilience to perturbation, and adaptability– from low-level biological primitives, allowing the system to assemble these primitives to achieve a desired target. This differs significantly from standard, top-down tissue modeling, where any higher-level biological properties must be explicitly designed and encoded into the system.

The ability to repair damage or injury is an emergent property of tissues evolved by the GA/ CellSim® system. Tissue phenotype results from interaction of genetically encoded elements with the environment defined by CellSim®, and improvements in phenotype arise through an iterative evolutionary process guided by a GA-based search strategy. Capability for self-repair was not specifically encoded in any gene, nor was this capability a factor in calculating the fitness during the GA-based search, but rather, capacity for self-repair arises from the biological primitives incorporated in CellSim® (e.g., molecular interaction, gene networks, feedback mechanisms, etc.). None of these biological primitives *explicitly* encodes the ability to repair damage, but when taken together they tend to make the process of construction robust to relatively large disruptions.

We are currently exploring ways to generate more complex self-repairing shapes and to incorporate biological primitives that support other tissue properties such as mechanical deformability.

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*As an extreme example of versatility, among certain reef fishes the largest female in a harem changes sex and becomes the dominant, functional male if the original dominant male dies or is removed [2]. Thus, changing the social environment triggers a complete reorganization of the (former) female body plan. By analogy, this could be seen as the equivalent of a house spontaneously reorganizing its floor plan and plumbing to accommodate an increased number of party guests as the holiday season approaches.

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References

[1] Sun, W. and P. Lal (2002) Computer Methods and Programs in Biomedicine 67: 85-103

[2] Robertson, D.R. (1972) Science 177:1007-1009

[3] Forbes, N. (2004) *Imitation of Life: How Biology Is Inspiring Computing*. MIT Press, Cambridge, MA, pp. 13-50

[4] Langton, C.G. (1989) In *Artificial Life*, vol. VI, C.G. Langton, ed., Addison-Wesley, NY, pp.1-47