Brief Review

Modern Physics Letters B, Vol. 20, No. 20 (2006) 1217–1231

World Scientific
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COMPUTATIONAL MODELING OF TISSUE SELF-ASSEMBLY

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Received 7 August 2006

As a theoretical framework for understanding the self-assembly of living cells into tissues, Steinberg proposed the differential adhesion hypothesis (DAH) according to which a specific cell type possesses a specific adhesion apparatus that combined with cell motility leads to cell assemblies of various cell types in the lowest adhesive energy state. Experimental and theoretical efforts of four decades turned the DAH into a fundamental principle of developmental biology that has been validated both *in vitro* and *in vivo*. Based on computational models of cell sorting, we have developed a DAH-based lattice model for tissues in interaction with their environment and simulated biological self-assembly using the Monte Carlo method. The present brief review highlights results on specific morphogenetic processes with relevance to tissue engineering applications. Our own work is presented on the background of several decades of theoretical efforts aimed to model morphogenesis in living tissues. Simulations of systems involving about 10^5 cells have been performed on high-end personal computers with CPU times of the order of days. Studied processes include cell sorting, cell sheet formation, and the development

of endothelialized tubes from rings made of spheroids of two randomly intermixed cell types, when the medium in the interior of the tube was different from the external one. We conclude by noting that computer simulations based on mathematical models of living tissues yield useful guidelines for laboratory work and can catalyze the emergence of innovative technologies in tissue engineering.

Keywords: Cell adhesion; differential adhesion hypothesis; tissue engineering.

1. Introduction

Starting from the beginning of the 20th century, theoretical models of living tissues have evolved along two distinct conceptual lines. The first of these views considers the tissue as a set of discrete, interacting cells, whereas the other treats it as a continuum, and monitors cell densities instead of individual cells.[18] Here we briefly describe a few of these models. The interested reader can find further details in the cited literature.

The continuum approach, promoted by Murray and Oster, uses the methods of continuum mechanics and allows for modeling tissues built of realistic numbers of cells (see Ref. 14 and references therein). The distribution of cells of various types throughout the tissue is described in terms of their densities, whereas their morphogenetic rearrangements are treated as fluxes. The method has been applied for studying developmental morphogenesis, dermal wound healing, contraction, scar formation and vasculogenesis.[18] The latter phenomenon may occur *in vivo* via two different mechanisms: (i) *vasculogenesis*, a term used for *de novo* vessel formation by the self-assembly of an endothelial cell population, and (ii) *angiogenesis*, the formation of capillary vessels by endothelial cell sprouting from preexisting vessels. (Endothelial cells represent the major component of small blood vessels and line the internal face of large ones. Besides endothelial cells, capillaries also feature attached pericytes, a cell type responsible for the stability of capillary vessels.)

Growing large, vascularized organ replacements in the laboratory is one of the major challenges of tissue engineering. Therefore, understanding the intimate mechanisms of vasculogenesis is vital for building perfusable tissue constructs. Starting from a cell population randomly distributed on the planar surface of a homogeneous extracellular matrix, the model of Murray *et al.*[14] predicts the emergence of interconnected filamentous structures of cells that are similar to a vascular network. The results are in good agreement with *in vitro* experiments on endothelial cells seeded on Matrigel.[11]

One of the most important principles of developmental biology, which inspired numerous discrete cell models, is the *differential adhesion hypothesis* (DAH).[20] It states that (i) cell adhesion corresponds to cell-type-dependent energies and (ii) the constituent cells of a tissue are motile enough to reach the lowest energy configuration. The DAH withstood the test of time, being confirmed by many experiments. This principle leads to a close analogy between true liquids and living tissues made of adhesive and motile cells, such as most embryonic and some artificial tissues.[3]

A considerable number of discrete cell models rely on DAH. Monte Carlo simulations of the large-N Potts model from statistical physics reproduced experimental findings regarding the segregation of different cell populations and the mutual engulfment of adjacent tissue fragments.[5] In this model the tissue is represented on a lattice, each cell spans several lattice sites, and has a unique identification number; the average number of sites per cell is maintained around a target value via an elastic energy term containing a Lagrange multiplier. The simulations are based on the Metropolis algorithm, accounting for cell migration and shape changes in systems made of up to several thousand cells.[4] These simulations suggested that cell motility may be ascribed to an effective, temperature-like parameter.[2]

Recent computational models of *in vivo* morphogenesis, besides DAH, also include chemical signaling, i.e. chemotaxis, cell differentiation and extracellular matrix production.[12,16] The process of culmination of the cellular slime mold *Dictyostelium discoideum* under the condition of scarce food supply, was simulated in two dimensions by combining the Glazier and Graner model[4] with a set of partial differential equations able to describe cAMP signaling. The model is defined by parameters characteristic for the subcellular level and is able to predict phenomena that involve the self-organization of thousands of cells. In this respect, this model bears the potential to characterize the morphogenetic impact of genes whose function is elucidated at the subcellular level.[12] Slime mold aggregation has also been described using a distinct, force-based, three-dimensional (3D) model, in which individual amoebae are treated as viscoelastic ellipsoids with type-dependent adhesion apparatus, intrinsic motility and cAMP-mediated signaling capacity.[16]

2. *In Silico* Tissue Engineering

Tissue engineering (TE) is a rapidly developing field of biomedical research, which aims to repair, replace or regenerate damaged tissues. It exploits biological morphogenesis, a self-assembly process that gives birth to a large variety of structures in living systems. TE employs a number of techniques meant to engage cells into forming tissues[10] (see also the web page `http://www.nsf.gov/pubs/2004/nsf0450/`). It is hard to overestimate the importance of the field, given that growing organs *in vitro* could solve the problem of transplantable organ shortage. TE evolved in close relationship with regenerative medicine, and proved successful in developing various functional organ modules. These may be used for *in vivo* tissue repair, may promote tissue regeneration, and can also be used for testing new drugs. The clinical success of TE depends on finding a suitable cell source, on optimizing scaffolds or hydrogels that support cell growth, differentiation and assembly, on building bioreactors able to provide physiological conditions for the engineered tissues, and, most importantly, on the development of techniques able to assure long-enough shelf life for the tissue construct to reach the patient.[6]

The success of the theoretical methods currently used to describe both *in vivo* and *in vitro* rearrangements of cell populations motivated recent attempts of modeling phenomena of interest in TE. In order to be efficient in screening alternative experimental designs and in offering hints for related laboratory studies, computational tissue engineering must properly account for the dynamics of cell populations in the presence of scaffolds and extracellular matrices that on the one hand guide cell behavior and, on the other hand, are subject to degradation and restructuring by cells. Several groups are presently engaged in this endeavor. For example, a recent computational model describes the movement of fibroblasts within an acellular dermal matrix of oriented fibers.[18] The simulations predict the dynamics of matrix invasion as a function of various parameters, such as fiber size distribution, packing density, and matrix morphometry. The scaffold is generated by a random walk algorithm and the fibroblast movement along the fibers is described by a five-state Markovian process of directional change. The hopping of cells between nearby fibers is simulated using the Monte Carlo method. The simulations are remarkable also from the point of view of the computational platform they used, a versatile 3D modeling and animation package, MAYA (Alias, Toronto, ON, Canada; `http://www.alias.com`). It incorporates an onboard programming language, along with physics simulation, visualization, and animation engines. The computational model is meant as a supplement for experimental efforts to streamline the workflow of matrix design.

In the context of bone and cartilage morphogenesis *in vitro*, hierarchical computational techniques have been employed to design anatomically shaped 3D scaffolds with controlled porosity and chemical composition via solid free-form fabrication.[7] The method proved useful for improving the mechanical properties of scaffolds and resulted in accelerated tissue formation.

Computer simulations, serving as proof-of-concept *in silico* experiments, may also speed up the development of new technologies. As an example, we present results related to the modeling of artificial organs built from spheroids containing tens of thousands of cells. The self-assembly of these cell aggregates resembles the fusion of adjacent liquid drops, thus justifying the name of "bioink" given to the cell aggregates used in bioprinters. Their computer-controlled, layer-by-layer deposition into a supportive hydrogel (also referred to as "biopaper") goes by the name of bioprinting.[9,13,15] Model assumptions and parameter estimations are based on experiments. Simulations of similar systems starting from new initial configurations, with same or modified conditions, are used to optimize future experiments. In the following we describe the employed computer simulation method along with some of the obtained results.

3. A Lattice Model of Living Tissues

We first build a lattice model of a system of living cells in a culture medium or an extracellular matrix, and then turn to simulate its evolution using the Metropolis

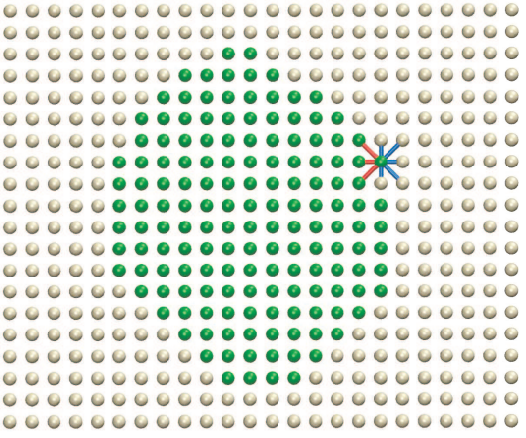

Fig. 1. A simplified, two-dimensional square-lattice model. Sites are occupied by cells (black), or volume elements of medium or extracellular matrix (light gray). A cell is considered to interact to the same extent with nearest or next-nearest neighbors. Cells interact with adjacent cells (light gray bond) and with the surrounding medium (dark gray bond). In order to avoid double-counting, only half of the depicted bonds are attributed to the given cell [see Eq. (1)].

Monte Carlo method.

Our goal is to describe the self-assembly of cells within tissue constructs made of hundreds of thousands of cells. Therefore, in contrast to the Glazier–Graner model,[4,5] our program focuses on the types of particles present on each lattice site rather than following cell shape changes or monitoring the position of individual cells.

For computational simplicity, we discretize the space and represent the biological system on a cubic lattice. Each lattice site is occupied either by a cell, or a similar-sized volume element of the embedding medium. Figure 1 depicts the 2D version of the model that enables us to explain the significance of the terms in the total interaction energy:

$$E = \sum_{i,j} \left[J(\sigma_{i,j}, \sigma_{i,j+1}) + J(\sigma_{i,j}, \sigma_{i+1,j+1}) + J(\sigma_{i,j}, \sigma_{i+1,j}) + J(\sigma_{i,j}, \sigma_{i+1,j-1}) \right]. \quad (1)$$

The occupancy of a given site,(i, j), is specified by a type index, $\sigma_{i,j}$, which can take two values, namely 0 for a medium (type 1) particle, and 1 for a cell (type 2) particle. A given cell interacts with its neighbors either directly, via cell adhesion molecules (e.g. cadherins) or indirectly by binding to extracellular matrix filaments via integrins. In our model adhesivities are associated to contact interaction energies also referred to as bond energies, which are determined by both the strength and dynamics of the involved chemical bonds. Each term on the right hand side of Eq. (1) can take the values $J(0,0) = -\varepsilon_{11}$, $J(1,1) = -\varepsilon_{22}$ or $J(1,0) = J(0,1) = -\varepsilon_{12}$. The ε's are positive quantities and represent the mechanical work needed to disrupt the corresponding bond.

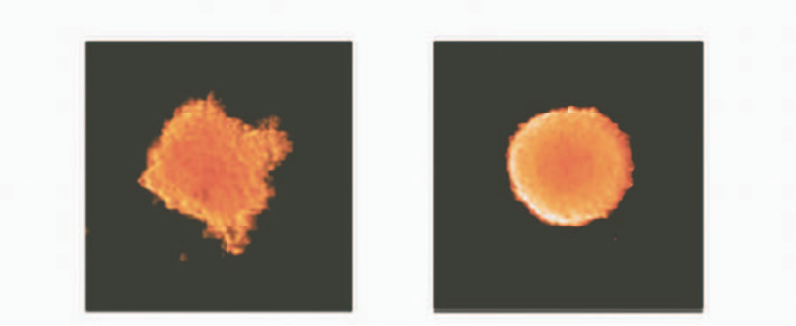

Fig. 2. Spontaneous rounding of an irregular tissue fragment made of CHO cells during 24 hours of incubation.

The second law of thermodynamics tells us that such a system will evolve towards the less structured, highest symmetry state that has maximum entropy. In the associated biological problem, however, we are dealing with an open system, so the principle of maximum entropy does not necessarily imply less structure in the emergent cellular pattern. That is why, for example, the differential adhesion hypothesis is not a direct consequence of the second law of thermodynamics. DAH has its origin in experiments like that of Fig. 2, showing that an irregular tissue fragment placed in a nonadhesive environment spontaneously rounds up, as a liquid droplet would.[3] This experiment indicates that the rearrangement of cells is dictated by interfacial forces: the tissue rounds up in order to minimize the area exposed to the tissue culture medium. (A sphere is the geometrical object of smallest surface area for a given volume).

The total interaction energy may be rewritten in terms of interfacial contributions. To this end, consider a configuration of N_1 particles of type 1, out of which N_1^I are located on the 1–2 interface while the rest, N_1^B, reside in the bulk. A similar partitioning may be done for the type 2 particles as well. Thus, Eq. (1) can be rewritten as

$$E = -\frac{1}{2}\left[N_1^B n_n \varepsilon_{11} + N_2^B n_n \varepsilon_{22} + \sum_{i_1=1}^{N_1^I}(n_{1i_1}\varepsilon_{11} + n_{2i_1}\varepsilon_{12}) + \sum_{i_2=1}^{N_2^I}(n_{2i_2}\varepsilon_{22} + n_{1i_2}\varepsilon_{12})\right] \tag{2}$$

where the factor 1/2 cancels the double counting of interacting pairs. The dummy index i_1 (i_2) runs over interfacial particles of type 1 (2), whereas $n_{1i_1}(n_{2i_1})$ stands for the number of type 1 (2) neighbors interacting with the type 1 particle labeled by i_1. A similar notation holds for the type 2 interfacial particles labeled by i_2.

The simulations are performed for systems of finite size, such that cells are coaxed to move within the medium. With these boundary conditions, it is technically convenient to maintain an immobile medium layer on the frontier of the system. The contribution to the interaction energy coming from this layer is constant and, therefore, it can be discarded. Thus we are dealing with particles lying in

the interior of the system with n_n significant neighbors ($n_n = 8$ in two dimensions and $n_n = 26$ in three dimensions); therefore $n_{1i_1} = n_n - n_{2i_1}$ and $n_{2i_2} = n_n - n_{1i_2}$, which leads to

$$E = -\frac{1}{2}\left[(N_1^B + N_1^I)n_n\varepsilon_{11} + (N_2^B + N_2^I)n_n\varepsilon_{22} + (\varepsilon_{12} - \varepsilon_{11})\sum_{i_1=1}^{N_1^I} n_{2i_1} + (\varepsilon_{12} - \varepsilon_{22})\sum_{i_2=1}^{N_2^I} n_{1i_2}\right]. \tag{3}$$

Both sums in Eq. (3) yield the total number of heterotypic bonds $\sum_{i_1=1}^{N_1^I} n_{2i_1} = \sum_{i_2=1}^{N_2^I} n_{1i_2} = B_{12}$. Indeed, the first is obtained by cumulating the numbers of type 2 particles in the significant neighborhood of each interfacial particle of type 1, whereas the second one is the sum of the numbers of type 1 particles around all type 2 particles from the 1–2 interface. Thus, the total adhesive interaction energy, both in 2D and 3D, becomes

$$E = \gamma_{12}B_{12} - \frac{1}{2}N_1 n_n \varepsilon_{11} - \frac{1}{2}N_2 n_n \varepsilon_{22} \tag{4}$$

where B_{12} is the number of 1–2 bonds, directly proportional to the area of the interface, and

$$\gamma_{12} = \frac{\varepsilon_{11} + \varepsilon_{22}}{2} - \varepsilon_{12} \tag{5}$$

is the interfacial tension parameter. During simulations that do not include cell proliferation, differentiation and death the last two terms on the right hand side of Eq. (4) are constant and, therefore, they may be omitted.

Canonical Monte Carlo simulations using $E = \gamma_{12}B_{12}$ yielded results in qualitative agreement with experiments on living tissue self-assembly.[9] This expression is remarkable, since it does not depend on the strengths of all types of interactions, but only on their combination, γ_{12}.

In the case of a complex tissue of several cell types and media, the total interaction energy, under the constraint of constant numbers of particles of each type, is given by

$$E = \sum_{\substack{i,j=1 \\ i<j}}^{T} \gamma_{ij} \cdot B_{ij}\,, \tag{6}$$

where T is the number of particle types in the system and $\gamma_{ij} = \frac{1}{2}(\varepsilon_{ii} + \varepsilon_{jj}) - \varepsilon_{ij}$ are the interfacial tension parameters. One can easily show that there are $T(T-1)/2$ independent γ_{ij} parameters.

The *interfacial tension*, σ_{12}, defined as the interaction energy corresponding to the unit area of the interface, may be obtained from γ_{12} by assuming that each of the n_n bonds formed between a cell particle and its neighbors stems form adhesive interactions acting on an average cell membrane area of S_c/n_n, where S_c is the cytoplasmic membrane area of a typical cell from the simulated population. Under this simplifying assumption, which does not take into account the eventuality of the clustering of adhesion molecules, one infers $\sigma_{12} = \gamma_{12}\, n_n/S_c$.

Tissue surface tension (TST) is defined as the energy of the unit area of interface between a biological tissue and cell culture medium; it is experimentally accessible via a specially designed parallel plate compression apparatus. During the last decade TST has been measured for several tissue types, and employed to predict the sorting behavior of heterotypic tissues.[3] In the framework of our lattice model the surface tension of a tissue is given by $\sigma = \varepsilon_{22}\, n_n/(2\, S_c)$. See Eq. (5) where the index 1 refers to cell culture medium and 2 to cells, and it has been assumed that ε_{11} and ε_{12} are negligible in comparison to the cell-cell work of adhesion, ε_{22}. Note that the latter may be assessed using the measured values of the tissue surface tension along with an estimate of the cell surface area.

4. Monte Carlo Simulations of the Self-Assembly of Cells into Tissue Constructs

A convenient way for studying energetically driven conformational changes of a system is the Monte Carlo method.[1] This name stands for a large collection of computational algorithms that involve the use of random numbers. It is important to pay attention to the choice of the random number generator — a numerical algorithm that actually generates pseudo-random numbers. Correlations between the terms of the generated sequence may have important impact on the results. Our simulations are based on a random number generator of L'Ecuyer with Baym–Durham shuffle.[17]

Tissue evolution is followed using a version of the Metropolis algorithm adapted for the biological problem at hand. The initial state is constructed based on the known composition and shape of the studied biological system. Then, a conformational change is made by identifying interfacial cells, picking one of them by chance and exchanging it with an adjacent, randomly chosen, medium particle. The corresponding change of adhesive energy, ΔE, is calculated, and the new conformation is accepted with a probability

$$P = \min\left(1, \exp\left(-\frac{\Delta E}{E_T}\right)\right). \tag{7}$$

The move is readily accepted if it leads to a decrease of the energy. However, in the opposite case, the move may also be accepted albeit with a probability less than one (given by the Boltzmann factor). In practice this decision is made by generating a random number, r, with uniform distribution between 0 and 1, and accepting the new conformation provided that $r < \exp(-\Delta E/E_T)$. A Monte Carlo step (MCS)

is defined as the sequence of operations during which each interfacial cell has been given the chance to move once.

Such an algorithm is suitable for the study of fluids with velocity-independent interactions,[19] therefore it provides a natural framework for investigating the consequences of tissue liquidity. Its technical implementation also requires creativity, since multiple decision steps can be made efficiently by relying on algebraic operations instead of nested *if* statements. For instance, in a system of T types of particles by assigning type indices as $\sigma_1 = 0$ and $\sigma_i = 100^{i-2}$ for $i = 2, \ldots, T$, the nature of the neighbors of a given site may be easily inferred from the sum of their type indices. Let us illustrate this in some particular situations: For the highlighted cell of Fig. 1 this sum would be 3, showing that the cell is surrounded by 3 cells and 5 medium particles. Had one of the adjacent cells been of another type, the sum would have been equal to 102, yielding one neighbor of type 3, two of type 2 and five of type 1.

From the biological point of view the above stochastic rules mean that a cell actively explores its neighborhood, being able to exchange position with adjacent cells or to reorganize the extracellular matrix from their vicinity. The latter process is known to involve both mechanical traction forces and enzymatic activity by matrix metalloproteases (MMPs). The DAH tells that cells are able to keep track of partial success in lowering their adhesive energy by building more/stronger bonds with their surroundings.

In the acceptance probability [Eq. (7)] E_T is a measure of cell motility, the analogue of the energy of thermal fluctuations in true liquids. It is referred to as the energy of biological fluctuations, being related to cytoskeleton-driven cell membrane ruffling, and has been estimated for certain cell types.[2] Our relevant model parameters are the ratios obtained by dividing the interfacial tension parameters by E_T. This implies that a highly cohesive tissue made of very motile cells is assumed to behave just as a less cohesive one which consists of less motile cells.

Finally, we demonstrate the simulation methods described above in two biologically relevant cases, namely cell sorting and directed self-assembly of cells into constructs of controlled shape.

It is a well known experimental fact that distinct cell types from a cell aggregate spontaneously segregate. Measurements have revealed that in segregated cell aggregates the most cohesive population occupies the central region, being surrounded by the less cohesive one (Fig. 3).

A measure of tissue cohesivity is its surface tension, an experimentally accessible quantity, which predicts the sorting hierarchy.[3] In the simulations of Fig. 4 we started with a 3D cell aggregate of linear size of 30 cell diameters, made of about 32000 cells of two different types, in roughly equal amounts, randomly intermixed.

Comparison with the experimental result allows to determine the relative magnitudes of the interfacial tension parameters. Note that the values given in the caption of Fig. 4 are consistent with the experimental observation that the cell type from the interior has higher adhesivity than that from the periphery. Indeed,

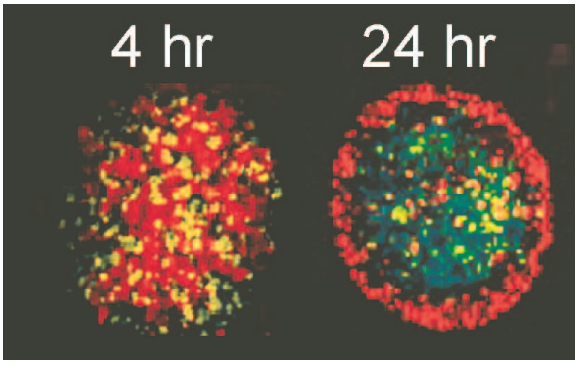

Fig. 3. Starting with a random mix of two distinct cell populations (red and green), within a day of incubation in a hanging drop assay the cells sort out, cells of the most adhesive type (green) being surrounded by those of the less adhesive one (red). The aggregate diameter is about 300 μm.

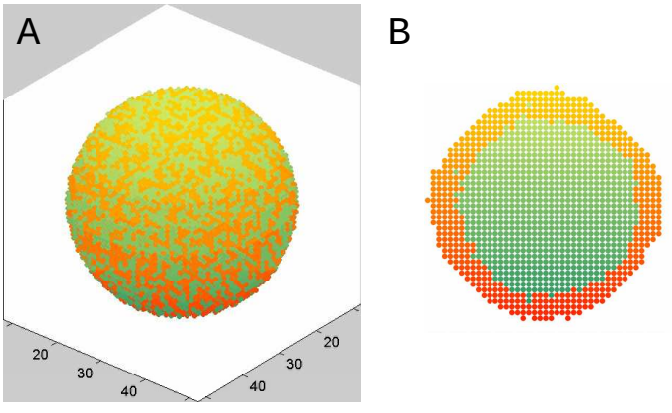

Fig. 4. Simulation of cell sorting in a 3D aggregate of 16 789 cells of type 2 (green) and 16 612 cells of type 3 (red). The surrounding medium (consisting of type 1 particles) is not shown. The initial state **(A)** contains a random mixture of the two populations, whereas the result of 2×10^5 MCS is a completely sorted equilibrium conformation of the system **(B)**, shown in cross-section. Compare with the experimental result of Fig. 3. The interfacial tension parameters, expressed in units of E_T are: $\gamma_{12} = 1.5,\ \gamma_{13} = 0.5,\ \gamma_{23} = 0.3$.

according to Eq. (5), $\gamma_{12} = \varepsilon_{22}/2$ and $\gamma_{13} = \varepsilon_{33}/2$ because the cells do not interact significantly with the cell culture medium (type 1); we conclude that the mechanical work needed to disrupt a bond between two model cells is three times lower in the case of the external (type 3) population ($\varepsilon_{33} = 1;\ \varepsilon_{22} = 3$.)

Once the parameters are estimated on the basis of experiments, the model may be employed for predicting possible outcomes of the spontaneous self-assembly of cells in a system of complex shape and composition.

In the evolving technology of organ printing, successive layers of cell aggregates

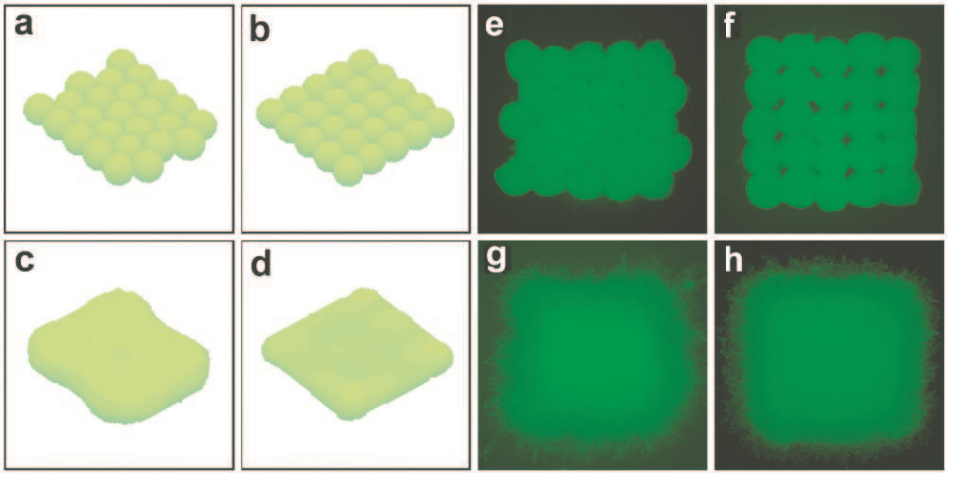

Fig. 5. Sheet formation depends on the initial configuration and the tissue-matrix interfacial tension. Two initial states, made of model cell aggregates, 925 cells each, packed in a hexagonal (**a**) and square lattice (**b**), during 250,000 MCS evolve into configurations shown in panels **c** ($\gamma_{cg}/E_T = 0.8$) and **d** ($\gamma_{cg}/E_T = 1.4$), respectively. For identical parameters, fusion from the hexagonal initial configuration is considerably faster. Similar structures of 25 aggregates of CHO cells (500 μm in diameter) were embedded in 1.0 mg/ml collagen type I (**e** and **f**). Compact sheets after 144 hours of incubation are shown in panels **g** and **h**.

and an embedding hydrogel are placed on top of each other and tissue liquidity is supposed to lead to subsequent fusion of the artificial tissue droplets, giving rise to constructs of desired shape. The *in silico* study of post printing cellular rearrangement may offer hints regarding the conditions needed to coax the cells to build the desired configuration. It has been shown that the properties of the supportive hydrogel are vital in this respect.[15] This has been verified both *in silico* and *in vitro*. Modeling results are shown in Figs. 5(a)–5(d), whereas the experimental validation is depicted in Figs. 5(e)–5(h). Spherical cell aggregates prepared from Chinese Hamster Ovary (CHO) cells transfected with N cadherins and histone-attached yellow fluorescent protein (for adhesion and fluorescence microscopy observations, respectively), were embedded in 1.0 mg/ml collagen type I hydrogel in 2D close packed and grid-like geometries. (For a detailed description of the protocol of manufacturing spherical aggregates, see Ref. 9).

Figure 6(A) depicts a model system of a "printed" tubular structure of two cell and two gel types. Suitable energetic conditions, expressed by the values of the interfacial tension parameters that incorporate cell-cell and cell-matrix adhesion energies lead to tissue conformations that are similar to blood vessels (Fig. 6(B)). The plot from Fig. 7 shows that our algorithm indeed generates conformations of lower and lower energy. Especially the first hundred MCS, corresponding to cell sorting and adjacent aggregate fusion, result in a sudden drop of the energy (Fig. 7, inset). This is followed by a regime of slow decrease, indicating that the

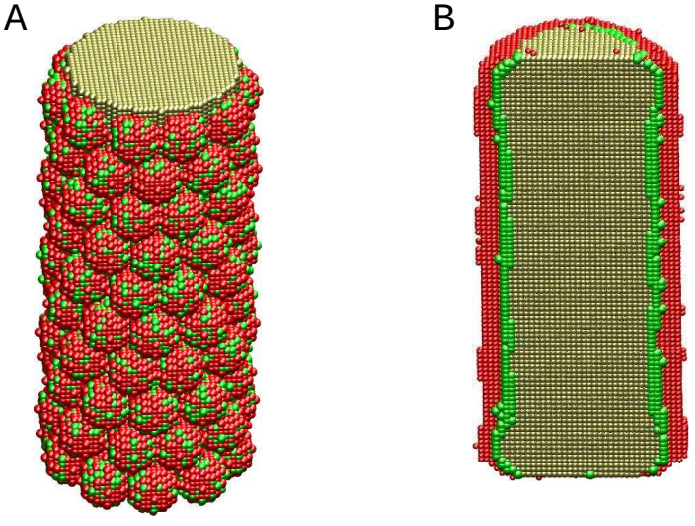

Fig. 6. Post-printing cell sorting and aggregate fusion in a blood vessel like construct. The initial state (**A**) consists of 100 cell aggregates, closely packed along a tube of gel (tan). Each cell spheroid contains 257 cells; about 30 % are model endothelial (green) cells, randomly intermixed with the model smooth muscle (red) cell population. The outcome of the 10^5 MCS simulation (**B**) indicates that spontaneous endothelialization is possible under suitable energetic conditions. In our model these are expressed by the values of the interfacial tension parameters [see Eq. (6)]: $\gamma_{12} = 1.8,\ \gamma_{13} = 1.2,\ \gamma_{14} = 0.7,\ \gamma_{23} = 0.7,\ \gamma_{24} = 1.2,\ \gamma_{34} = 0.4$. Illustrations rendered with the program VMD.[8]

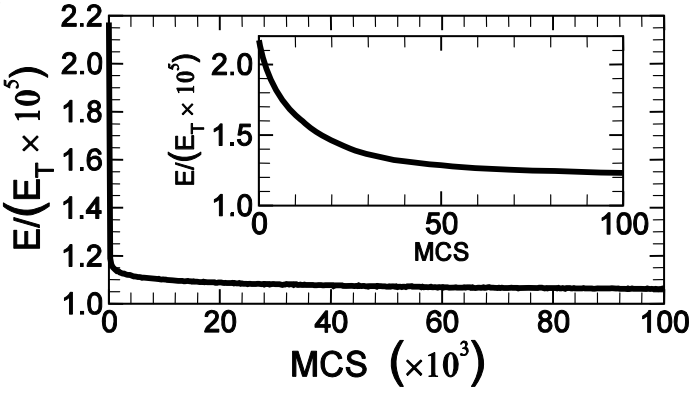

Fig. 7. The total adhesive energy expressed in units of the biological fluctuation energy, E_T, versus number of elapsed MCS during the simulation of Fig. 6.

tubular structure of Fig. 6(B) is a long-lived metastable state. According to this *in silico* experiment we expect that, provided that the technical difficulties of building such systems in the laboratory can be overcome, the self-assembled tube can be transferred into a pulsed-flow bioreactor that will provide biomimetic conditions for tissue maturation.

5. Conclusions and Outlook

A large variety of theoretical models aim towards understanding how cells organize into tissues. Due to the diversity and complexity of biological tissues, these models, rather than being universally valid, are in fact able to account only for a limited range of phenomena in specific tissue types by shedding light on specific developmental processes, e.g. *in vivo* tissue remodeling and *in vitro* morphogenesis. The importance of modeling in regenerative medicine and tissue engineering cannot be overlooked, since computer simulations are relatively quick and inexpensive ways of testing working hypotheses and designing new experimental setups.

Living tissues are viscoelastic materials. On a time scale of days most embryonic tissues and artificial tissue constructs, obtained by the self-assembly of adherent cells, mimic the behavior of highly viscous fluids. This property, referred to as tissue liquidity, may be exploited in tissue engineering for controlling the spreading of cells on biocompatible materials in scaffold-based approaches as well as for building living structures of desirable shape and composition by bioprinting and biopatterning. Tissue liquidity may be understood in the light of the DAH, which implies that the rearrangements of the constituent cells result from their motility and from their tendency of establishing the largest possible number of strong bonds with their neighbors.

In the present mini-review we have presented a brief overview of the biological tissue models employed in computational tissue engineering, with particular emphasis on a specific, DAH-based lattice model of the self-assembly of living cells into artificial tissue structures. The model has been validated by comparison to *in vitro* experiments on aggregates of genetically engineered CHO cells. The segregation of cell populations during cell sorting has also been reproduced in the simulation, showing that, in spite of its simplicity, our *in silico* approach captures the essential features of tissue self-assembly.

As a particular application of the model, Monte Carlo simulations were performed to study spontaneous structure formation after computer controlled, layer-by-layer deposition of cellular spheroids into hydrogels.

Such studies facilitated recent developments in the emergent technology of bioprinting[9, 13, 15] — a new technique that makes possible the organization of cells and biomolecules in three dimensions with desired anatomical shape and local density that mimic their distribution in organs and, therefore, promoting their functionality. The efficiency of bioprinting hinges on the development of new hydrogels which can be co-deposited with cells or cell aggregates without affecting the physiological parameters of the latter. Controlled degradation of the supportive biomaterials via physico-chemical or biological mechanisms is also required in order to make room for the cells to produce their own ECM.

Refinements of the current *in silico* approaches may be done by implementing more structural details into the models and by improving the methods of parameter estimation. To this end one may rely on targeted experiments and on the wealth

of available proteomics and genomics data. In order to meet the needs of tissue engineering, future models will have to address problems such as scaffold/hydrogel biodegradability, prediction of biomechanical properties of the fabricated tissue constructs and characterization of nutrient and gas transport throughout tissue maturation in specially designed bioreactors and after implantation into the host organism.

Acknowledgments

This work was supported in part by NSF FIBR — 0526854, VIASAN 313/2004 and CEEX 11/2005.

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