SIMULATING ARTIFICIAL TISSUES. A HEURISTIC TOOL IN TISSUE ENGINEERING?

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Abstract. Tissue engineering is a rapidly developing field of biomedical research that aims to repair, replace or regenerate damaged tissues. It exploits biological morphogenesis, which is an example of self-assembly, an ubiquitous natural phenomenon that gives birth to a large variety of structures in both living and inanimate systems. Computer programs that incorporate well-established principles of developmental biology are able to predict the evolution of certain living tissues. This encouraging fact raised the question if computational biology may draw reliable guidelines for tissue engineering. Relying on the state of the art of the literature and on our own results, the present review tries to answer this question. The available data leads us to the conclusion that a computational approach to tissue engineering may successfully complement experimental work.

Key words: bioprinting, bioassembly, self-assembly.

INTRODUCTION

The field of tissue engineering (TE) encompasses a large variety of techniques designed to coax cells to form tissues [10]. One of its long-term goals, that of growing organs *in vitro*, would solve the problem of transplantable organ shortage. Along the way, evolving in close relationship with regenerative medicine, TE proved successful in developing various functional organ modules, which in the near future may be used for *in vivo* tissue repair, or as promoters of regeneration; furthermore, they could be used for testing new drugs. Some products are already on the market. However, the successful large-scale application of TE in the clinic hinges on the solution of several problems regarding (1) a suitable cell source, (2) optimal scaffolds that support cell growth, differentiation and assembly, (3) bioreactors able to provide physiological conditions for the engineered tissues, (4) appropriate techniques for product preservation and transport [4].

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Theoretical models of living tissues have evolved during the last 75 years along two distinct lines of abstraction, viewing the tissue either as a set of discrete interacting cells or as a continuum, in which cell densities are monitored as opposed to individual cells [15].

The question arises if computer simulations may play a constructive role in solving some of the problems TE is facing to date. While trying to point out the uses of *in silico* experiments in TE, the present paper presents a far from complete survey of relevant results stemming from mathematical modeling and computer simulations of biological morphogenesis. It will be argued that at least points (2) and (3) of the above list of problems may be addressed computationally using experimentally motivated approaches.

THEORETICAL MODELS OF LIVING TISSUES

One of the early successes of the theoretical biology of living tissues dates back to 1981, when Odell and coworkers have constructed a model of the morphogenetic folding of epithelial sheets [13]. The epithelium is described as a monolayer of adherent cells which also cling to a basal lamina and possess a contractile cytoskeleton. Computer simulations based on this model reproduced experimentally observed shapes.

Several discrete cell models have been inspired by Steinberg's differential adhesion hypothesis [16] (DAH), which states that (i) cell adhesion involves energies that depend solely on cell types and (ii) cells are motile enough to reach the lowest energy configuration. During the last four decades DAH has been confirmed by numerous experiments and grew into a fundamental principle of developmental biology [5]. This line of thinking leads to a close analogy between true liquids and living tissues made of adhesive and motile cells (such as most embryonic tissues and some artificial ones).

Several types of computer simulations have been built on the foundations of the DAH. These were used to test its predictions regarding the sorting out of two different cell populations or the mutual engulfment of two adjacent tissue pieces. Monte Carlo simulations based on the Potts model from statistical physics turned out to precisely reproduce experimental findings and suggested that cell motility may be described by an effective temperature-like parameter [2, 3]. 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 and account for cell migration and shape changes in systems made of up to several thousand cells.

Differential adhesion, chemical signalling, chemotaxis, cell differentiation and extracellular matrix production have been incorporated in computational models of *in vivo* morphogenesis [11, 14]. The process of culmination of the cellular slime mold *Dictyostelium discoideum* was simulated in two dimensions by complementing the Glazier and Graner model [2, 3] with a partial differential

equation system accounting for cAMP signalling [11]. The model is defined by parameters characteristic to the subcellular level and is able to predict phenomena that involve the self-organization of thousands of cells. In this respect, it bears the potential to characterize the morphogenetic impact of genes whose function is elucidated at the subcellular level [11]. 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 signalling capacity [14].

The continuum approach pioneered by Murray and Oster (see [12] and refs. therein) allowed for treating realistic numbers of cells by using the methods of continuum mechanics. The distribution of cells of different types throughout the tissue is described in terms of their densities, whereas their morphogenetic rearrangements are treated as fluxes. The method has been successfully applied in various biological contexts, such as embryonic pattern formation, wound healing in the dermis, wound contraction, scar formation and vasculogenesis. The latter is of special importance from the point of view of TE, since growing large, vascularized organ replacements in the laboratory is one of the major challenges of the field. Understanding the intimate mechanism of vasculogenesis may help developing strategies for building tissue replacements that incorporate a web of interconnected capillary vessels. Starting from a cell population randomly distributed in a homogeneous extracellular matrix, the model predicts, via matrix remodeling and cell migration, the formation of a pattern similar to a vascular network. The results are in good agreement with experiments performed using endothelial cells seeded in Matrigel [12].

Recently, hybrid models have been constructed, which describe scar formation after dermal wounding by investigating extracellular matrix dynamics and tissue regeneration in a combined framework that treats cells as discrete objects and the matrix as a continuum [1].

In order to be efficient in screening alternative experimental designs and in offering hints for follow-on laboratory studies, computational tissue engineering must account for the dynamics of cell populations in the presence of scaffolds and extracellular matrices, which guide cell behavior and are also subject to degradation and cell activity-based restructuring. An important first step toward this ambitious goal has been made by Semple et al. by developing a computational model for studying the movement of fibroblasts into an acellular dermal matrix made of oriented fibers [15]. The simulations predict the pace of matrix invasion versus various parameters of scaffold architecture, such as fiber size distribution, packing density, and morphometry. The scaffold is generated by a random walk algorithm and the fibroblast movement along the fibers is described by a five-state Markov process of directional change, which proved suitable for treating lymphocyte motility on planar surfaces. The hopping of cells between nearby fibers

is simulated using the Monte Carlo method. Besides addressing practical TE needs, this approach is remarkable also from the point of view of the utilized software tool: a 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 for streamlining the workflow of matrix design [15].

Computer simulations, serving as proof-of-concept *in silico* experiments, may also speed up the development of new technologies. As an example, we mention our own modeling results regarding the possibility of using aggregates of several thousand cells as building blocks of artificial organs. The self-assembly of these "bioink" particles is a key step in the evolving technology of organ printing [7, 8, 9].

We simulated living tissue evolution by the Monte Carlo method, using a lattice model inspired by the DAH [16]. It was also assumed that cells remodel the adjacent extracellular matrix. Aiming to deal with systems of the order of hundred thousand cells, at variance with the model due to Glazier and Graner [2, 3], we neither considered shape changes, nor monitored each individual cell. An element of similarity is the way how cell motility is described by the Metropolis algorithm, which gave good results in the Potts-model-based studies of cell sorting.

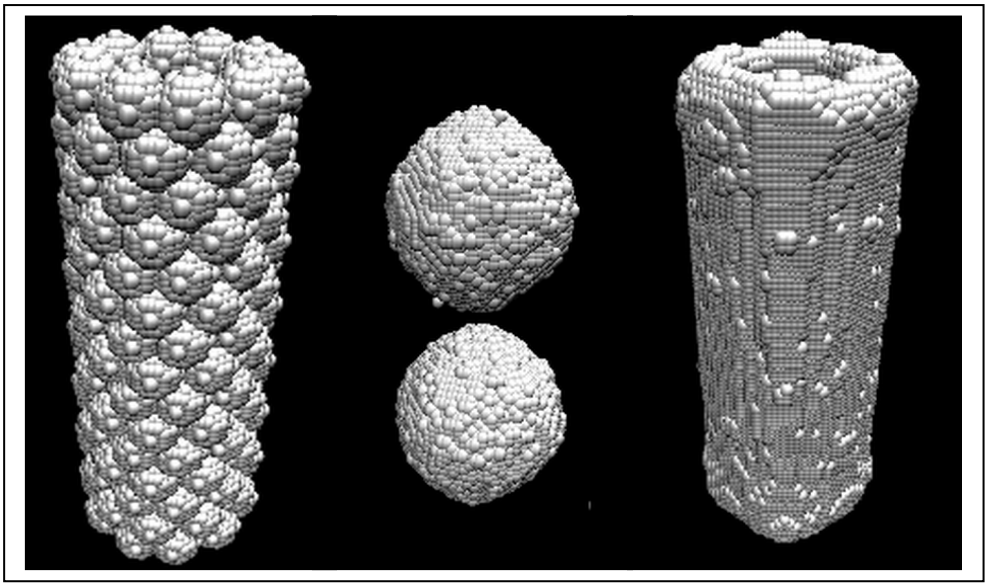

Fig. 1. The initial configuration of 15 closely packed rings made of 10 aggregates of 257 cells each (left), the results obtained in runs of 2×10^5 MCS if the interfacial tension parameter was 40% (middle) and 200% (right) of the biological fluctuation energy (the analogue of the energy of thermal fluctuations from statistical physics).

To investigate model predictions, we developed our own simulation software, written in C^{++}, whereas tissue configurations were visualized using VMD 1.8.2 (Theoretical Biophysics Group, University of Illinois at Urbana-Champaign, IL, USA, http://www.ks.uiuc.edu/Research/vmd/) [6].

In order to validate the model, theoretical results were tested against experiments performed on aggregates of CHO cells with genetically engineered adhesion apparatus [7].

Our *in silico* study of biological morphogenesis is illustrated here by the example of a tubular tissue structure, an architectural element of many organs. The results of two representative runs of 2×10^5 Monte Carlo steps (MCS) are depicted in Fig 1. The outcome of the pearling instability, explained by Rayleigh in the case of liquid columns, is shown in the middle panel of Fig. 1. It corresponds to a low interfacial tension. Based on this observation, we suggest that the manifestation of tissue liquidity in a permissive, motility-enhancing extracellular matrix may be responsible for the clogging of tissue engineered blood vessels. On the other hand, simulations reveal that appropriate values of the interfacial tension allow for optimal assembly of the initially spherical tissue fragments, leading to long-lived tubular structures as depicted in Fig. 1, right panel. If the interfacial tension is extremely high, the rearrangement of cells is hampered and the adjacent cell aggregates do not fuse (not shown). Such a behavior was observed experimentally by embedding the aggregates in agarose gel.

Similar computational studies were performed to test the feasibility of cell sheet fabrication and for studying shape and pattern formation in systems assembled from cell aggregates made of multiple cell types [9]. The results indicate that tissue liquidity may be exploited in a novel approach to tissue engineering based on bioprinting.

CONCLUSIONS

Several arguments point towards the feasibility of *in silico* tissue engineering as a complementary approach to *in vitro* investigation. The steady boost of computing power has made desktop computers suitable for studying relevant problems in tissue engineering. For example, recent works [7,9,15] demonstrate that the issue of scaffold and bioreactor design may be addressed theoretically, leading to application-oriented computer simulations, which run on high-end personal computers, with CPU-times of the order of days. The scale-up of system size and complexity is made possible by the emergence of cluster, grid and distributed computing. Various tissue models constructed so far have withstood the test of time and, in some cases, have led experimental efforts by shedding light on new properties of developing or healing tissues. The explosion of biological

information, which took place in the post-genome era, gave birth to large databases hosting data on genes, proteins, cells and tissues. New data mining techniques are being developed, with the potential of improving tissue models. In the light of the above facts we conclude that computational tissue engineering is an exciting research environment, with the potential of attracting much interest in the near future.

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