Computational study of the self-assembly of two different cell populations in contact with a biomaterial

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Abstract. The organisation of a heterotypic multicellular system is intensely studied in developmental biology, tissue engineering and regenerative medicine. To address this problem, we have created a computational model of a biological system made of two cell populations of various cohesivities, and simulated its evolution on the surface of biomaterials of different adhesivities. To this end, it was necessary to extend our SIMMMC application with algorithms that treat two cell types. We have observed, in accordance with experiments that, depending on the strength of cell-substrate adhesion, different structures emerge by the self-assembly of the two cell populations. The agreement with experimental results validates the extended version of the SIMMMC application, suggesting that this tool might offer useful insights for tissue engineers.

Keywords. Tissue engineering, cell aggregate, biomaterial, cell sorting, adhesion, cohesion, modeling, simulation.

Introduction

During embryonic development, multiple types of cells give rise to distinct compartments. The emergence of interfaces between different cell populations is an essential feature of morphogenesis [1]. The ability of understanding and controlling the spatial distribution of several cell populations of different types offers rational approaches to tissue engineering, which has the goal to create three-dimensional tissue constructs of controlled geometry and composition [2, 3]. Steinberg suggested that the self-assembly of different cell populations is driven by interfacial energy minimization, and formulated the differential adhesion hypothesis (DAH), which states that cells move in their environment trying to reach the minimal energy configuration [1,4]. According to DAH, mixed populations of mobile cells rearrange in such a way that the more cohesive cells coalesce, being surrounded by the less cohesive cells, a phenomenon known as cell sorting [1]. Using the SIMMMC (SIMulations based on the Metropolis Monte Carlo method) application, here we study the organization of two cell populations of different cohesions – consisting of a cell aggregate of two types of cells– placed on the surface of biomaterials with various adhesivities.

SIMMMC is an application developed by the authors that simulates the evolution of multicellular systems in the vicinity of biomaterials, on the basis of the Metropolis Monte Carlo (MCMC) algorithm.
In previous work with SIMMMC we identified the optimal chemotactic conditions that lead to a rapid and uniform seeding, varying the chemotactic strengths at constant energetic and geometric input parameters. Our aim was to investigate the potential role of chemotaxis in enhancing the cell seeding of tissue engineering scaffolds, and the obtained results converged to the real situation [5].

Here we extend SIMMMC to study the self-assembly of two different cell populations in contact with a biomaterial. This work aims, on one hand, to validate the SIMMMC application, and, on the other hand to offer useful information for tissue engineers who seek to control the organization of cells in tissue structures made of several types of cells and biomaterials.

1. Methods

We developed a computational model of a biological system that consists of a multi-cellular aggregate immersed in cell culture medium, situated on the flat surface of a biomaterial. The system is represented on a rectangular lattice, with nodes separated by one length unit, equal to one cell diameter. The biomaterial is represented as a rectangular slab of 100 x 100 x 3 lattice sites. The OZ axis is the longitudinal axis of the system. The multi-cellular aggregate, made of 2 types of cells, of equal concentrations, randomly mixed, is represented by a sphere with the radius \( g_{1844} \) units.

An element of the sphere is either a type 1 cell, or a type 2 cell, specified by the value of the cell type index. We assumed that type 1 cells are more cohesive than type 2 cells. We used SIMMMC to simulate the rearrangements of two cell populations of different cohesion on the surface of biomaterials of low, medium and high adhesion.

The total adhesion energy of the system, \( E \), is given by:

\[
E = B_{s0}y_{s0} + B_{s1}y_{s1} + B_{s2}y_{s2} + B_{o1}y_{o1} + B_{o2}y_{o2} + B_{12}y_{12},
\]

where \( B_{s0}, B_{s1}, B_{s2}, B_{o1}, B_{o2}, \) and \( B_{12} \) are the number of bonds between the elements of the system and \( y_{s0}, y_{s1}, y_{s2}, y_{o1}, y_{o2}, \) and \( y_{12} \) are the interfacial tensions between the elements of the system: \( s \)- substrate, \( 0 \)- medium, \( 1 \)- type 1 cell, \( 2 \)- type 2 cell) [7]. For example, \( B_{s1} \) is the number of bonds between type 1 cells and substrate particles.

According to the MMC algorithm, a biologically relevant random move is made, which is accepted with the probability \( P = \min \left( 1, \exp \left( \frac{-\Delta E}{k_BT} \right) \right) \). Here, \( E_T \) is the biological analogue of the energy of thermal motion, and \( \Delta E \) is the change in the adhesion energy of the system [7]. One Monte Carlo step (MCS) is the set of operations that gives all interfacial cells a chance to migrate. An elementary move consists in swapping a cell with a neighbour of another type. To avoid the double rearrangement of the 1-2 interface, a type 2 cell is allowed to switch position with any of its 26 (up to third order) neighbours (type 1 cells or medium particles), whereas a type 1 cell is allowed to switch position only with medium particle (type 0) neighbours.
2. Results

The model of the biological system under study is represented in Figure 1 using the Visual Molecular Dynamics (VMD) program [6].

![Figure 1](image1.png)

Figure 1. The model of a multi-cellular aggregate, made of two types of cells (type 1 – red spheres, type 2 – blue spheres), situated on the surface of a biomaterial (grey spheres): shown are a 3D view (left) and a vertical cross-section of the aggregate (right).

Table 1 presents the values of the input parameters used in our simulations, as well as the number of elapsed MCS. To characterize the cohesion among cells and the adhesion between cells and the substrate, we used energetic parameters that convey a quantitative measure of the interactions between the components of the system: \( \varepsilon_{11} \), for instance, is the mechanical work needed to separate two type 1 cells that are bound together. The interfacial tensions from Eq. (1) are expressed in terms of these parameters as follows [7]:

\[
\gamma_{s1} = \frac{1}{2} \varepsilon_{11} - \varepsilon_{s1} \quad \text{and so on for other interfaces that involve the substrate}
\]

and

\[
\gamma_{12} = \frac{1}{2} (\varepsilon_{11} + \varepsilon_{22}) - \varepsilon_{12} \quad \text{(and similar relationships hold also for interfaces that involve the cell culture medium)}
\]

Table 1. Values of input parameters in representative simulations.

<table>
<thead>
<tr>
<th>( \varepsilon_{11} )</th>
<th>( \varepsilon_{22} )</th>
<th>( \varepsilon_{12} )</th>
<th>( \varepsilon_{s1} )</th>
<th>( \varepsilon_{s2} )</th>
<th>MCS</th>
<th>Simulation set, Figure</th>
</tr>
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<td>0.8</td>
<td>0.9</td>
<td>1.7</td>
<td>1.4</td>
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<td>200 000</td>
<td>II, Fig. 3</td>
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<td>1.4</td>
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<td>0</td>
<td>50 000</td>
<td>III, Fig. 4</td>
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</table>

Generated using VMD [6], Figures 2A, 3A, and 4A depict the configurations of the system obtained after a certain number of MCS, in three different input conditions. To characterize the arrangement of the two cell populations on the surface of the biomaterial, we monitored the number of bonds between type 1 cells and the substrate, \( B_{s1} \), as well as between type 2 cells and the substrate, \( B_{s2} \) (Figures 2B, 3B, 4B).

![Figure 2](image2.png)

Figure 2. A representative result of simulation set 1: A. The configuration of the system after 10^7 MCS – vertical cross-section; B. The number of bonds between type 1 cells and the substrate (red line) and type 2 cells and the substrate (blue line). Parameters are given in the first row of Table 1.
3. Discussion

To validate the SIMMMC application, we simulated the organization of hepatic and endothelial cells, observed in liver tissue engineering experiments [8]. When placed on surfaces covered with different levels of type 1 collagen (a coating that favours cell attachment), endothelial cells and hepatocytes give rise to topologically different structures (Figure 5). The more cohesive (type 1) cells of our simulations correspond to hepatocytes, whereas type 2 cells correspond to endothelial cells.

Figure 3. A representative result of simulation set II: A. The configuration of the system after $10^5$ MCS - vertical cross-section; B. The evolution of the number of bonds between the type 1 cells and the substrate (red line) and type 2 cells and the substrate (blue line). Parameters are given in the second row of Table 1.

Figure 4. A representative result of simulation set III: A. The configuration of the system after $3 \times 10^4$ MCS - vertical cross-section. B. The evolution of the number of bonds between type 1 cells and substrate (red line) as well as between type 2 cells and the substrate (blue line). Parameters are given in the third row of Table 1.

Figure 5. Hepatocyte (blue)/Endothelial Cell (green) Sorting [8]. Reproduced with permission from the Proceedings of the National Academy of Sciences.
At high levels of collagen, when cell-substrate adhesion is strong, both endothelial and liver cells spread on the surface of the substrate. This configuration is similar to the one shown in Figure 2A. Strong adhesion favors the attachment of almost all cells to the substrate, at similar rates (Figure 2B). The difference in cohesion drives their self-assembly within the monolayer: the more cohesive cells segregate in the center, being surrounded by the less cohesive cells, as observed experimentally in two-dimensional aggregates of hydra cells [9]. On medium levels of collagen, the endothelial cells form a layer on the surface of the biomaterial and the hepatocytes form a layer on top of the endothelial cell layer. At intermediate adhesion, such a behavior emerged also in simulations (Figure 3). At low levels of collagen, a core of liver cells forms, which is covered by a crust of endothelial cells [8].

Thus, at weak adhesion, the cells sort as they would do in the absence of the biomaterial [1,4]. According to Figure 4A, such an outcome is observed also in simulations. Unlike in experiments [8], however, the emerging aggregate is not permanently attached to the surface of the biomaterial (Figure 4B). In other respects the SIMMMC program has precisely reproduced the experimental results.

4. Conclusions

We have modeled a biological system composed of a heterotypic cell aggregate placed on a biomaterial. Starting from this model, using the SIMMMC application, we simulated the rearrangement of two cell populations of different cohesivities on the surface of biomaterials of different adhesivities. Using VMD [6], the outcomes of simulations were analyzed qualitatively, via suggestive visualizations. Moreover, they were analyzed also quantitatively, by monitoring the number of bonds between different constituents.

Comparing the results of simulations with those of experimental studies [1,4,8,9], we conclude that the proposed algorithm accurately reproduces most features of the self-assembly of two cell populations of different cohesivities, placed in the vicinity of biocompatible materials, thereby validating the SIMMMC application.

References