User Centred Networked Health Care
A. Moen et al. (Eds.)
IOS Press, 2011

doi:10.3233/978-1-60750-806-9-882

Cell seeding of Tissue Engineering Scaffolds studied by Monte Carlo simulations

Andreea ROBU[a,1], Adrian NEAGU [b], Lacramioara STOICU-TIVADAR[a]
[a] *University "Politehnica" Timişoara, Romania*
[b] *Center for Modeling Biological Systems and Data Analysis, Victor Babeş University of Medicine and Pharmacy, Timişoara, Romania*

Abstract. Tissue engineering (TE) aims at building multicellular structures in the laboratory in order to regenerate, to repair or replace damaged tissues. In a well-established approach to TE, cells are cultured on a biocompatible porous structure, called scaffold. Cell seeding of scaffolds is an important first step. Here we study conditions that assure a uniform and rapid distribution of cells within the scaffold. The movement of cells has been simulated using the Metropolis Monte Carlo method, based on the principle that cellular system tends to achieve the minimum energy state. For different values of the model parameters, evolution of the cells' centre of mass is followed, which reflects the distribution of cells in the system. For comparison with experimental data, the concentration of the cells in the suspension adjacent to the scaffold is also monitored. Simulations of cell seeding are useful for testing different experimental conditions, which in practice would be very expensive and hard to perform. The computational methods presented here may be extended to model cell proliferation, cell death and scaffold degradation.

Keywords. differential adhesion, dynamic cell seeding, scaffold, tissue construct

1. Introduction

Tissue engineering (TE) is a relatively new field of biomedical research. Closely related to regenerative medicine, TE develops new therapies for patients who suffered tissue damage [2, 7].

A widely used approach to TE consists in culturing cells on a porous scaffold made of a biocompatible and biodegradable material. Cells are harvested from the patient, expanded in Petri dishes, and seeded onto scaffolds. The optimization of cell seeding is essential for the development of functional tissue constructs in vitro [1, 2]. It has been shown that if the cell seeding is uniform, the development of tissue constructs is more rapid and their mechanical properties are closer to the ones of native tissues. The mechanical properties of tissue constructs are largely due to the synthesis of extracellular matrix (ECM) – a web of proteins produced by cells. ECM production depends on the quality of cell seeding. If cell seeding is uniform, the culture medium equally reaches all the cells in the scaffold, providing gas and nutrient transfer to them.

[1] Corresponding Author. Andreea Robu, Faculty of Automation and Computers, Blvd. Vasile Parvan, No. 2, 300223, Timisoara, Romania; E-mail: andreea.robu@aut.upt.ro

Thus, a proper cell development and cell proliferation is ensured. Currently, the mechanical resistance of tissue constructs grown in the laboratory is about one order of magnitude below the corresponding native tissues [7, 8].

The objective of this study is to find the optimal conditions that lead to a uniform and rapid distribution of cells in the scaffold.

The basic principle that underlies this study is the differential adhesion hypothesis (DAH) proposed by Steinberg, which states that constituent cells of a tissue tend to reach configuration of lowest energy of adhesion; that is, cells tend to establish largest possible number of strong bonds with their environment [4, 5]. Cells interact with each other due to cohesion forces, and adhere to scaffold via adhesion forces. Thus, the self-assembly of cells into multicellular constructs is governed by the interaction energy between cells and by the interaction energy between cells and the scaffold [4, 5].

2. Methods

The studied model system consists of a cell suspension located near a porous scaffold, bathed in culture medium. The model is built on a cubic lattice (of 50×50×150 nodes). The *Oz* axis is the longitudinal axis of the system. The length unit, equal to one cell diameter, is the distance between two adjacent nodes. The cell suspension occupies the region $z < z_0$ (with $z_0 = 70$), where each node of the network is occupied either by a cell or by a medium particle. In the region $z \geq z_0$ each node is occupied either by an immobile (scaffold) particle or by a medium particle; this region models the scaffold, with pores filled with culture medium, and, eventually, by cells [3, 5].

The total adhesion energy of a system composed of t types of cells in the vicinity of a substrate can be brought to the form [5]:

$$E = \sum_{\substack{i,j=0 \\ i<j}}^{t-1} \gamma_{ij} \cdot B_{ij} + \sum_{i=0}^{t-1} \gamma_{is} \cdot B_{is} \qquad (1)$$

where B_{ij} is the number of links between two particles (of type i and j), B_{is} is the number of links between the cells of type $i \in \{0,1,...,t-1\}$ and the substrate; $\gamma_{ij} = (\varepsilon_{ii} + \varepsilon_{jj})/2 - \varepsilon_{ij}$ is the cell-cell interfacial tension, whereas $\gamma_{is} = \varepsilon_{ii}/2 - \varepsilon_{is}$ is the cell-substrate interfacial tension [5]. To simulate the evolution of the cellular system in the vicinity of the scaffold, we used the Metropolis Monte Carlo algorithm. Running Monte Carlo Steps (MCS) consists of exchanging a cell position with another cell or a culture medium particle from its vicinity [3, 5].

The current study is based on Monte Carlo simulations performed for different values of the following model parameters: (i) the cohesion energy between cells, (ii) the adhesion energy between cells and scaffold, (iii) the radius of pores and (iv) the radius of the orifices that connect the pores. As output parameters we monitored (i) the centre of mass of all cells, (ii) centre of mass of seeded cells and (iii) the concentration of the cells remained in suspension. The centre of mass of seeded cells is an indicator of cell distribution within the scaffold; its dependence on elapsed MCS is a measure of the rate of cell seeding. Since experiments on dynamic cell seeding of scaffolds monitor the concentration of the cell suspension adjacent to the scaffold [2], we also plotted this parameter versus the elapsed MCS.

3. Results and Discussions

Table 1 presents the values of the input parameters that we used in the simulations, and the values obtained for the output parameters; it also points to the relevant figures. The input parameters values were selected for the current study on empirical basis, after many previous tests that shown which are the optimal energy values and the relevance of the scaffold' porosity for an uniform cell seeding.

Table1. Values of input and output parameters in representative simulations.

Cell-cell interaction energy	Cell-scaffold interaction energy	Radius of pores	Radius of circular orifices	MCS	Plateau of z_{CM} for seeded cells	Plateau of z_{CM} for all cells	Plateau of fraction of cells in suspension	Set of simulations, Figure
0	0.6	5	2	80 000	110	90	0.2	I, Fig. 1a,1b,1c
0;0.4;0.8	0.6	5	2	80 000	110,110,100	90,90,60	0.2;0.2;0.5	II, Fig. 2a,2b,2c
0	0.6	8	2;3; 4;5	80 000	110;110; 100;100	90;90; 80;60	0.2;0.2; 0.3;0.5	III, Fig. 3a,3b,3c
1	0.25	5	2	80 000	75	35	0.9	IV, Fig. 4a,4b,4c

The volume percent concentration of the cells in the initial suspension was 1%. As shown on Fig. 1a, in about 7×10^4 MCS a stationary state is reached, in which the centre of mass of seeded cells is very close to the centre of mass of the scaffold, $z_{\text{CM scaffold}} = 110$ (Fig. 1a, upper curve). This indicates that the distribution of the cells in the scaffold is uniform (see also the snapshot in Fig. 1c). The centre of mass of all cells reaches a plateau at $z_{CM} \cong 90$ because a part of the cells remain in suspension. In Fig. 1b we observe that already at 2×10^4 MCS about 75 % of the cells penetrated the scaffold, and soon a plateau is reached with 20% of the cells remaining in suspension. However, the plateau of z_{CM} is reached later because cells rearrange inside the scaffold.

In experiments, the cell suspension is permanently homogenized (by magnetic stirring); therefore, the vast majority of the cells penetrate the scaffold. In our simulations, however, the mobility of the cells is described by the same algorithm, both in suspension and in the scaffold, so part of the cells will remain in suspension (Fig. 1c). Further refinements of the model should include the possibility to ascribe a larger motility for cells (and aggregates of cells) in suspension.

In the second set of simulations, with parameters given in the second row of Table 1, we varied the cohesion between cells. For a cell-cell interaction energy of 0.8 cell aggregates emerge (Fig. 2c), and the penetration of cells into the scaffold is slower. Note, however, that the cell-substrate interfacial tension is still negative, (–0.2), and

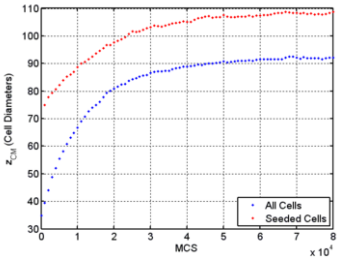

Fig.1a The centre of mass of all cells
The centre of mass of seeded cells

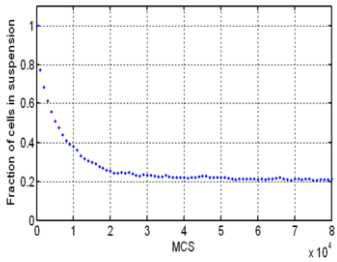

Fig.1b Cell concentration in suspension (Table 1, row 1)

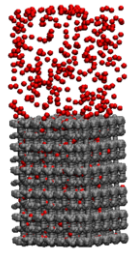

Fig.1c Final configuration represented using VMD[9]

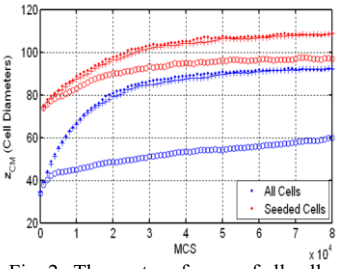

Fig. 2a The centre of mass of all cells
The centre of mass of seeded cells

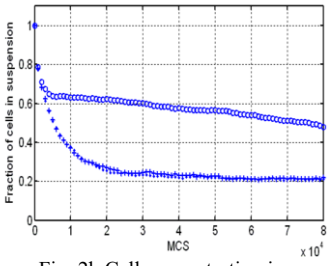

Fig. 2b Cell concentration in suspension (Table 1, row 2)

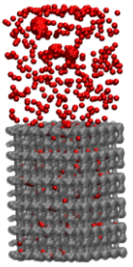

Fig.2c Final configuration represented using VMD[9]

cells enter the scaffold, albeit slowly, while also preserving cell-cell contacts. Figure 2b shows that after 8×10^4 MCS more than half of cells are still in suspension.

In the third set of simulations (parameters in Table 1, row 3), we varied the radius of the orifices between pores. Surprisingly, an increase of the radius of orifices from 2 to 3 cell diameters did not influence the seeding rate (Fig 3a, crosses and dots) and the final extent of seeding (the plateau of the plots shown as + sings and dots on Fig. 3b). Moreover, as the radii of the orifices increased, the fraction of seeded cells decreased; circles (squares) on Fig. 3b refer to orifice radius of 4 (5) cell diameters.

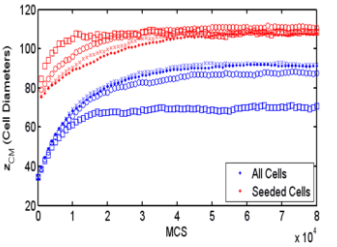

Fig. 3a The centre of mass of all cells
The centre of mass of seeded cells
(Table 1, row 3)

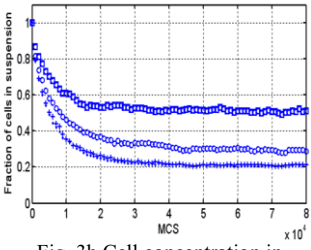

Fig. 3b Cell concentration in suspension (Table 1, row 3)

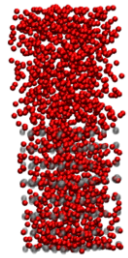

Fig.3c Final configuration represented using VMD [9]

In the fourth simulation (parameters in Table 1, row 4) the attraction between cells is higher than twice the cell-scaffold attraction, making the cell-scaffold interfacial energy positive. Our simulations show clearly that the emergent configuration is a result of a tug-of-war between cell-cell and cell-substrate interaction. This has been suggested earlier on the basis of a careful experimental study [6]; our approach brings quantitative arguments for the correctness of this observation.

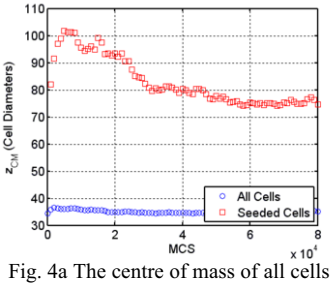

Fig. 4a The centre of mass of all cells
The centre of mass of seeded cells
(Table 1, row 4)

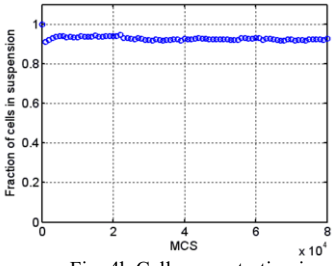

Fig. 4b Cell concentration in suspension (Table 1, row 4)

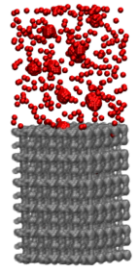

Fig. 4c Final configuration represented using VMD[9]

4. Conclusions

This work presents a lattice model and a computational algorithm able to evaluate energetic and geometric factors that may be tuned to assure optimal cell seeding.

Scaffold pore sizes and the diameter of the orifices between pores influence cell seeding only in extreme conditions: if the orifices are small (comparable to the cell diameter), or if they are large (exceeding half of the pore diameter), such that the scaffold is not contiguous and does not offer enough biomaterial to be attached to.

If cells do not adhere to each other, but they adhere to the scaffold, the seeding is rapid and cell distribution is uniform. If the cell-cell interaction energy is nonzero, but small enough to ensure a negative cell-scaffold interfacial tension, uniform distribution is reached, but the process is slower. Seeding is severely hampered if the cell-cell interaction energy is larger than twice the cell-substrate interaction energy, rendering the cell-scaffold interfacial tension positive. Moreover, if the cell-cell interaction energy is high, regardless of the interaction between the cells and the scaffold, cells tend to aggregate and their penetration into the scaffold is slowed down drastically.

Although it accounts for the competition between cell-cell and cell-substrate interaction energies, our study of the impact of cell aggregation on the rate of cell seeding is not accurate, since the present algorithm is unable to describe the fast movement of cell aggregates in the stirred suspension. Future developments of the computational framework proposed here need to incorporate a hybrid algorithm that differentiates between individual cell motility and the movement of cells and aggregates of cells with the flow of cell culture medium. Such a development is especially appealing, since it would enable one to simulate also perfusion cell seeding [1]. Also, future models might account for cell proliferation, cell death and scaffold degradation.

References

[1] Francioli, S.E. Candrian, C. Martin, K. M. Heberer, Martin, I. Barbero. A. Effect of three-dimensional expansion and cell seeding density on the cartilage-forming capacity of human articular chondrocytes in type II collagen sponges. *Journal of Biomedical Materials Research*. Part A. (2010) 95(3):924-931.

[2] Vunjak-Novakovic Gordana, B. Obradovic, I. Martin, P.M. Bursac, R.Langer and L.E.Freed DynamicCell Seeding of Polymer Scaffolds for Cartilage Tissue Engineering, *Biotechnology Progress* Volume 14, Issue 2, 1998, 193–202

[3] Robu, A. P. Neagu, A. Stoicu-Tivadar, L. A computer simulation study of cell seeding of a porous biomaterial, Computational Cybernetics and Technical Informatics (ICCC-CONTI), 2010 International Joint Conference, ISBN: 978-1-4244-7432-5, 225-229

[4] Foty Ramsey, A. Malcolm S. Steinberg, The differential adhesion hypothesis: a direct evaluation, *Developmental Biology*,Volume 278, Issue 1, 1 February 2005, 255-263

[5] Neagu, A. Kosztin, I. Jakab, K. Barz, B. Neagu, M. Jamison, R. Forgacs, G. Computational Modeling of Tissue Self-Assembly, *Modern Physics Letters* B, Volume 20, Issue 20, (2006), 1217-1231

[6] Ryan, P.E. Foty, R.A. Kohn, J. and Steinberg, M.S. Tissue spreading on implantable substrates is a competitive outcome of cell– cell vs. cell–substratum adhesivity, *Proc. Natl. Acad. Sci.* U.S.A. (2001) 98(8):4323-4327.

[7] Griffith L.G. and Naughton, G. Tissue engineering - current challenges and expanding opportunities, *Science* 295 (5557) (2002), 1009

[8] Semple, J.L. Woolridge N.and Lumsden, C.J. In vitro, in vivo, in silico: Computational systems in tissue engineering and regenerative medicine, *Tissue Engineering* 11 (3-4) (2005), 341-356.

[9] Humphrey, W. Dalke, A. Schulten, K. VMD – Visual Molecular Dynamics, J. Mol. Graphics 14, (1996) (33-38) (http://www.ks.uiuc.edu/Research/vmd/).