SIMMMC – AN INFORMATIC APPLICATION FOR MODELING AND SIMULATING THE EVOLUTION OF MULTICELLULAR SYSTEMS IN THE VICINITY OF BIOMATERIALS

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Abstract. Understanding the evolution of multicellular systems near biocompatible materials is important both for the *in vitro* creation of implantable tissue constructs and for understanding tumor invasiveness. We have developed the SIMMMC (SIMulation using Metropolis Monte Carlo method) application for modeling and simulating the evolution of multicellular systems in the vicinity of biomaterials. This application creates computational modeling possibilities for certain artificial tissue structures, as well as for the simulation of their evolution. The application simulates the behavior of a variety of biological systems using algorithms based on the Metropolis Monte Carlo (MMC) method. It takes into account the cohesion between cells, the adhesion between cells and biocompatible materials, as well as chemotaxis. The algorithms used by SIMMMC incorporate results of recent research regarding cell motility in three-dimensional systems made of cells and biomaterials. In this work, we present the functionalities and the architecture of the application, the activity diagrams and the implementation characteristics of the application, the user interface and representative results of simulations. The SIMMMC application and the proposed algorithms were validated by comparison with experimental data from the literature. Allowing to simulate shape changes of multicellular constructs in contact with biomaterials, SIMMMC might be a useful instrument for the optimization of *in vitro* tissue fabrication.

Key words: tissue engineering, biological systems, computational modeling, simulation, application.

INTRODUCTION

Tissue engineering proposes to create implantable multicellular structures that substitute or foster the regeneration of injured tissues in the human body [2]. Cell spreading on a biocompatible substrate and the reorganization of several cell populations in tissue structures are ubiquitous in tissue engineering. Their in-depth understanding would help the *in vitro* creation of multicellular structures that recapitulate the structure and function of native tissues [3, 9].

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Although many facets of morphogenesis have been deciphered by developmental biologists [12, 13, 14], the complexity of the process calls for computational modeling [4, 5, 10, 11]. Using computer simulations, one can gradually increase the complexity of the model, establishing thereby a hierarchy of mechanisms involved in a given biological process [6]. In tissue engineering, the *in silico* studies are among the few alternatives to expensive and time consuming trial-and-error experiments.

To assist the *in vitro* studies and to minimize their number, we have developed a modeling and simulation application called SIMMMC (an acronym that stands for SIMulation using Metropolis Monte Carlo methods). The application models various multicellular systems used in laboratory experiments and simulates their evolution in the vicinity of biomaterials, being based on Metropolis Monte Carlo method (MMC).

MATERIALS AND METHODS

SIMMMC was implemented in the Visual Studio.Net 2008 development environment, by using the Visual Basic.Net language. The 3D visualization of the modeled biological systems was accomplished by using the molecular graphics software Visual Molecular Dynamics (VMD) [1].

For a quantitative analysis of the simulation results, a MATLAB software package has been developed that provides information of interest on tissue engineering optimization techniques.

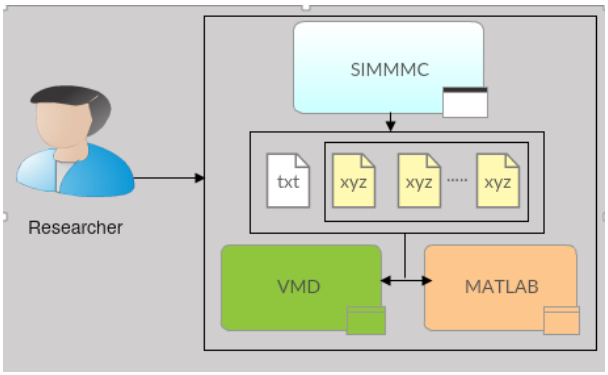

Fig. 1. Tools used for studying the evolution of multicellular systems in the vicinity of biomaterials.

As illustrated in Figure 1, SIMMMC generates a text file with the simulation parameters, and several xyz files that contain the initial configuration of the

biological system as well as the successive configurations obtained after running the simulation. The xyz files are loaded into both the VMD for a qualitative analysis of the results and the MATLAB in order to follow the evolution of certain indicators of interest.

RESULTS

SIMMMC is designed to easily create computational models for different types of biological systems. Based on these models, SIMMMC enables the user to accurately simulate the evolution of a live cell population in the vicinity of biocompatible materials. SIMMMC is based on original algorithms, in which the Metropolis Monte Carlo method is adapted according to the results of recent research on cell motility in three-dimensional systems composed of cells and biomaterials.

The functionalities of the application are divided into two main categories, related to modeling and simulation.

Regarding the modeling of biological systems, the application allows to: (i) generate the model of a cell aggregate located on the flat surface of a biomaterial; (ii) generate the model of a cell suspension located near a porous scaffold with spherical pores; (iii) generate the model of a cell suspension located near a porous scaffold with cubic pores; (iv) generate the model of a cell aggregate located in the vicinity of a porous scaffold with spherical pores.

In what concerns the simulation of the evolution of the model systems, the application allows the study of cell spreading on the surface of biomaterials and the study of cell seeding into porous scaffolds, taking into account different geometric, energetic and chemical conditions.

SIMMMC can simulate (i) the spreading of a cell aggregate made of a single type of cells on the surface of a biomaterial; (ii) the evolution of a cell aggregate made of two different cell populations, located next to a biomaterial surface; (iii) the seeding of a cell suspension containing one or two different populations of cells in a porous scaffold; (iv) the seeding of a cell suspension into a porous scaffold having incorporated a chemoattractant; (v) the seeding of a cell aggregate into a porous scaffold, and (vi) the seeding of a cell aggregate into a porous scaffold having incorporated a chemoattractant. A simulation consists of running a certain number of Monte Carlo steps (MCS) specified by the user. One MCS represents the sequence of operations that gives each cell a chance to make a movement [7].

The SIMMMC informatic application consists of three main modules; its architecture is shown in Figure 2. The first module is dedicated to the generation of model systems, the second module is the one that links the generation module with the simulation module, and the third one is the one that simulates the evolution of multicellular systems.

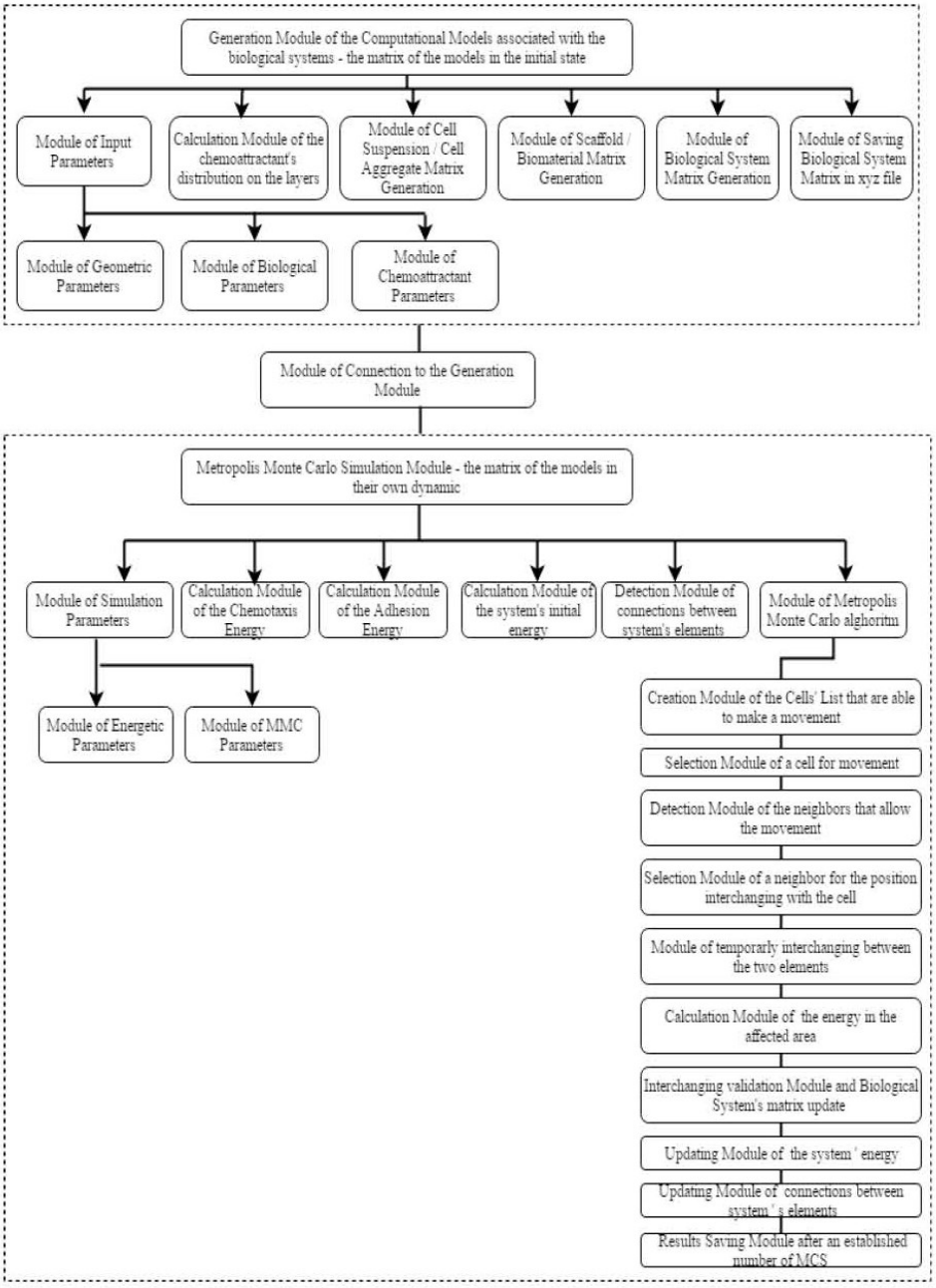

Fig. 2. The architecture of the SIMMMC application.

In order to emphasize the workflow of the three modules of the application, we have used a representation based on the UML activity diagrams (UML is an acronym for Unified Modeling Language, which is a standard language for describing models and specifications for a software). These represent the activity sequences of the SIMMMC informatic application, as well as the ways of decision taken during the events comprised in the activities.

Figure 3 shows the UML activity diagram associated to the module that generates the computational models of certain biological systems. The situation when the porous scaffolds also incorporate a chemoattractant has also been taken into consideration.

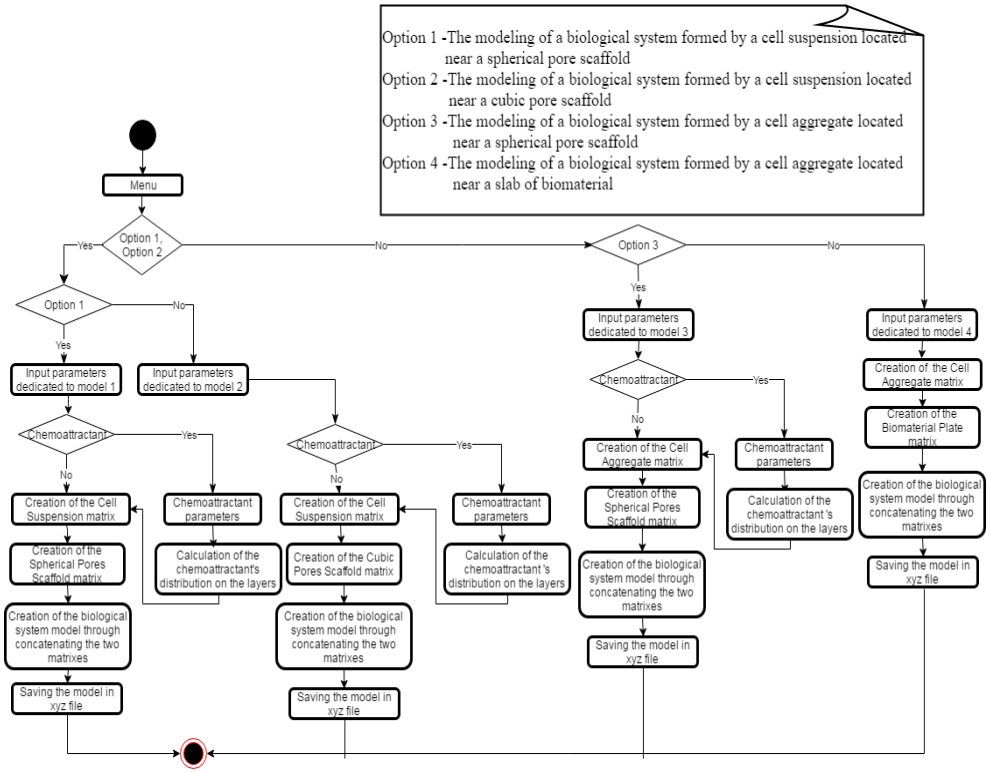

Fig. 3. The UML activity diagram of the module that generates computational models associated to the biological systems.

The computational models generated by this application allow the control of the biological system architecture. The user has the option to create a model of a parallelepipedic slab made of a biocompatible material. Scaffolds of different sizes can be generated, with spherical pores of different radii, connected by circular

holes of different radii. Moreover, the application can create models of scaffolds with cubic pores of different sizes. The application enables to model the biological component of the system by describing cellular aggregates of different dimensions, or cell suspensions composed of a single type of cells or of two types of cells in various proportions.

Figure 4 depicts models of different porous scaffolds with controlled architecture.

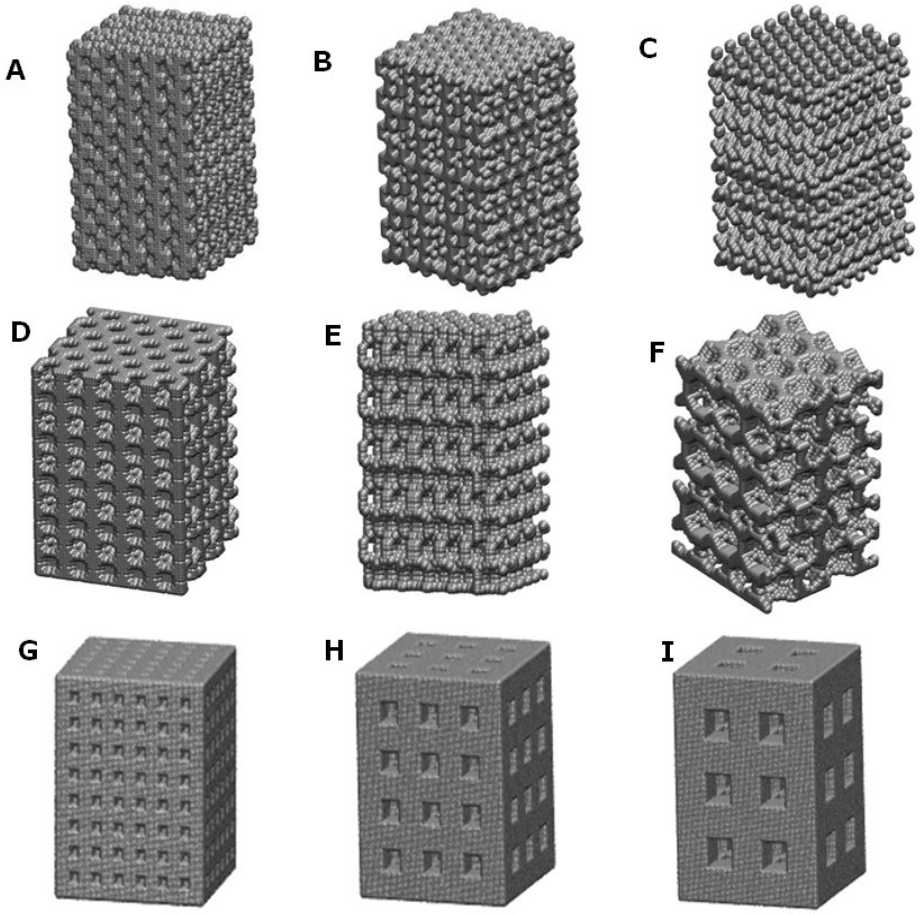

Fig. 4. 3D computational models associated with spherical porous scaffolds: *Rpor* = pore radius; *Rorif* = radius of the holes that connect adjacent pores. (A) *Rpor* = 3, *Rorif* = 1; (B) *Rpor* = 4, *Rorif* = 1; (C) *Rpor* = 4 *Rorif* = 2; (D) *Rpor* = 5, *Rorif* = 1; (E) *Rpor* = 5, *Rorif* = 2; (F) *Rpor* = 8, *Rorif* = 2; Panels G to I represent computational models of scaffolds with cubic pores of side length, *L*: (G) *L* = 5; (H) *L* = 10; (I) *L* = 15.

Models of different multicellular systems represented in SIMMMC are depicted in Figure 5.

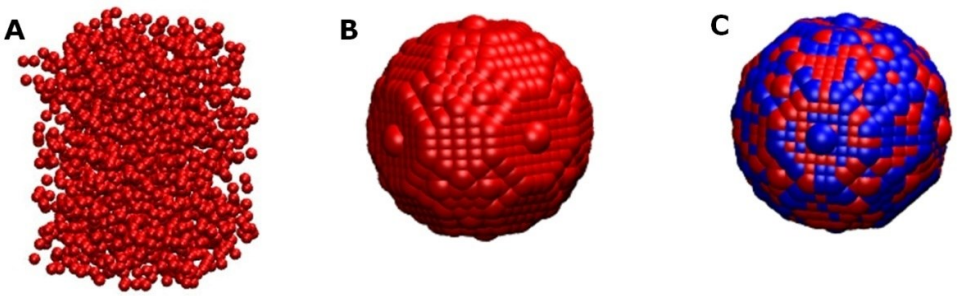

Fig. 5. The 3D computational model associated to a cell suspension of one type of cells (A). 3D computational model of a cell aggregate with the radius of 15 cell diameters, composed of a single type of cells (B). Two types of cells (C).

The structure of the biological systems modeled in 3D space is presented in Figure 6A.

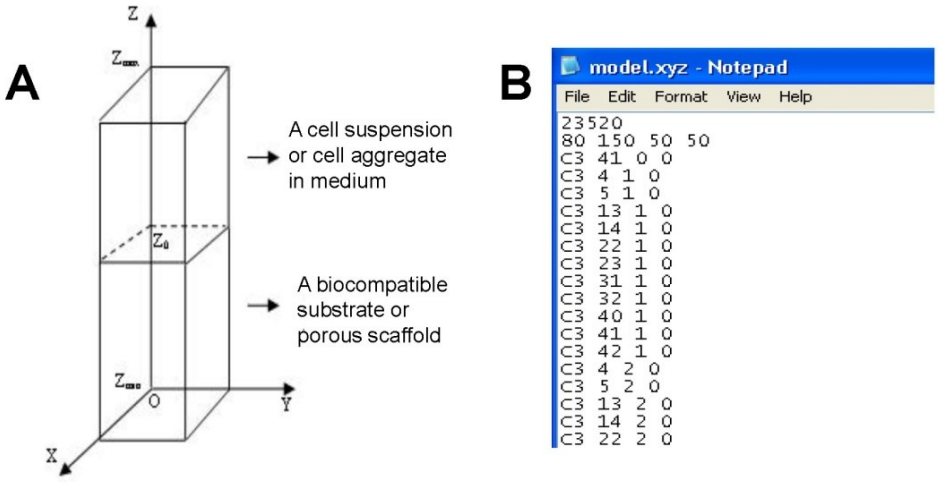

Fig. 6. (A) The structure of the biological system modelled in 3D: a multicellular system in the vicinity of a scaffold/biomaterial surface. (B) A typical xyz file, where the initial configuration of the system is saved.

The elements of the 3D lattice associated to the biological system are saved in xyz files (Fig. 6B) in the following manner: starting from the third line (the first

two are excluded because they contain general data about the model), the type of element is specified (C1 – for a type 1 cell, C2 – for a type 2 cell, C3 – for a biomaterial particle), along with its x, y, and z coordinates.

Figure 7 exemplifies graphical representations of several models of biological systems.

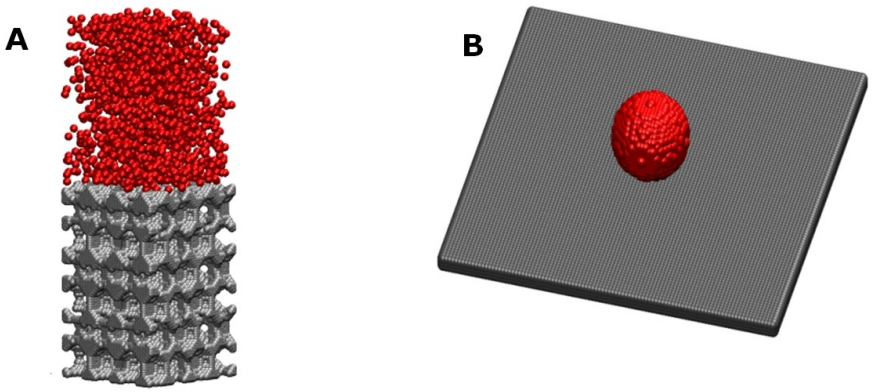

Fig. 7. (A) The computational model of a biological system composed of a cell suspension in the vicinity of a porous scaffold. (B) The computational model of a biological system consisting of a cell aggregate situated on the flat surface of a biomaterial slab.

The logical flux of the simulation algorithm of the evolution of multicellular systems is presented in Figure 8.

Due to the importance of the cell self-organization in the vicinity of certain biomaterials, SIMMMC has been developed so as to allow the modeling and simulation of the evolution of certain artificial tissue structures that include biomaterials as well, taking into account the interactions between cells and biomaterials, besides the cell-cell and cell-medium interactions.

The first step of the simulation consists in taking from the keyboard the energetic parameters that describe the degree of interaction between these elements, the number of intended MCS, as well as the number of MCS performed between two successive savings of the system's configuration.

The algorithm developed for the calculation of the adhesion energy is based on an extended formula that also takes into account the interaction of cells with the scaffold particles [7].

In the context of a multicellular system (suspension/cell aggregate) situated in the vicinity of a porous scaffold that also contains a chemoattractant substance, the total energy of the system is the sum of the adhesion energy and the energy associated to the chemotaxis phenomenon [4, 8].

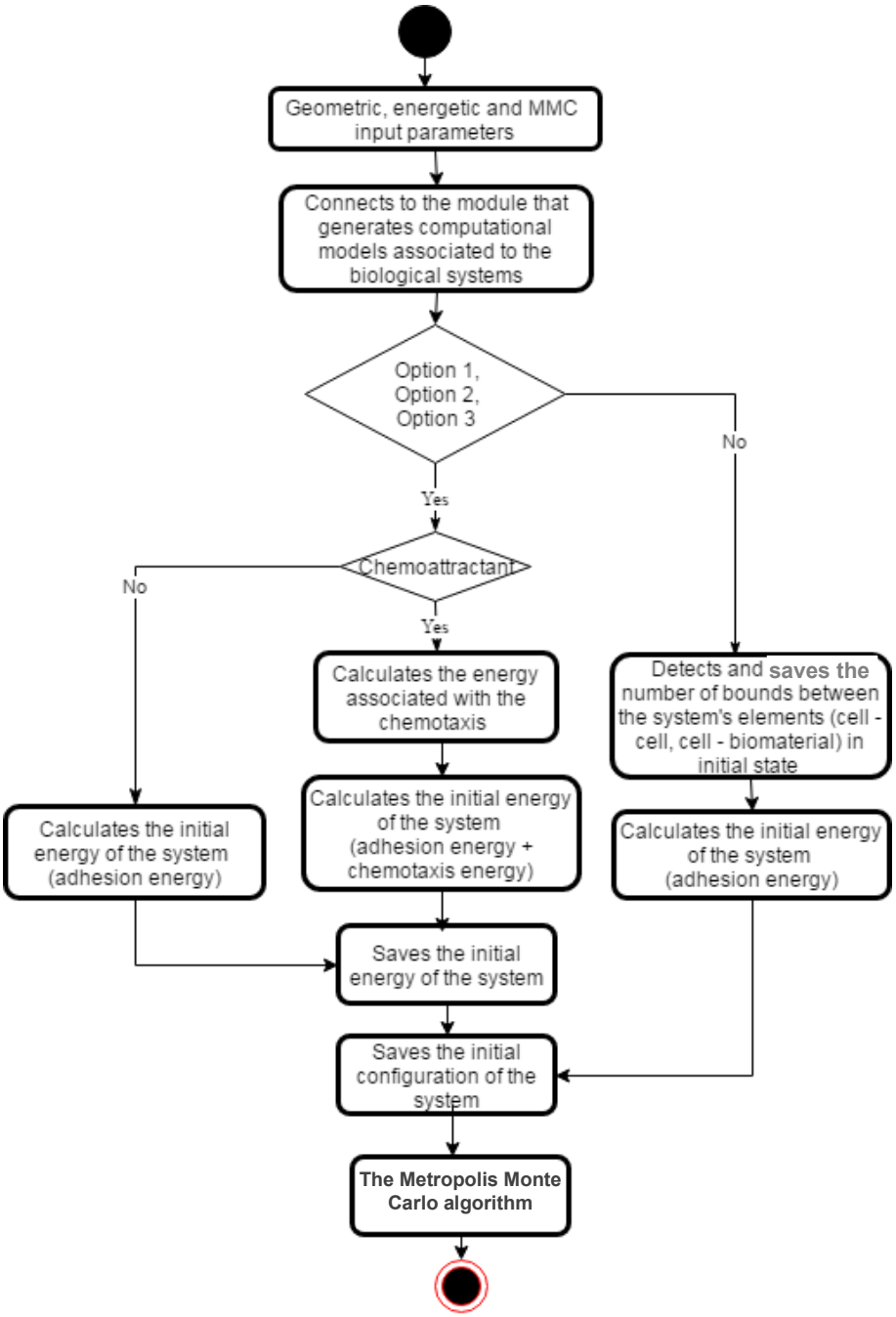

Fig. 8. The UML activity diagram of the Metropolis Monte Carlo computational simulation module.

Figure 9 presents the UML activity diagram associated with the implementation module of the Metropolis Monte Carlo algorithm. The sequence of operations necessary for running a MCS requires, at the beginning, the detection of all the cells in the system that have the possibility to migrate. A movement implies the interchange of the position of a cell with a neighbor of another type. Interactions of the same extent are considered between first, second and third order neighbors. (On a cubic lattice, each site has 6 first order neighbors, located at a distance of one lattice spacing, 12 second order neighbors at a distance of $\sqrt{2}$ lattice spacings, and 8 third order neighbors at a distance of $\sqrt{3}$ lattice spacings.) Hence, in our model each particle is considered to interact with up to third order neighbors (a total of 26 particles). It is considered that type 2 cells have the possibility to migrate in the place of any type 1 cell or medium particle from their neighborhood, whereas type 1 cells have the possibility to migrate only in the place of medium particles from their neighborhood. (This rule helps to avoid swapping a given pair of dissimilar neighbors twice).

For computational efficiency, the algorithm does not recalculate the total adhesion energy of the system for each migration attempt; it only computes the energy in the zone affected by the movement, before and after the interchange had taken place.

If the change in energy, ΔE, is smaller or equal to 0, the movement is accepted. Otherwise, a random number between 0 and 1 is generated, and if it is smaller than $\exp\left(-\frac{\Delta E}{E_T}\right)$ the movement is accepted; if not, it is rejected. In other words, the movement is accepted with a probability $P = \min\left(1, \exp\left(-\frac{\Delta E}{E_T}\right)\right)$. Here E_T is the biological analog of the energy of thermal fluctuations [5]. After each movement, the lattice associated to the system is updated along with the total energy of adhesion.

When the scaffold also contains a chemoattractant substance, the algorithm also computes the change in the energy associated to the chemotaxis phenomenon. This calculation is also restricted to the area affected by the movement. In this case, the change in the system's energy, ΔE, is the sum between the change in the adhesion energy and the change in the energy associated to chemotaxis [8].

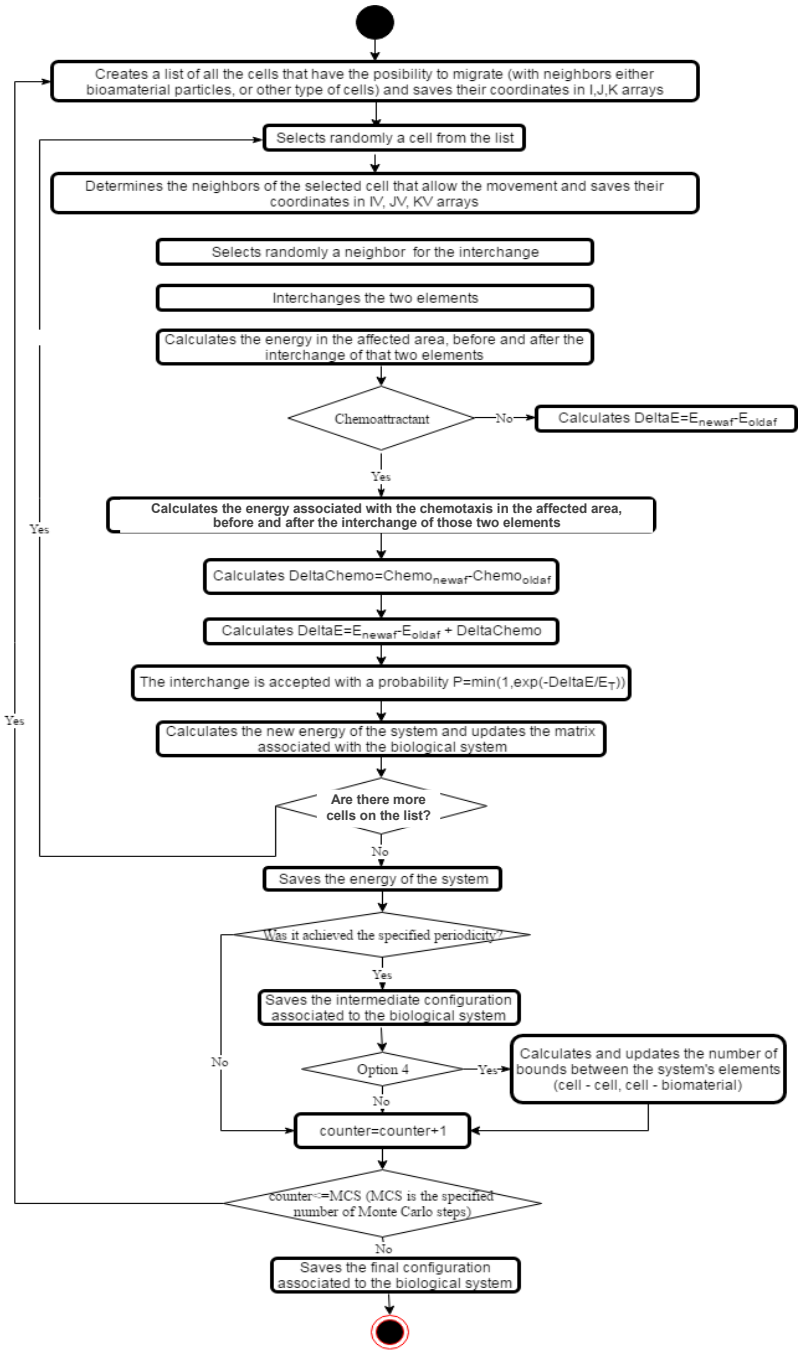

Fig. 9. The UML activity diagram of the Metropolis Monte Carlo algorithm.

During a simulation, the configurations of the system are saved periodically in xyz files, after running a predetermined number of MCS, thereby monitoring the evolution of the biological system.

The SIMMMC application has a user-friendly interface, which allows to easily introduce the values of model and simulation parameters.

The main window of the application contains 4 options associated with the 4 biological systems that can be modeled: a cell suspension in the vicinity of a porous scaffold with spherical pores; a cell suspension in the vicinity of a porous scaffold with cubic pores; a cell aggregate in the vicinity of a porous scaffold with spherical pores, and a cell aggregate situated on a flat biomaterial surface (Fig. 10).

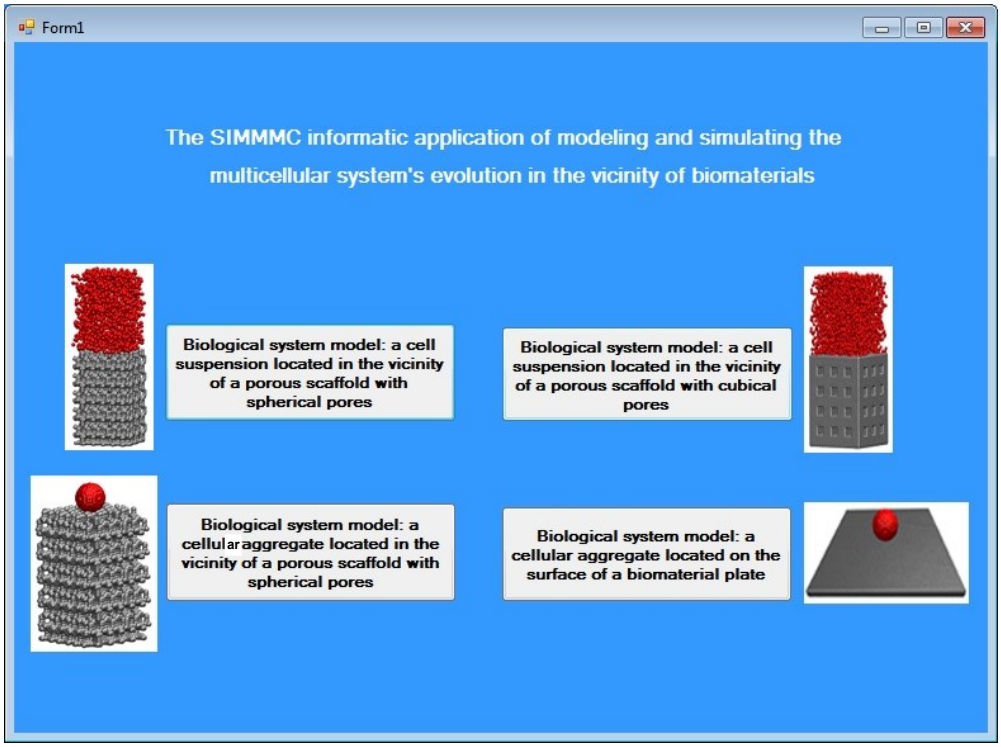

Fig. 10. The menu of the SIMMMC application.

According to the selected option, a window will be opened which allows the user to introduce the customized model parameters for the selected biological system (Fig. 11).

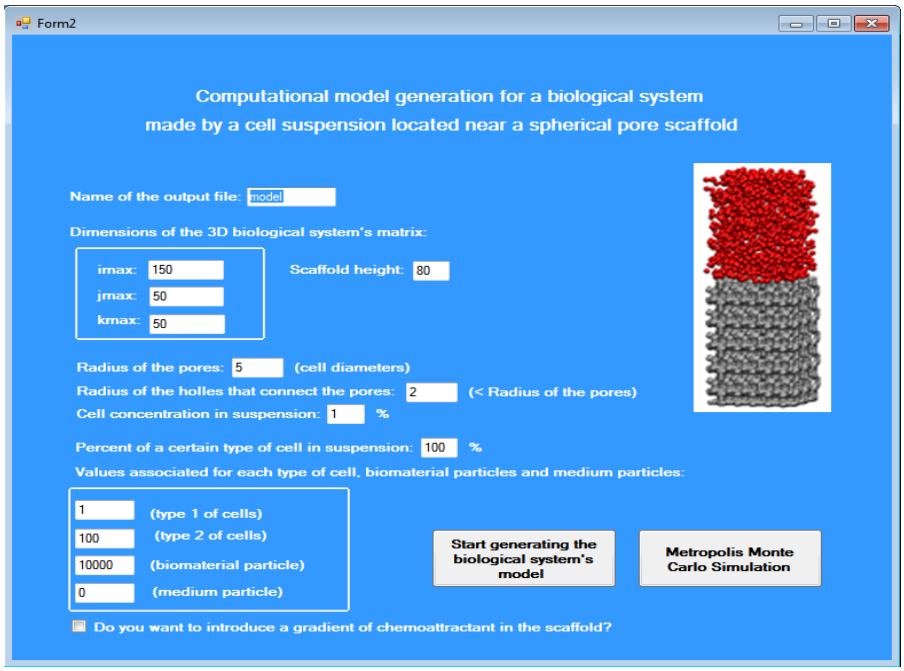

Fig. 11. The graphical interface for the introduction of the model parameters for a cell suspension in the vicinity of a scaffold with spherical pores.

If the option for introducing a chemoattractant gradient in the scaffold is selected, a window will be opened which allows for setting the value of the chemoattractant concentration, $C1$, at the top layer of the scaffold (the point of minimum concentration), the value of the chemoattractant concentration, $C2$, at the bottom layer of the scaffold (the point of maximum concentration) as well as the chemotactic strengths associated to type 1 and type 2 cells (Fig. 12).

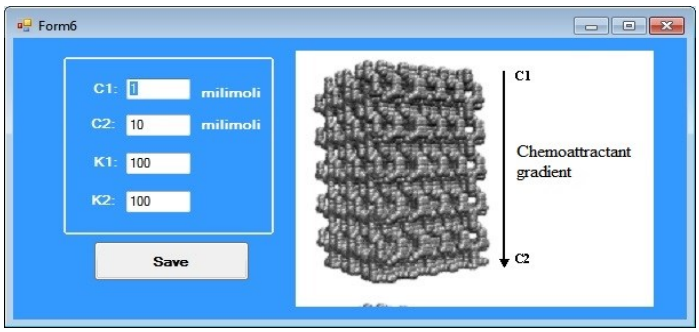

Fig. 12. The graphical interface for the introduction of chemotaxis parameters.

Once the model system is created, the user has the possibility to start the simulation of the multicellular system's evolution. The graphical interface of the simulation module is presented in Figure 13.

After the completion of the specified number of MCS, the application displays the duration of the simulation in days, hours, minutes, and seconds.

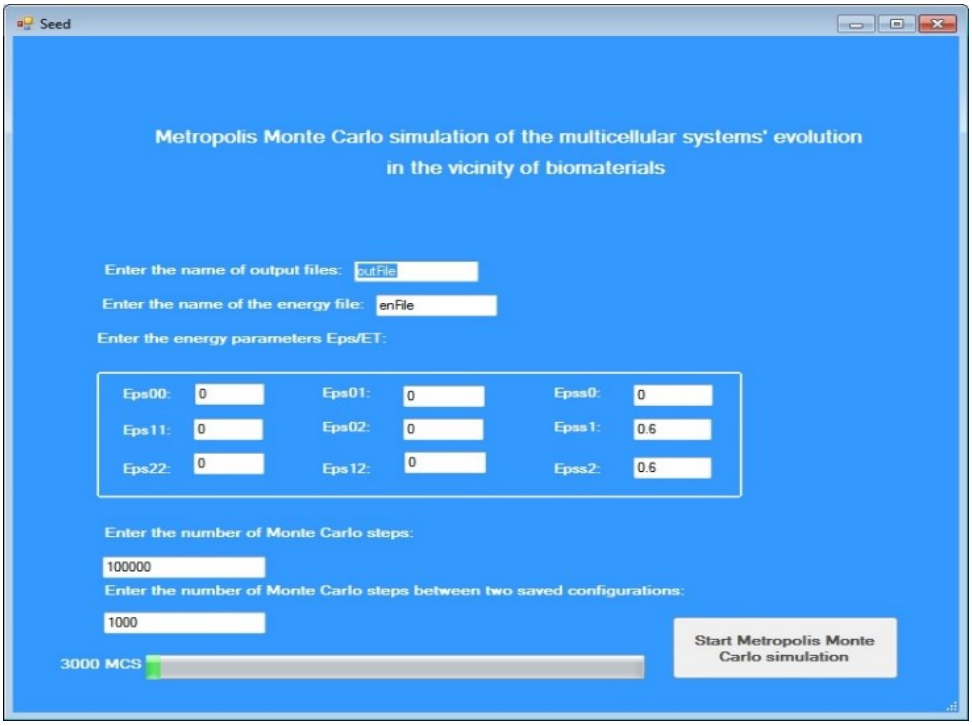

Fig. 13. The graphical interface for the introduction of simulation parameters.

The application also offers the possibility to run an extra number of MCS starting from the final configuration obtained after the previous usage. This option is extremely useful for running long simulations of previously uninvestigated systems.

As an example of a typical SIMMMC study, here we present the results of simulations of a cell suspension in the vicinity of porous scaffolds (Fig. 14).

For all 4 simulations of Figure 14, we considered the cell-cell cohesion energy equal to 0 and the cell-biomaterial adhesion energy equal to 0.6.

The configurations represented in Figure 14 are the outcomes of simulations aimed identifying optimal geometric and energetic conditions for successful cell

seeding. To enable the quantitative analysis of the results, our team has developed MATLAB algorithms for (i) calculating the number of cells attached to the biomaterial, (ii) fitting the data regarding the number of cells attached to the biomaterial *versus* the number of elapsed MCS, (iii) monitoring the rate of cell spreading on the substrate according to various energetic parameters, (iv) calculating the number of cells attached to the substrate, (v) counting the number of connections between various interfacial elements (type 1 cell – type 2 cell, type 1 cell – substrate, type 2 cell – substrate), (vi) computing the cell fraction from the suspension, (vii) monitoring the center of mass of the cells that populated the scaffold, and (viii) monitoring the total energy of adhesion.

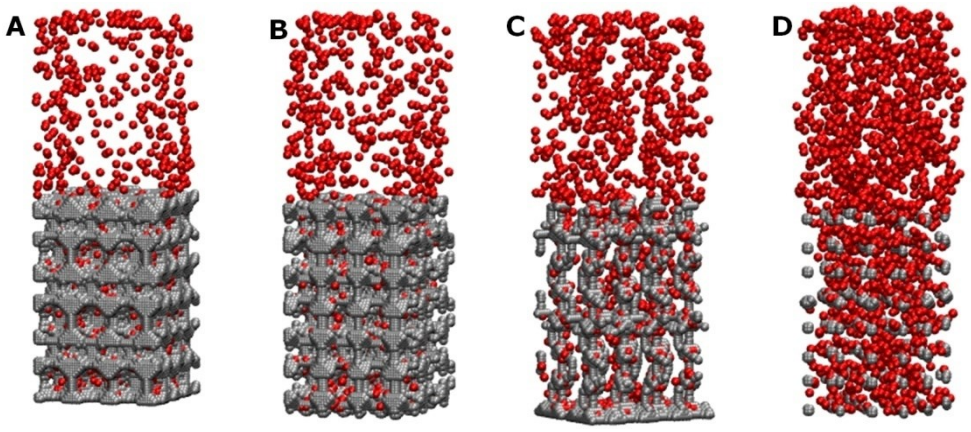

Fig. 14. The configurations obtained after running 150 000 MCS for a cell suspension in contact with scaffolds of various porosities (R is the pore radius, whereas r is the radius of the window that connects adjacent pores). (A) $R = 8$, $r = 2$; (B) $R = 8$, $r = 3$; (C) $R = 8$, $r = 4$; (D) $R = 8$, $r = 5$.

Analyzing the results of these simulations, we observed that, surprisingly, an increase of the radius of the connecting windows, r, from 2 to 3 cell diameters while maintaining the radius of the pores, R, did not influence either the seeding speed or the percentage of cells that entered the scaffold.

CONCLUSIONS

Computational methods are regarded as useful instruments for tissue engineering because they enable researchers to test working hypotheses and to optimize experimental procedures, thus reducing the costs of developing new technologies.

This paper presented SIMMMC, an informatic application specifically designed to model the evolution of multicellular systems in the neighborhood of biocompatible materials. The model takes into account physical interactions, as well as chemical signaling. The application offers a user-friendly graphical interface for setting up the simulation in close correspondence with the geometry of the biological system under study. The application is flexible, easily allowing the development of new modules that generate computational models for other types of biological systems.

SIMMMC is an integrated application of single cell resolution that allows for modeling and simulating the evolution of artificial tissue structures that also include biomaterials. SIMMMC takes into account cell-cell, cell-medium, and cell-substrate interactions, as well as chemotaxis.

Although the SIMMMC application has been validated by comparison with several experiments of *in vitro* morphogenesis [6], it is important to be aware of its underlying assumptions, which assure its efficiency at the cost of a simplified, low resolution, lattice representation of the biological system. Therefore, SIMMMC is unable to describe shape changes and motility of individual cells within a tissue construct. Moreover, relying on the MMC algorithm, it models a pathway towards energy minimization rather than the time evolution of the system.

Nevertheless, SIMMMC is faster than other informatic applications built for modeling multicellular processes, such as the subcellular element model (ScEM) [10] or CompuCell3D [15]. Therefore, it enables the user to simulate spontaneous shape changes of tissue engineered structures that contain millions of cells.

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