Physica A 395 (2014) 247–254

Contents lists available at ScienceDirect

Physica A

journal homepage: www.elsevier.com/locate/physa

Dynamics of cell aggregates fusion: Experiments and simulations

Gilberto L. Thomas [a], Vladimir Mironov [c], Agnes Nagy-Mehez [d], José C.M. Mombach [b,*]

[a] Departmento de Física, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil
[b] Departmento de Física, Universidade Federal de Santa Maria, Santa Maria, Brazil
[c] Renato Archer Information Technology Center, Campinas, Brazil
[d] Regenerative Medicine and Cell Biology, Medical University of South Carolina, Charleston, USA

HIGHLIGHTS

- Experiments of cell aggregates fusion are performed using human adipose derived stem cells.
- The process of fusion is simulated using the cellular Potts model with aggregates reaching up to 10,000 cells each.
- The simulations imply that individual cell motion corresponds to an anomalous diffusion.
- Cell diffusion has a strong influence on the dynamics of aggregates fusion.

ARTICLE INFO

Article history:
Received 18 October 2013
Available online 26 October 2013

Keywords:
Bioprinting
Aggregates fusion
Stem cells
Potts model

ABSTRACT

Fusion of cell tissues is an ubiquitous phenomenon and has important technological applications including tissue biofabrication. In this work we present experimental results of aggregates fusion using adipose derived stem cells (ADSC) and a three dimensional computer simulation of the process using the cellular Potts model with aggregates reaching 10,000 cells. We consider fusion of round aggregates and monitor the dimensionless neck area of contact between the two aggregates to characterize the process, as done for the coalescence of liquid droplets and polymers. Both experiments and simulations show that the evolution of this quantity obeys a power law in time. We also study quantitatively individual cell motion with the simulation and it corresponds to an anomalous diffusion.

1. Introduction

The study of fusion of cell aggregates is important in biofabrication and its *in silico* modeling can help the design of bioengineered tissues. Fusion is also important in different developmental processes including heart formation [1,2].

Aggregates of cells or multicellular spheroids behave similarly to liquids in long time scales: they round to minimize their surface and fuse or envelop each other when placed in contact [3,4]. In these types of experiments the spheroids are grown in culture medium containing all required cell nutrients. They have been used to model tumors [5,6] and more recently used as 'building blocks' for organ tissue engineering [7]. In this case, a tissue is fabricated from several spheroids that are entrapped in hydrogel, forming a three dimensional matrix. The expected result would be the fusion of the aggregates forming a uniform tissue. Hence, a detailed description of cell aggregates fusion is a necessary first step to yield the adequate control

* Corresponding author. Tel.: +55 55 32209521.
E-mail addresses: glt@if.ufrgs.br (G.L. Thomas), jcmombach@ufsm.br (J.C.M. Mombach).

0378-4371/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.physa.2013.10.037

necessary for biofabrication. To acquire control of these processes we investigate the physics subjacent to the observed cell behavior. Cells in an aggregate interact in complex ways that involve (i) adhesion molecules that may differentially attach to different cell types, (ii) active motion caused by the cytoskeleton activity and (iii) the cortical tension originating from the cytoskeleton structure that causes cells resist to deformations. In terms of rheological properties aggregates present simultaneously elastic, viscous and plastic properties [8]. For example, aggregates generated by random mixtures of two or more cell types undergo a sorting process where the final configuration is one layer of a tissue enveloping the other (engulfment), resembling the phase separation in pure physical systems due to interfacial free energy minimization [9,10]. Forgacs and coworkers have studied fusion using a simplified simulation where cells are represented as dots, which is an oversimplification of a real cell [11]. More recently, Rieu and coworkers (see supporting information in Ref. [8]) experimentally determined the speed with which the contact area increases during the fusion of two cell aggregates.

Today, there is a growing experimental and theoretical consensus that a balance between cell adhesion and cortical tension, which determines the shapes of cells in epithelia, can play a relevant role in the dynamics of cell aggregates and tissue organization [9,12–14]. In this work we present new experimental data on fusion and we study with numerical simulations the influence on the process of several mechanisms including cell adhesion, motility, cortical tension and the aggregate number of cells.

2. Methods

2.1. Experiments

Cell culture: adipose tissue derived stem cells (ADSC) from Invitrogen were used for this experiment. Cells were multiplied in MesenPro RS basal medium (Invitrogen) supplemented with 2% MesenPro RS growth supplement (Invitrogen), 1% Penicillin/Streptomycin (Invitrogen), 1% L-glutamine (Invitrogen), 1% Amphotericin B (Sigma-Aldrich). Cells were maintained in a humidified incubator at 37 °C in 5% CO_2–95% air atmosphere, with the medium changed every second day. Cells from first passage were used in experiments.

Tissue spheroid production: 32 tissue spheroids were produced in parallel by the traditional hanging drop method. Cells from confluent ADSC layers were harvested using 0.25% Trypsin/EDTA for 5 min in a humidified incubator. They were counted and the concentration of cell suspension was readjusted to 10^6 cell/ml (25,000 cell/25 μl). From this suspension droplets of 25ml were placed on the lid of Petri dishes that were inverted and incubated at 37 °C in humidified incubator for 48h. During this period of time, tissue spheroids formed at the bottom of the droplets due to gravity. Then the tissue spheroids were juxtaposed in hanging drops with fresh medium barely touching each other. The fusion of tissue spheroids was followed up for 2 days and pictures were taken with a Dage-MTI RC300 camera mounted on a Leica MZ12 stereomicroscope at every 4 h.

2.2. Numerical simulation

The simulation model was developed using the CompuCell3D 3.6 simulator [15] of the cellular Potts (CPM) which is available for download from: http://www.compucell3d.org

The model is defined as follows. A label is attributed to each site of a discrete lattice with dimensions starting from $L_x \times L_y \times L_z = 63 \times 63 \times 106$ to $L_x \times L_y \times L_z = 74 \times 74 \times 312$ voxels. Cells or external medium are represented by domains composed by neighboring labels with the same value. The energy between cell–cell or cell–medium interactions is proportional to the shared interface length with energy $E_{\tau\tau'}$ per unit area, where τ specifies cell type and/or medium. The energy (in arbitrary units) used in the Monte Carlo process over the cubic lattice is defined as

$$E = \frac{1}{2} \sum_{\langle ijk,i'j'k' \rangle} E_{\tau_{ijk}\tau'_{i'j'k'}} \times I_{\tau_{ijk}\tau'_{i'j'k'}} + \lambda_V \sum_{\tau} (V(\tau) - V_T)^2 + \lambda_S \sum_{\tau} (S(\tau) - S_T)^2, \quad (1)$$

where $E_{\tau_{ijk}\tau'_{i'j'k'}}$ is the interfacial energy per unit area between neighboring lattice sites labeled by τ_{ijk} and $\tau'_{i'j'k'}$ and $I_{\tau_{ijk}\tau'_{i'j'k'}}$ is the area of interface between them that depends on the range of interaction among lattice sites. The sum in the first term extends to third-nearest neighbors that decreases significantly lattice energy anisotropy. The last energy terms constrains the volume V and surface S [12,13,16] of each cell to predefined target values $V_T = 125$ voxels and $S_T = 190$ pixels, with intensity controlled by λ_V and λ_S, respectively. The last term in Eq. (1) models the cell cortical tension. External medium is represented by an unconstrained generalized cell occupying all the empty space. The initial state of the simulation is two spherical aggregates of identical cells touching each other (see Fig. 1). The system evolves using a Monte Carlo process with a Metropolis dynamics explained as follows. A site σ of the lattice and one of its 6 nearest neighbors are randomly chosen to test local boundary energy minimization. If the two sites are of different types, then we propose that the site assumes the neighboring label value σ' and accept it with probability P determined by the Metropolis algorithm: a variation in energy ΔE is accepted with probability 1, if $\Delta E \leq 0$. If $\Delta E > 0$ a second random number, r, is generated and the change of label occurs if $e^{-\Delta E/T} > r$, otherwise the label is not changed. T is a fluctuation parameter that controls cell motility and is kept constant during the simulation. Higher T implies higher motility and vice versa. This process is repeated indefinitely. The unit of time, one Monte Carlo step (MCS), is defined as the number of exchange attempts equal to the total number of sites of the lattice. Only cell