STUDY ABOUT CELL ADHESION KINETICS ON SOLID BIOMATRICES

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Abstract. Cell adhesion process on solid biomatrices is demonstrated to influence dramatically the *in vitro* morphogenesis of artificial tissues, because it involves all the key factors of early tissue formation: cells, matrix and the physiological interactions between them. To evaluate the efficiency of this process, many authors proposed static parameters like final cell density, cell distribution, and final cell viability in different seeding techniques, i.e. static, circular flow or direct perfusion seeding. We studied the cell adhesion dynamics using a homemade optical density-meter under various experimental conditions. A modified fluorimetric cuvette was used where solid collagen type I matrices are exposed directly to the 3T3 murine cells under continuous agitation with a magnetic stirrer. The optical density data are digitally recorded by an optoelectronic device (Texas Instruments). Through computer processing, the variation of the cell suspension's concentration during the entire process can be observed, results being expressed either in optical density decrease per minute, or in number of cells seeded per minute. The experimental seeding curves were analyzed in terms of a sequential 2-phase kinetics model. Results showed a good correlation between prediction and experimental curves.

Key words: cell seeding, adhesion, collagen biomatrices.

INTRODUCTION

Being sustained by the most recent achievements of the cell stem biotechnology, tissue engineering and regenerative medicine becomes little by little an important domain of medical research, since it promises to solve the difficult problem of organ or tissue replacement. The main philosophy is to extract stem cells from the patient having major organ function deficiency, to culture them in the lab, to seed certain 3D scaffolds, to grow the resulting fragments into specific bioreactors and, finally, to replace the defected organ with the engineered one [8, 13]. The success of such therapy, rather limited for the moment, is promising enough for the patients, and also for the researcher, who is highly focused to increase the benefits and to reduce the shortcomings. For example, one of the main limitations is that immune response is not so much diminished after implant, as

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expected, even though the cells source is the same. The experience showed that cultured stem cells lose their histocompatibility pattern, so they become non-self at the end of the artificial tissue/organ synthesis. Though, by controlling the initial culture conditions, including isolation, seeding and construct growing stages, there are hopes to overcome this problem [3, 4, 10].

The research endeavor in this area is more often conducted in a trial-and-error fashion, meaning that there is no time for fundamental approaches. The literature is highly abundant in papers focused on the matrix quality regarding biocompatibility and other architectural features, and also, abundant in describing clinical studies, either on humans or pets, concerning the outcome of such implant methods. Thus, reliable results were more reported for simple structured organs, like epithelia, gallbladder, large vessels, bone or cartilage, while for parenchymatous organs, with complicated 3D architecture, solutions are far from being conclusive [4, 10].

Among other important processes involved in tissue engineering, cell seeding on solid matrices is thought to play a crucial role in the formation of the future 3-dimensional organ, since it involves all the key factors of tissue formation: cells (type, metabolic activities, division rate, viability, etc.), matrix (chemical structure, porosities, architecture) and biophysical interactions (biophysics of cell-cell or cell-matrix adhesion). During the seeding process, cells and matrix are for the first time together, and this initial encounter has to be decisive. To characterize this process, static parameters like final cell density, cell distribution or cell viability were proposed, in different seeding techniques, i.e. static seeding, circular-flow seeding or direct perfusion seeding [12]. In our study, we evaluate a dynamic parameter, the cell seeding velocity that we consider to have significant relevance, since it represents a direct indicator for both, cell-cell or cell-matrix interactions. The cells used were fibroblasts, chosen as models for adherent cells [11]. We used collagen matrices that are known to specific stimulate integrin expression in contact cells [5, 6].

MATERIALS AND METHODS

Cell line: immortalized murine skin fibroblasts (NIH-3T3) were cultured in DMEM supplemented with 2 mM glutamine, 10% fetal bovine serum and antibiotics at 37 °C in 5% CO_2 humidified incubator. Before the experiment, cells were trypsinized, washed and centrifuged twice at 1000 rpm (200 g) and finally resuspended in supplemented CO_2-independent medium (GIBCO) (CIM), prior to optical density measurement.

3D matrices: type I collagen 3D scaffold (Poneti Ltd., Bucharest, Romania) were supplied as white sponges of 10×10×5 mm^3, packed in double polyethylene bags, γ-rays sterilized with 25 Gy. For experiments, pieces of 3×3×3 and 5×5×5

mm^3 were sliced, weighed and previously rinsed in supplemented CIM for 15–20 minutes prior seeding.

Method principle. A home-made optical density-meter was developed to quantify the dynamics of the cell seeding process on collagen solid biomatrices. The optical density of the cell suspension will decrease in time due to the cell number lowering. Thus, the cell seeding velocity can be evaluated as the number of cells that go away from suspension (are sequestered into the matrix) during the seeding process:

$$v_{\text{seed}} = -\frac{\mathrm{d}\, c_{\text{sc}}}{\mathrm{d}\, t} = -\frac{\mathrm{d}\, N_{\text{sc}}}{V \cdot \mathrm{d}\, t} \tag{1}$$

where c_{sc} is the cell concentration in suspension, N_{sc} is the cell number, V is the suspension volume and t represents time. The calibration curve (Fig. 1) shows a direct dependence of the optical density (OD) on cell concentration in the concentration domain used in our experiments ($1 \div 3 \times 10^6$ cells/ml). These calibration experiments were made with a CoulterCounter device to control the cell number and a Zeiss Spectrophotometer to measure the optical density at the wavelength of 450 nm, used also in the seeding protocols.

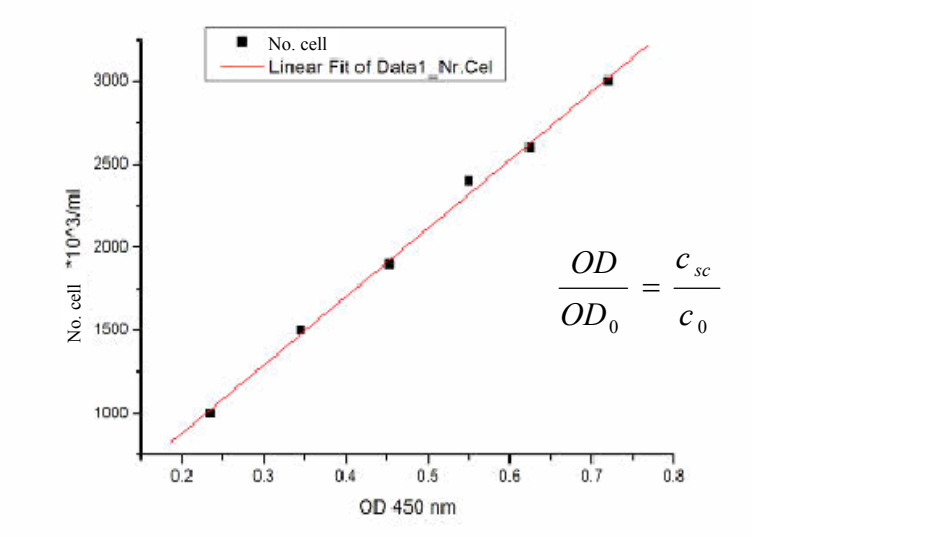

Fig. 1. Calibration curve shows the linear dependence of cell concentration on optical density at 450 nm.

Thus, one can express the cell seeding velocity with the following formula:

$$v_{\text{seed}} = -K \frac{\mathrm{d}\, OD}{\mathrm{d}\, t}, \text{ with } K = \frac{c_0}{OD_0} \tag{2}$$

where c_0 and OD_0 represent the initial cell concentration and the corresponding optical density of the suspension.

Experimental setup. A home made optical density-meter was used, which contains a variable light source, a thermostated sample holder, the seeding micro-reactor (Fig. 2), the photodetector and the data acquisition system connected to a computer. The seeding micro-reactor is thermostated at 37 ºC through a metallic coating connected to an external water thermostat. All the experimental devices are sterilized and the cell suspensions are also prepared in sterile conditions. Cells must be kept alive for the entire monitoring session since the adhesion process on solid biomatrices has been shown to last for several hours [1, 7].

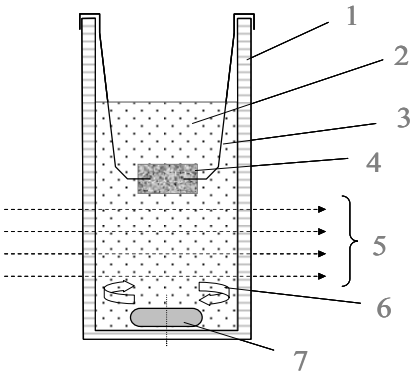

Fig. 2. Seeding micro-reactor – is a modified fluorimetric plastic cuvette of 10×10×40 mm^3; 1. transparent wall; 2. cell suspension; 3. stainless steel wire; 4. solid matrix; 5. light beams; 6. induced circular flow; 7. magnetic stirrer.

The optical density data are recorded by an OPT101 optoelectronic device (Texas Instruments) plugged to the PC-Scope PCS500 electronic interface (Velleman Instruments) connected to a PC. Through computer processing, the variation of cells suspension concentration recorded during the entire seeding process can be observed. Results are expressed either as cell suspension optical density over time, or as normalized number of attached (seeded) cells over time.

RESULTS

We present two examples of seeding curves obtained with opposite experimental conditions (Fig. 3):

A. one with a less number of cells (0.78×10^6 ml^{-1}) and a much bigger matrix (5×5×5 mm^3, ~ 8 mg dry) and

B. the second, with an initial high concentration of cells (2.86×10^6 ml^{-1}) and a small piece of matrix (3×3×3 mm^3, ~ 3 mg dry).

The control sample (C) is a graph recorded in the absence of the matrix and demonstrates that cells are not attachable to the cuvette walls and do not adhere

each other significantly due to the circular flow stress induced by the stirrer. Each data point represents the average value of 10 min continuous recording at 1/second sampling rate.

The mathematical analysis of the two data sets, realized with the OriginPro 7.5 program (OriginLab Corp., USA) (Fig. 4), allowed us to observe that the seeding process is very likely a simple consecutive 2 stage process. Both experimental sets data fit, with good accuracy, a simple sequential kinetics:

$$N \xrightarrow{k_1} A \xrightarrow{k_2} AS \tag{3}$$

where N is the number of non(yet)-adherent cells, A – the number of cells that are able to adhere and AS are the seeded cells. The kinetic constants k_1 and k_2 are time dependent according to the following equation [2]:

$$[AS] = N_0 \cdot \left(1 + \frac{k_2}{k_1 - k_2} \cdot e^{-k_1 t} + \frac{k_1}{k_2 - k_1} \cdot e^{-k_2 t} \right) \tag{4}$$

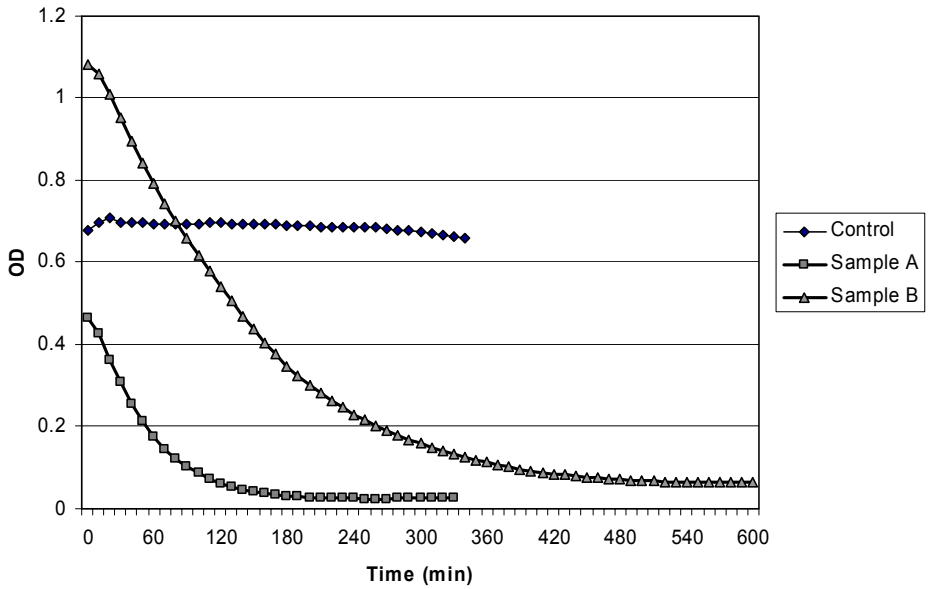

Fig. 3. Optical density curves obtained at 450 nm; *sample A* – low cell number (0.76×10^6 ml^{-1}), big matrix ($5\times5\times5$ mm^3); *Sample B* – high cell number (2.86×10^6 ml^{-1}), small matrix ($2\times2\times2$ mm^3), *Control* – cell suspension (1.5×10^6 ml^{-1}) without matrix; each data point represents the average value of 10 min continuous recording at 1/second sampling rate.

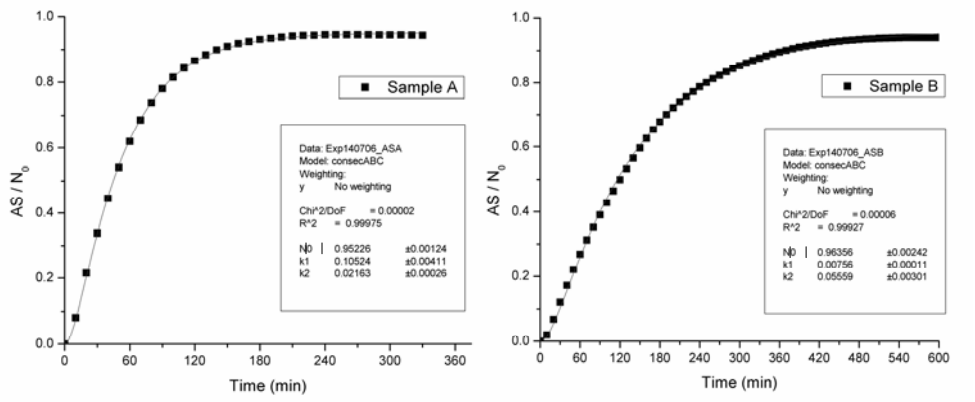

Fig. 4. Experimental seeding curves fitted with the simple sequential 2-stage model (the ratio AS/N_0 represents the normalized number of seeded cells); *sample A* – low cell number (0.76×10^6 ml^{-1}), big matrix ($5\times5\times5$ mm^3); *sample B* – high cell number (2.86×10^6 ml^{-1}), small matrix ($2\times2\times2$ mm^3); each data point represents the average value of 10 min continuous recording at 1/second sampling rate.

The fitting parameters are presented in the following table:

Table 1

Simple sequential model fitting parameters

	Sample A	Sample B
measured cell number ($\times10^6$ ml^{-1})	0.76	2.86
$\|N0\|$ – seeding ratio	0.95226	0.96356
k_1	0.10526	0.00756
k_2	0.02163	0.0556
R^2	0.99975	0.99927

CONCLUSIONS

The proposed experimental setup proves to be extremely versatile and allows for a simple proof of the fact that initial cell concentration and scaffold surface induce significant changes in the seeding dynamics.

The simple sequential 2-stage kinetics model gives high correlation values to experimental data and it can be taken into account for understanding the early steps of the seeding process. In this model, the k_1 constant is a global measure of the integrin expression process. This cellular parameter has to depend on the probability of exposure to collagen stimulation which is significantly higher for

sample A than for sample B ($k_{1A} >> k_{1B}$). The k_2 constant can be regarded as a measure of the cell-matrix interaction and it is in the same order of magnitude for the two samples. After sufficient integrin molecules expression on the membrane surface, cells are trapped into the matrix with the same velocity, weighed only by the instant cell density on matrix surface unit.

Computed ratio, $|N0|$, of seeded cells at the end of the process over initial number of cells in suspension shows that almost all initially suspended cells were finally attached to the matrix. The differences may represent dead or not functional cells.

This kinetics model analysis proves to be very useful for the evaluation of some important seeding parameters like regeneration of integrins after Trypsin/EDTA treatment or the matrix bioavailability. Also it can help to provide reliable data about the molecular mechanism of different seeding promoters or modulators.

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