COMPUTATIONAL STUDY OF CELLULAR ASSEMBLY ON HYDROPHOBIC/HYDROPHILIC MICRO-PATTERNS

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Abstract

Cell migration and assembly play an important role in many biological systems. We develop simple one-dimensional stochastic model of cell behavior on chemically patterned surfaces that is based on three key parameters: speed of cell movement (motility) across substrate, probability of cell adhesion to substrate, and probability of cell division on substrate when adhered on substrate. Amount of adhered cells on hydrophobic and hydrophilic regions is calculated as function of time (number of cycles up to 2000). The model is correlated with in-vitro data obtained within 48 h in real time. We show that this simple stochastic model with the three parameters (where cell motility is the most important one) can describe with high accuracy the experimental data and thereby explain the observed preferential cell assembly on hydrophilic/hydrophobic micro-patterns (up to 200 μm width).

Keywords: SAOS-2 cells, cell movement, adhesion, stochastic computer simulations

1. INTRODUCTION

Cell migration and assembly play an important role in many biological systems. Cell migration is required for embryonic development [1], wound healing [2] and immune responses [3]. Accurate understanding of cell migration requires good knowledge of biological, chemical and mechanical processes [4]. Those processes can be better understood by using mathematical models, which focus only on the most important factors. Mathematical modelling is widely used in life sciences. Modelling can be applied to explain single cell movements (motility) [5, 6] and how cell adhesion influences cell movement [7]. Models can also be used to explain cell communication pathways [8] and collective cell movements [9].

Properties of substrate play crucial for cell migration, assembly as well as viability and proliferation. For instance, suitably adjusted nanoscale topography of nanocrystalline diamonds promoted differentiation of osteoblastic cells [10]. Osteoblastic SAOS-2 cells also exhibited different adhesion and viability properties on hydrophobic hydrogen-terminated nanocrystalline diamond (H-NCD) or hydrophilic oxygen terminated nanocrystalline diamond (O-NCD). When such surfaces are combined into micro-arrays, osteoblastic, fibroblastic, as well as HeLaG can self-assemble on the O-NCD patterns [11]. Proposed mechanism for such behavior were different cell adhesion strength between the cells and the substrate and between protein layer and the substrate [12], possibly related with different conformation of protein molecules [11] or aggregates from the cell medium [13]. However, unambiguous conclusion could not be made.

Here we show that the self-assembly mechanism of cells on chemically micro-patterned substrates can be elucidated by a simple, one-dimensional stochastic model that is based on three key parameters: speed of cell movement (motility) across substrate, probability of cell adhesion to substrate, and probability of cell division on substrate when adhered on substrate. The model is compared and validated with in-vitro data obtained within 48 h in real time.

2. EXPERIMENTAL DETAILS

2.1 Computational Model

The model used for computer simulations is a 1D stochastic model. Large amount of cells (N=1200) is evenly distributed over the sample segment, length of which corresponds to interval 1 to N, i.e. [1, N]. The segment is divided into three pairs of hydrophobic (H) and hydrophilic (O) stripes. The model uses three parameters: speed of cell movement (S), probability of cell adhesion (P), and probability of cell division (D). They are defined separately for H and O regions.

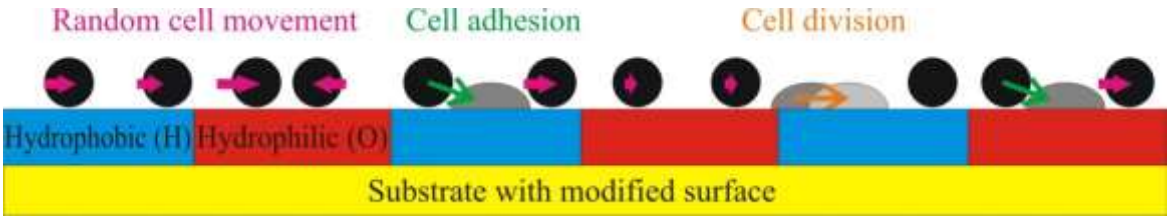

Fig 1 Schematic model of cell movement, attachment and division processes on the sample segment with three pairs of hydrophobic/hydrophilic stripes.

The concept of the stochastic model is schematically shown in Fig.1. At the first computational step, random movement of each cell takes place from starting point (x) within the range [$x-S_H/2$, $x+S_H/2$] for the cells on hydrophobic part and within segment [$x-S_O/2$, $x+S_O/2$] for the cells on hydrophilic part. If the cell leaves the segment during movement it is placed back to the segment (to nearest position 1 or N). At the second step each cell is attached to the surface with probability P_H or P_O respectively. If the cell is attached to the surface it cannot move anymore in the next cycles. At the third step, each adhered cells is divided into two cells with probability D_O or D_H depending on the underlying surface. In case of subsequent division there will be three cells placed in the same spot. The cycle of movement, adhesion, and division processes is repeated many times. Two thousands cycles were found enough to achieve equilibrium when practically all the cells adhered. Computer simulations were performed using Matlab software on HP pro 3400 computer with Intel® Core™ i5-2400 processor.

2.2 In-vitro experiments

For validation of the computational model, time-resolved cell culture experiments were performed on nanocrystalline diamond samples. NCD sample preparation and SAOS-2 cells growth procedure were made according to the protocol [11]. Diamond was chosen because its surface can be made hydrophobic and hydrophilic using plasma treatment. Moreover, it is transparent and this is a key feature for real-time optical measurements. The NCD layers were prepared on 10x10 mm glass substrates by microwave-plasma chemical vapor deposition [14]. Stripes of 200 μm width treated with hydrogen (hydrophobic) and oxygen (hydrophilic) plasma were created on the surface by photolithographic masking.

Phase contrast image sequences were acquired on microscope Nikon TE2000E (lens - Plan Fluor 4x, N.A.=0.13) with cultivation chamber Okolab Boldline for sustaining the cell culture for 48 h. Image sequence intervals were 1 minute for the first 2 h of cultivation (cell seeding) and 10 minutes for the rest of the experiment up to 48 h. Medium was supplemented with 15% FBS during whole experiment. The number of adhered cells on each type of surface was counted for each frame of the image using image background equalization and thresholding in ImageJ (NIH) and NIS-Elements (Laboratory Imaging) software. The adhered cells appeared as black spots on lighter background.

Cell doubling times were calculated using Doubling time calculator (http://www.doubling-time.com/). Cell number data entered in the calculator were taken from 5 time points (2 h, 6 h, 10 h, 24 h and 48 h after seeding).

SAOS-2 cells were fixed using 4% paraformaldehyde in PBS after the 48 h cultivation. Cells were then fluorescently stained for actin (Phalloidin+AF488, Life Technologies). Multipoint images of stained cells on NCD sample were taken using microscope Nikon TiS with 4x lens (NA=0.13) and stitched together using NIS-Elements software (LIM).

Initial cell plating density was ~15000 cell/cm^2 [11]. Three pairs of 200 μm stripes on the sample thus correspond to 1.2 mm long segment with 1800 cells within the segment and 550 cells within the field view of optical microscope. In computations there is 1200 cells on the segment width 1200, each stripes has width 200. Two thousand cycles for 48 h correspond to 1.44 min/cycle. The experimentally measured cell movement speed is approximately 120-150 μm/h, i.e. in the model S=6-7. The experimentally measured ratio S_H/S_O within 1 min interval is 1.23. The probabilities of cell attachment (P_O and P_H values) were defined based on experimentally measured amount of round (non-adhered) cells on the sample from exponential decay fit as 1/decay constant. The values are P_O=0.016, P_H=0.014. Such values provide > 99% probability that the cell will attach to the surface during 2000 cycles. Experimentally measured average values of doubling time on the sample with stripes are T_O=33 h (in the model D_O=0.0008) and T_H=117 h (D_H=0.0002).

3. RESULTS AND DISCUSSION

Fig. 2a show amount of cells on hydrophobic and hydrophilic stripes as function of time as determined from the real-time cell culture experiments on 200 μm stripes. There is clearly different trend and amount of cells after 48 h on each type of surface. Fluorescence microscopy image in Fig. 2b shows experimentally obtained cell pattern, which was formed on the NCD sample with 200 μm H/O-terminated stripes after 48 h. There are several cells on H-NCD; however, most of them are small, round and not well-adhered. Practically all cells on O-NCD are well adhered and spread on the pattern. This is in agreement with previous studies [11]. The experimental pattern also shows very sharp border, which is in agreement with model-based computed data.

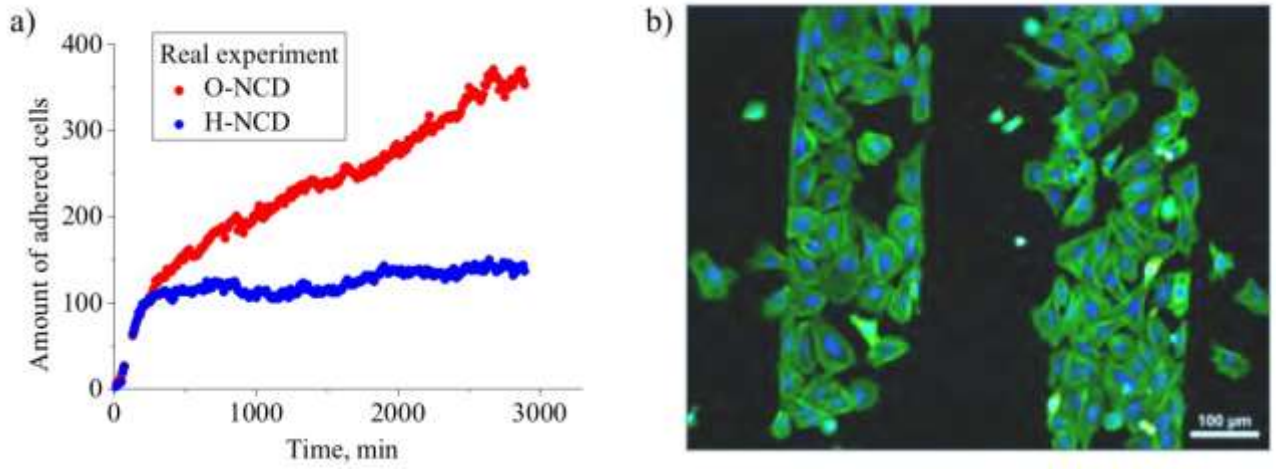

Fig. 2 Amount of adhered cells on hydrophobic surface (blue curve) and hydrophilic surface (red curve) in (a) real in-vitro experiment on 200 μm stripes. (b) Optical fluorescence microscopy image of cells on the NCD sample with 200 μm hydro-phobic/philic stripes. Green color corresponds to immunostained actin filaments (cytoskeleton), blue color corresponds to cell nuclei.

The two experimental curves were approximated by the stochastic model using the five fixed parameters measured experimentally (S_H/S_O, P_O, P_H, D_O, D_H) and only one adjustable parameter (S_O) in order to achieve

closest possible ratio A_O/A_H (amount of cell) to experimental result where A_O/A_H=2.58 after 48 h. Fig. 3a represent the best approximation of the experimental results (Fig. 2a) with the parameters S_O=57, S_H=70, P_O=0.016, P_H=0.014, D_O=0.0008, D_H=0.0002. Note that S_O=57 value is much higher than expected value (S~6-7). This is due to the fact that "theoretical" cells stochastically move back and forth a lot, while "experimental" cells generally have some preferential direction of movement. However, mean travel distance of all cells in the computation is 91, in good agreement with experimental value 85 μm (85 in the model).

Fig. 3b show how cells were distributed over the sample after 2000 cycles. Blue and red lines represent average amount of cells on each stripe. The average ratio A_O/A_H=2.64 > 2 indicates that the pattern was formed. Sharp difference in the amount of cells at the boundary of the H/O-regions is observed theoretically. Note that no additional assumption was required for appearance of such sharp boundary, just the employed model parameters.

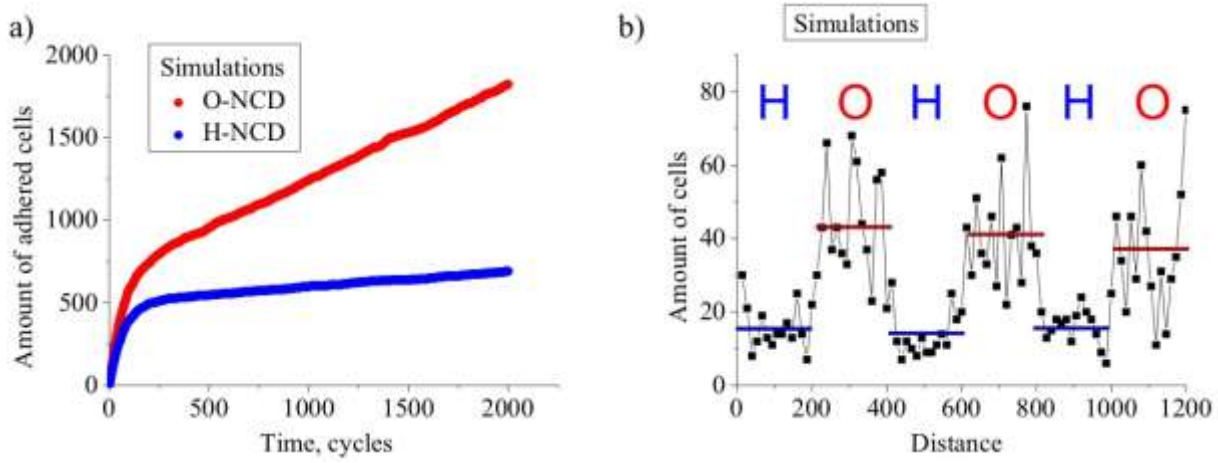

Fig. 3 (a) Amount of adhered cells on hydrophobic surface (blue curve) and hydrophilic surface (red curve) in computational model with calibrated parameters. (b) Computed line profile of cell amount across the stripes.

In the case of absence of cell adhesion (P_O=0, P_H=0) amount of adhered cells is zero in the model. Fig. 4a illustrates computational result without taking into account cell movement (S_O=0, S_H=0). The resulting ratio of cell amount is A_O/A_H=1.86, which is slightly below our empirical threshold (2), and the pattern was thus not formed. However, the shape of the curves is quite similar to experimental curves, indicating high impact of cell division on pattern formation. Fig. 4b illustrates computational result without taking into account cell division (D_O=0, D_H=0). The resulting ratio of cell amount is A_O/A_H=1.39, which is below our empirical threshold (2), and the pattern was thus not formed. Amount of adhered cells saturates in case of absence of cell division. This was not observed experimentally and shows how the model can elucidate individual involved parameters, which is difficult to do in in-vitro experiments.

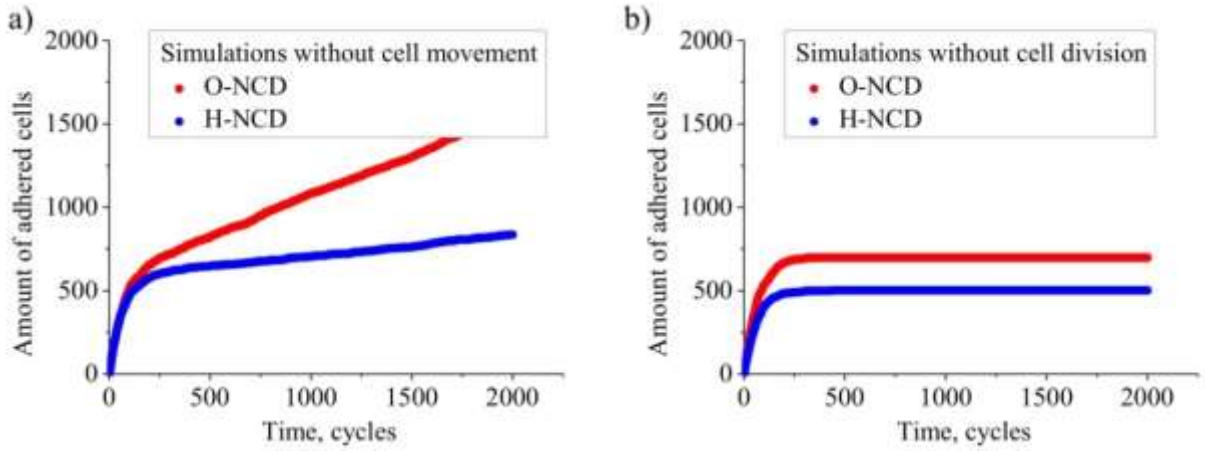

Fig. 4 (a) Computation without cell movement taken into account. (b) Computation without cell division taken into account.

Based on those computation results we can tell that cell movement and cell division are required for pattern formation. The 4x faster cell division (D_O vs D_H) on the hydrophilic surface is practically enough to generate the patterns; however, at least small difference in cell motility ($S_H/S_O = 1.23$) is necessary to indeed generate the patterns and make them clearly pronounced. Moreover, in the model as well as in real experiment cell proliferation can occur only after cell adhesion; therefore, cell adhesion is also required for pattern formation.

The results shows that the three chosen parameters are accurately represent and describe cell behavior on micro-patterned sample. The cell motility, cell adhesion and cell division have high impact on improving ratio A_O/A_H and quality of selective cell assembly. Additional parameters such as cell interaction or communication or cell movement after adhesion or division will make the model more complicated, but will not drastically change the result of simulation experiments.

4. CONCLUSIONS

In this work the simple yet biologically realistic and accurate model based on cell movement, adhesion and proliferation was demonstrated. The model fits well the observed self-assembly of cell micro-patterns on hydrogen and oxygen terminated diamond. The model elucidated that the main factor for micro-pattern formation is the difference in cell motility and proliferation on hydrophobic and hydrophilic surface. Moreover, the model was also able to reproduce formation of sharp border of the cell culture on the boundary of stripes, in agreement with experiment. The model is universal and can be applied on different materials and cells.

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