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Fractal properties of biophysical models of pericellular brushes can be used to differentiate between cancerous and normal cervical epithelial cells

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ABSTRACT

Fractal behavior is found on the topographies of pericellular brushes on the surfaces of model healthy and cancerous cells, using dissipative particle dynamics models and simulations. The influence of brush composition, chain stiffness and solvent quality on the fractal dimension is studied in detail. Since fractal dimension alone cannot guarantee that the brushes possess fractal properties, their lacunarity was obtained also, which is a measure of the space filling capability of fractal objects. Soft polydisperse brushes are found to have larger fractal dimension than soft monodisperse ones, under poor solvent conditions, in agreement with recent experiments on dried cancerous and healthy human cervical epithelial cells. Additionally, we find that image resolution is critical for the accurate assessment of differences between images from different cells. The images of the brushes on healthy model cells are found to be more textured than those of brushes on model cancerous cells, as indicated by the larger lacunarity of the former. These findings are helpful to distinguish monofractal behavior from multifractality, which has been found to be useful to discriminate between immortal, cancerous and normal cells in recent experiments.

1. Introduction

The fractal concept introduced by Mandelbrot [1] is commonly invoked in the description of biological systems, where the repetition of patterns at different scales is frequent. In physiology, fractality has been used for decades [2,3] in the analysis of complex patterns that can be found in neuronal and cardiac activity [4–7], arterial and blood vessel networks [8–11] and bronchial trees [12,13], for example. Much of the physio – pathological research related to the fractal analysis of images has focused on correlating the fractal dimension (FD) of the structures or patterns present in such images with the health of cells. Among the extensive research in this field, there are works that relate the value of the FD to the presence of cancer [14–22]; studies carried out at the macro- and micro-scale for samples of colon [16], breast [17,18], skin [19], cervical cells [20,21], and even white blood cells [22] establish a difference between the FD of cancerous and normal tissues. From these results, it has been possible to distinguish healthy cells from cancerous cells [16,17,20,22], define a relation with tumor growth [18,23], trace the progression towards cancer [21] and measure its invasiveness [24].

In the context of the current understanding of the molecular mechanisms of cancer, medical imaging remains one of the most commonly used routes towards diagnosis. The implementation of fractal analysis for medical imaging has the potential of becoming a strong tool to yield precise diagnosis. The FD of an image can be estimated using several methods, such as box – counting, correlation, and Fourier analysis, among others [25]. Atomic force microscopy (AFM) has been widely used in cancer research to characterize mechanical properties that can discriminate between cancerous and normal cells [26–28]. Recently, it has been applied to the generation of topography and adhesion maps of individual cervical epithelial cells, wherein the FD of such images is calculated by Fourier analysis [20,21]. The results show that the FD of cancerous cells tends to be higher than that of their healthy counterparts, with the differences likely due to the topographical features arising from the molecular brushes on the cell's surface. These brush – like structures coating the cell's surface are composed of complex macromolecules (microvilli, microtubules, microridges) tethered to the cell membrane, and results obtained with AFM show that their mechanical response can be measured separately from that of the cell's surface [26]. Furthermore, these experiments reveal that the brush on normal cervical cells (NCC) is made up of an approximately monodispersed array of chains, while cancerous cervical cells (CCC) are covered by a brush with at least two characteristic lengths. It is also argued that the grafting density on NCC brushes is lower than that on CCC brushes [26]. However, previous studies did not

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address aspects such as brush stiffness/softness, which is hypothesized to matter as cancer progresses [27], or the physicochemical environment of the cells, an important aspect to investigate since most experiments are carried out *in vitro*. Also, it is crucial to determine to what extent are the fractal properties of brushes dependent on image resolution, so that a confidence margin can be established when assessing distinctions between cancerous and normal cells.

2. Models and methods

Here we report predictions of geometric properties of brushes that help distinguish between model NCC and CCC using numerical simulations. The models are solved using the dissipative particle dynamics (DPD) method [29,30]. The CCC brush model is a tri-modal brush made up of end-grafted bead – spring linear chains of three different values of the polymerization degree: 294 short, 82 medium-sized and 33 large chains made of $N_1 = 5$, $N_2 = 30$ and $N_3 = 42$ DPD beads, with grafting densities of $\Gamma_1 = 1.74\,nm^{-2}$, $\Gamma_2 = 0.49\,nm^{-2}$ and $\Gamma_3 = 0.20\,nm^{-2}$, respectively. The NCC brush model is a monodispersed brush made up of 130 chains of $N = 27$ beads and a grafting density of $\Gamma = 0.78\,nm^{-2}$. Brush models with these characteristics have been used previously [31] to reproduce accurately the mechanical response of brushes on human epithelial cervical cells under the AFM probe [26]. The novelty of the models introduced here is an added force between consecutive bonds, which controls the persistence length of the chains. By varying this three – body force one can control the local rigidity of the chains to define soft and stiff brushes. The motivation for considering brushes with different stiffness comes from tests carried out on mammary epithelial cells, where Young's modulus for cells at different tumorigenic phases is found to be lower as cancer progresses [27]. It is argued that the stiffness of the cells depends on their microenvironment. Here, the environment change is modeled as the change of the brush – solvent interactions, hence our brushes are modeled under good and bad solvent conditions. To complete the model, the brushes are confined by an explicitly curved surface made of DPD beads, to mimic their interaction with a nanosized AFM probe. Full details are provided in the Supplementary Information (SI).

Polymer brushes are created from polymer chains at relatively high grafting density; polymer chains are made up of linear sequences of monomeric beads, joined by freely rotating, harmonic springs:

$$\boldsymbol{F_S} = -k_s(r_{ij} - r_0)\hat{\boldsymbol{r}}_{\boldsymbol{ij}}, \tag{1}$$

where k_s is the spring constant, and r_0 is the equilibrium position [32]. To model the chain's stiffness or softness, a three – body force acting between three consecutive beads is added [33]:

$$F_A = k_\theta \sin(\theta_{ijk} - \theta_0), \tag{2}$$

where k_θ is the constant for the angular forces, [34,35]. The relative distance between adjacent beads and the unit vector joining them are represented by the symbols r_{ij} and $\hat{\boldsymbol{r}}_{\boldsymbol{ij}}$, respectively. The equilibrium angle is $\theta_0 = 180°$ and θ_{ijk} is the angle between two adjacent bonds, respectively. Appropriate values for the parameters in Eqs. (1) and (2) are chosen that prevent bond – crossing; for the Hookean spring the value $k_s = 100\,k_BT/r_c^2$ [35] is used for all the chains in each system. For the angular force, two values of the constant k_θ are used to model the rigidity of the chains, namely $k_\theta = 10\,k_BT/r_c$ for soft chains and $k_\theta = 100\,k_BT/r_c$ for stiff chains. The systems are confined by two parallel surfaces perpendicular to the z–direction. The cell's surface ($z = 0$), is modeled by an effective, linearly decaying force given by:

$$\boldsymbol{F_{wall}} = \begin{cases} a_{iw}(1 - z_{iw}/z_c)\hat{\boldsymbol{z}} & z_{iw} \le z_c \\ 0 & z_{iw} > z_c \end{cases}, \tag{3}$$

with a cutoff length z_c and z_{iw} being the distance between the i-th particle and the surface, $\hat{\boldsymbol{z}}$ is their unit vector, and a_{iw} is the maximum intensity of the force [36]. On the opposite side of the simulation box is placed the surface of the AFM probe, which is a semi sphere made up of DPD particles frozen in space and curvature radius equal to $R = 0.8\,L_x$. The non - bonding conservative DPD force is:

Table 1
Interaction parameters a_{ij} of the conservative force DPD and the force of the implicit surface representing the cell surface. k_BT and r_c are expressed in reduced DPD units and represent energy and length, respectively. Bead species are: S = solvent, H = chain's head, T = chain's tail, P = AFM probe and C = cell's surface.

$a_{ij}\,[k_BT/r_c]$	S	H	T	P	C
S	78	79.3(85)	79.3(85)	140	100
H	79.3(85)	78	78	140	60
T	79.3(85)	78	78	140	100
P	140	140	140	78	0*
C	100	60	100	0*	0**

*Since the distance is larger than the cutoff radius.
**Because the cell's surface is implicit.

$$\boldsymbol{F_{ij}^C} = \begin{cases} a_{ij}(1 - r_{ij}/r_c)\hat{\boldsymbol{r}}_{\boldsymbol{ij}} & r_{ij} \le r_c \\ 0 & r_{ij} > r_c \end{cases}, \tag{4}$$

where a_{ij} is the repulsion parameter between beads i and j and r_c is the cutoff distance, set to $r_c = 1$. The former depends on the coarse –graining degree (the number of water molecules grouped into a DPD particle); for a coarse – graining degree equal to three, $a_{ii} = 78.0\,k_BT/r_c$, where k_B is Boltzmann's constant, and T is the absolute temperature. Table 1 shows the values of the interaction parameter for the various pairs of particles. Full additional details can be found in the SI.

The FD is calculated by Fourier analysis, following the procedure used to process images of human cervical epithelial cells [20,21]. The procedure requires the calculation of the two –dimensional Fast Fourier Transform of the image as follows:

$$F(u, v) = \frac{\sum_{x=0}^{N_x-1}\sum_{y=0}^{N_y-1} z(x, y)\exp\left[-i2\pi\left(\frac{ux}{N_x} + \frac{vy}{N_y}\right)\right]}{N_xN_y}, \tag{5}$$

where z(x, y) is the height of the brush at the pixel (x, y), and N_x and N_y are the number of pixels in the x and y directions, respectively. Then, the magnitude of $F(u, v)$ is transformed into polar coordinates and averaged over the angle:

$$A(Q) = 1/\pi\int_0^\pi F(Q\cos\varphi, Q\sin\varphi)d\varphi. \tag{6}$$

Q is the inverse of the lateral size L of the geometrical features on the image. Linear behavior of $A(Q)$ on log-log scale ($A(Q) \sim Q^b$) is a signature of fractality. We extracted images with $L_x = L_y = 14\,nm$, which leads to $Q_{min} \approx 0.071\,nm^{-1}$. The FD, α, as suggested by Dokukin et al. [20], is defined as $\alpha = 2 - b$, so that for flat surfaces $b = 0$ ($\alpha = 2$), while $b = -1$ ($\alpha = 3$) for infinitely rough surfaces, as limiting cases.

3. Results and discussion

The very concept of fractality implies that it is possible to find self – similar structural features on images upon magnifications on any scale. However, in practice, finding structures that are invariant over large orders of magnification is impossible; this empirical fact restricts the scale ranges where fractality can be observed. In image processing such ranges are generally restricted by the resolution of the image, since the search for patterns on scales smaller than the size of a pixel becomes meaningless. We calculate the FD for each image generated from DPD simulations at four resolutions: 1024 × 1024, 512 × 512, 256 × 256 and 128 × 128 pixels ($N_x \times N_y$). Fig. 1 shows some representative examples of height images of the brushes, at a resolution of 1024 × 1024 pixels; in the SI we describe the procedure followed to generate the images.

The FD obtained at different resolution for soft brushes is presented