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Opinion paper

Combined effect of surface nano-topography and delivery of therapeutics on the adhesion of tumor cells on porous silicon substrates

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ABSTRACT

Porous silicon is a nano material in which pores with different sizes, densities and depths are infiltrated in conventional silicon imparting it augmented properties including biodegradability, biocompatibility, photoluminescence. Here, we realized porous silicon substrates in which the pore size and the fractal dimension were varied over a significant range. We loaded the described substrates with a $PtCl(O,O'-acac)(DMSO)$ antitumor drug and determined its release profile as a function of pore size over time up to 15 days. We observed that the efficacy of delivery augments with the pore size moving from small (~8 nm, efficiency of delivery ~0.2) to large (~55 nm, efficiency of delivery ~0.7). Then, we verified the adhesion of MCF-7 breast cancer cells on the described substrates with and without the administration of the antitumor drug. This permitted to decouple and understand the coincidental effects of nano-topography and a controlled dosage of drugs on cell adhesion and growth. While large pore sizes guarantee elevated drug dosages, large fractal dimensions boost cell adhesion on a surface. For the particular case of tumor cells and the delivery of an anti-tumor drug, substrates with a small fractal dimension and large pore size hamper cell growth. The competition between nano-topography and a controlled dosage of drugs may either accelerate or block the adhesion of cells on a nanostructured surface, for applications in tissue engineering, regenerative medicine, personalized lab-on-a-chips, and the rational design of implantable drug delivery systems.

1. Introduction

Porous silicon (PSi) is a nano-material in which conventional silicon is modified through electrochemical procedures to contain a layer of pores within its structure [1,2]. The artificially introduced network of pores may vary in size and shape to a large extent and the distribution of these in the porous matrix can be finely adjusted on changing few parameters of the fabrication process, including etching time, current intensity, active etchant concentration, temperature of the process [1,2]. Depending on pore size, shape and density the micro-structure of PSi reveals an increased surface to volume ratio, up to 500 folds, and this increment is responsible for a number of properties of PSi including, but not limited to, biodegradability under physiological conditions [3], biocompatibility [4,5], hydrophobicity [1], photoluminescence [6]. Taken together, these and other not described properties render PSi a biomaterial, that is, a substance which can be engineered to take a form which, alone or as part of a complex system, may be used to direct the course of any therapeutic or diagnostic procedure. Porous silicon and its more sophisticated evolutions have been employed for time [7,8] and space [9] resolved drug delivery systems, for the separation of the low molecular weight content of a mixture for spectroscopic and spectrometric analysis [10–14], for the sequencing of DNA [15] or nanoparticles [16]. In few cases, porous silicon substrates with a fixed [2] or smoothly variable pore size [17] were used to verify the adhesive behavior of cells as a function of surface topography. Recalling that, according to the IUPAC definition [3], surfaces with a pore size smaller than 2 nm, comprised between 2 and 50 nm, and larger than 50 nm, are categorized as micro-porous (MiP), meso-porous (MeP) and macro-porous (MaP) silicon, respectively, results presented in [2,17] indicate that nano-scale surface topography with feature sizes in the low MeP regime accelerates cell growth and adhesion. In line with the presented results, in [18] it has been demonstrated that porous silicon chips (with a small pore size ranging from 8 nm to 75 nm and large fractal dimensions up to $D_f \sim 2.8$ nm) may boost the assembly of neuroblastoma N2A cells into highly clustered networks in comparison to un-etched silicon. Fractal dimension is a non-conventional parameter that describes a surface

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over multiple scales [1]. It provides in a sole number a measure of the complexity of a pattern, given as the amount of change of the details in a pattern to its scale. Fractal dimension may describe more efficaciously than roughness the topography of pattern. A technical description of fractals and the significance of fractal dimension may be found in references [1,2] and in the Materials and methods.

Here, we demonstrate the fabrication of MeP and MaP Si substrates in which the average pore size varies in the 10–60 nm range, and the fractal dimension transitions from $D_f \sim 2.8$ for the substrates in the low MeP regime, to $D_f \sim 2.6$ for the intermediate MeP regime, to $D_f \sim 2.7$ for the MaP structures, where the substrates are here reported in order of pore size. The described substrates were loaded with a $PtCl(O,O'-acac)(DMSO)$ antitumor drug ($MW \sim 408$) and its secretory profile was determined through different pore sizes. This permitted to verify the rate of drug delivery and the duration of therapeutic efficacy over time up to 15 days. The described devices were used as substrates for culturing MCF-7 breast cancer cells. Cell adhesion was verified on the described substrates with and without the administration of the antitumor drug. This permitted to untangle how the competition between nano-topography and a controlled dosage of drugs may either accelerate or block the adhesion of cells on a nanostructured surface, for applications in tissue engineering, regenerative medicine, personalized lab-on-a-chips, and the rational design of implantable drug delivery systems.

2. Materials and methods

2.1. Fabrication of the porous surfaces

Porous silicon substrates were obtained by anodization of silicon following the methods reported in [2,18] and here briefly recapitulated. We used p-type, (100) silicon wafers with a low $0.05-0.10\ \Omega$/cm resistivity as a substrate. The samples were cleaned with acetone and ethanol to remove possible contaminants. We obtained different pore morphologies on changing the parameters of the process. For substrates in the low MeP regime (average pore size <15 nm), we used a combination of HF, D.I. water, and ethanol in the electrolyte where the components of the solution stand in the $(1:1:2, v/v/v)$ ratios. In this case, the current density was adjusted as 20 mA/cm^2 and maintained for 5 min at 25 °C. Differently, for substrates in the high MeP regime (average pore size >15 nm) we used a mixture of HF, D.I. water, and methanol for the electrolyte in which these parts stand in a proportion of $(5:3:2, v/v/v)$. For this configuration, the current density was adjusted as 4 mA/cm^2 for 5 min at 25 °C. Finally, MaP silicon samples were fabricated applying a current density of 4 mA/cm^2 for 4 min at 25 °C to an electrolyte mixture of HF, D.I. water and DMF $(9:1:115, v/v/v)$. After electrochemical etching, the samples were rinsed in D.I. water, ethanol, and pentane with steps of 4 min. The porous substrates were baked at 200 °C for 6 h to assure hydrophilicity.

2.2. Atomic Force Microscopy characterization of the samples

The structure of the porous substrates was quantitatively determined using Atomic Force Microscopy (combined Raman-AFM Witec alpha300 RA). The samples were imaged using an intermittent, non-contact modality over a sampling area of 500×500 nm^2 at room temperature. Ultra-sharp silicon tips (ACLA-SS, AppNano) with a curvature radius at the tip less than 5 nm were used. The final images were averaged over multiple measurements (at least four), each of them performed at a scanning rate of 1 Hz. The images (1024×1024 points in size) were corrected using the Witec Project 2.10® software. The characteristic power spectrum (PS) and then the fractal dimension were derived for all the substrates (Fig. 2).

2.3. SEM characterization of the samples

The porous samples were verified using SEM. A Dual Beam (SEM-FIB) FEI Nova 600 NanoLab system was used for all the acquisitions, in which the beam energy was adjusted to 15 keV and the electron current accordingly to 0.14 nA. The pores, where the average pore size is in some cases comprised in the low nano-meter range, were resolved using a specific imaging modality, named mode 2, whereby sample can be magnified over 2.5 million times.

2.4. Fourier analysis and fractal dimension of the substrates

Fractals are entities that are too irregular to be described by conventional geometry. They display the following properties [1]: (fractals) (i) have a fine structure, that is, they reveal details on arbitrarily small scales; (ii) can be generated by short algorithms (perhaps recursively); (iii) exhibit a fractal dimension D_f strictly greater than the classical topological dimension: that is to say, the fractal dimension of a surface is generally greater than 2. In addition to this, self-affine fractal surfaces are hierarchical, that is, they are generated by the repetition of the same structure over multiple scales. Here, we used the methods described in [19,20], to analyze the AMF images of the samples (Fig. 2) and extract from each image the power spectrum $C(q)$ which reads

$$C(q) = \frac{H}{2\pi}\left(\frac{h_o}{q_o}\right)^2\left(\frac{q}{q_o}\right)^{-2(H+1)}, q>q_o \quad (1)$$

where q is the wave-number, λ is the wavelength (and notice that $q = 2\pi/\lambda$), q_o is the lower cut-off wavenumber and thus $\lambda_o = 2\pi/q_o$, and $h_o = \sqrt{2}$ Rrms. Moreover, we assume that the considered fractal surface is self-affine. In Eq. (1), H is the Hurst coefficient. In a diagram in which we display the logarithm of $C(q)$ against the logarithm of q, $C(q)$ takes the form of a line with a slope β for $q > q_o$. In this dimensionality range, $\beta = 2(H+1)$ and the fractal dimension $D_f = (8-\beta)/2$.

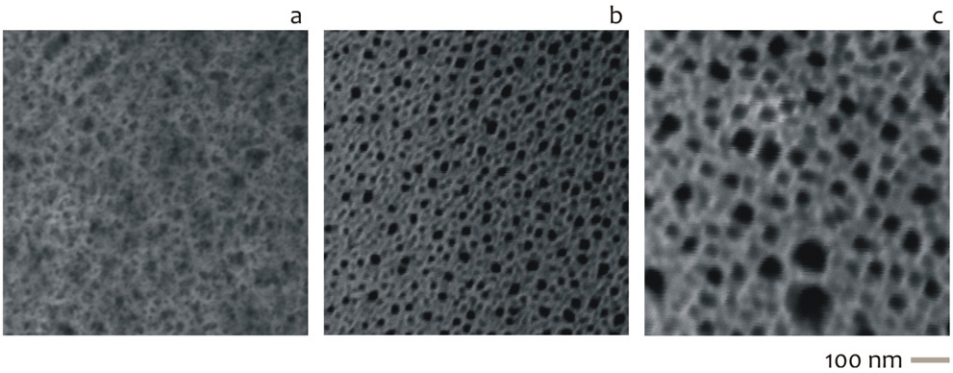

Fig. 1. SEM micrographs reveal the morphology of porous silicon. Small pore Meso-Porous (SP MeP) silicon, with an average pore size of $S = 8 \pm 3$ nm (a); large pore Meso-Porous (LP MeP) silicon, where $S = 20 \pm 4$ nm (b); and Macro-Porous silicon, with $S = 55 \pm 9$ nm (c).