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Design and characterization of surfaces presenting mechanical nanoheterogeneities for a better control of cell–material interactions

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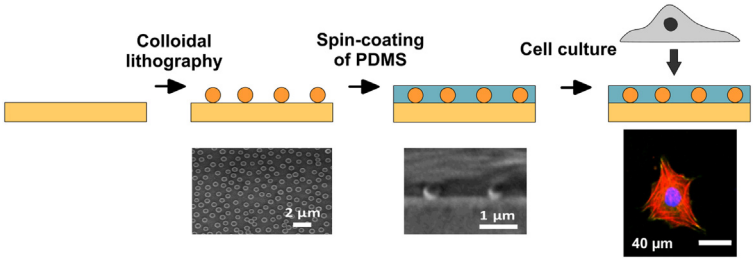
HIGHLIGHTS

- Colloidal lithography was used to create a rigid topographic nanostructure.
- This topography was covered with soft PDMS to create a surface mechanical contrast.
- Obtained surfaces had a limited topography and homogeneous surface chemistry.
- Mechanical contrast was evaluated using atomic force microscopy.
- Mechanical nanoheterogeneities are shown to direct MC3T3 cell response.

GRAPHICAL ABSTRACT



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ABSTRACT

Development of microfabrication technologies allowed creating surfaces with subcellular topographic and chemical features, which led to an improved control of cell reaction in contact with biomaterials. More recently, it was discovered that cells were also sensitive to mechanical properties of their substrate. This study aims at evaluating cell behavior in contact with surfaces presenting mechanical cues at the nanometer scale. However, control of the mechanical properties of such surface remains challenging. The adopted strategy consists in creating rigid topographic features prepared by colloidal lithography of 500 nm silica particles on a glass substrate, and covering it with a soft layer of polydimethylsiloxane (PDMS) by spin-coating. X-ray photoelectron spectroscopy analysis shows that the PDMS layer ensures a homogeneous surface chemistry, while spin-coating parameters can be adjusted to obtain a limited topography. Presence of mechanical contrast is confirmed by force–distance measurements obtained by atomic force microscopy. Finally, morphology and proliferation of MC3T3 preosteoblasts cultured during 3 days on the obtained substrates is shown to be influenced by the presence of mechanical heterogeneities at the subcellular level.

1. Introduction

One of the main objectives in cell engineering is to direct cell behavior *in vitro* toward an organization similar to the one encountered *in vivo*. This is especially important because cell function strongly depends on cell organization and tissue structure. With the improvement of microfabrication technology during the last decades, more and more materials defined at a subcellular scale were tested as biomaterials. On the one hand, the micro- and nanotopography allows controlling to a certain extent multiple cell parameters such as adhesion, morphology, migration and differentiation, which already led to the development of *in vivo* applications [1]. For example, the effect of micrometer-size groove on neuronal cells has been widely studied in order to guide and stimulate axonal

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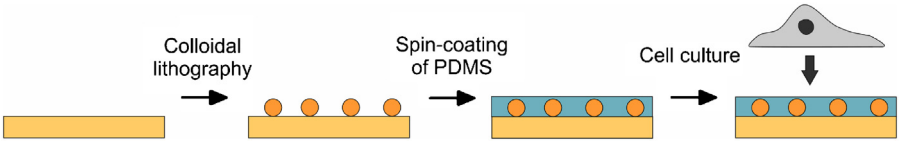

Fig. 1. Strategy used to design surfaces presenting mechanical nanoheterogeneities for the control of cell behavior.

regeneration [2,3]. On the other hand, the development of surfaces presenting subcellular chemical heterogeneities has led to a fine control of, among others, cell adhesion and morphology. Most of the surface chemistry effects are linked to the adsorption of proteins recognized by cells, typically fibronectin or collagen, or to the grafting of cell-binding oligopeptides such as the well-known RGD sequence. In this way, cell shape control has been shown to strongly influence the division axis of epithelial cells [4] which could have implications on tissue engineering strategies. At a nanoscale level, modification of the spacing of RGD cues from 58 nm to 73 nm dramatically changed cell adhesion and morphology and helped understanding cell adhesion processes [5].

More recently, different cell lines were shown to be influenced by mechanical properties of the substrate. Many different cell types such as fibroblasts [6–8], muscular cells [9,10] or endothelial cells [11] were shown to develop a larger projected area on stiffer substrates compared to softer ones. This increase in cell spreading with stiffness was observed on materials with Young moduli ranging from 1 kPa to 1 MPa. It is worth noting that this range varies in function of the cell type and the surface chemistry [12]. Interestingly, in several cases, cells cultured on a substrate with a given stiffness had a behavior similar to the one observed *in vivo* in tissues having the same stiffness [13,14]. For example, mesenchymal stem cells cultured on substrates with different stiffnesses corresponding to the ones of nervous, muscular and bone tissues showed a morphology and expressed differentiation markers typical of cells of the corresponding tissue [14].

Cell reactions on substrates presenting mechanical heterogeneities at the supracellular scale were studied as well. Higher cell spreading and cell density were observed on the stiffer areas in agreement with observations made on homogeneous substrate of varying stiffness. Several cell lines including fibroblasts [15–18], myoblasts [16] and epithelial cells [15,17] were shown to move toward stiffer areas, a phenomenon known as durotaxis [19]. It was shown for fibroblasts that a stiffness gradient of 30–40 kPa over a length of 50 μm is needed to direct cell motility [18]. Nevertheless, size of stiffer areas was always larger than cell size or at least similar to it. Moreover, most of the time, stiffness differences were depending on the presence of a cross-linking agent in different concentrations, and as a consequence, surface chemistry was also varied. In many studies, it is thus difficult to attribute the observed effects solely to the heterogeneity in terms of mechanical properties.

The aim of this study is to analyze cell behavior on substrates presenting mechanical heterogeneities at a subcellular scale keeping a strictly constant chemistry along the sample surface and limiting the presence of topographic features as much as possible. The strategy used is depicted in Fig. 1. First, a topography is created by colloidal lithography using silica particles with a diameter of 500 nm. Then, these rigid colloids are covered with a soft layer of polydimethylsiloxane (PDMS) using spin-coating. The rigid colloids situated underneath the elastomer layer bring localized mechanical contrast compared to the soft PDMS matrix. The stiffness of the PDMS layer may be tuned using different cross-linker agent concentrations.

Surface chemistry, topography and mechanical properties are characterized using X-ray photoelectron spectroscopy (XPS), scanning electron microscopy (SEM) and atomic force microscopy (AFM). Finally, MC3T3 preosteoblasts are grown on the obtained substrates as well as on control samples devoid of colloids, and their response to mechanical nanoheterogeneities is investigated.

2. Materials and methods

2.1. Materials

Glass substrates were squares of ~1.2 cm-side cut from microscope slides (Marienfield, Germany). Polyallylamine hydrochloride (PAH, M_w ~56,000 g/mol), sodium hydroxide and bovine serum albumin (BSA; essentially fatty acid free) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Silica colloids (9.83 wt.%, nominal diameter 500 nm) were purchased from Polysciences Inc. (Warrington, PA, USA). Hexane (99%) and absolute ethanol were purchased from VWR international (Leuven, Belgium). Polydimethylsiloxane (PDMS) Sylgard184 silicone elastomer kit was purchased from Dow Corning (Barry, UK). Water was ultrapure water (18.2 MΩ/cm) provided by Elga Purelab Ultra device (Veolia, UK).

2.2. Colloidal lithography

The silica colloid deposition protocol was described previously [20]. Briefly, glass substrates were cleaned in piranha solution (H_2SO_4 cc:H_2O_2 (30%); 7:3), rinsed thoroughly, and immersed in vertical position for 30 min in a PAH 10^{-5} M solution at pH 11 (adjusted with sodium hydroxide). The samples were then rinsed 6 times with water, gently dried under nitrogen, immersed in vertical position in a 0.1 wt.% suspension in water of 500 nm silica colloids for 2 h, rinsed six times with water, immersed in an albumin solution (30 μg/mL) for 30 min, rinsed three times with water, three times with isopropanol and dried in air overnight. Note that the albumin layer helps preventing colloid aggregation. All samples were examined by SEM (Digital Scanning microscope 982 Gemini, Leo Electron Microscopy, UK) without adding any metal coating, in order to confirm a proper colloidal distribution before being further used.

2.3. PDMS samples and thin films

Sylgard184 PDMS includes two components: the base and the curing agent, which must be mixed and cured at 95 °C for 1 h in order to form a three-dimensional PDMS matrix. Bulk PDMS stiffness for different base:curing agent ratios was determined macroscopically using a method described by Pelham and Wang [15]. Briefly, PDMS strips of about 15 mm × 80 mm × 4 mm were elongated with different loads ($F_\perp$) and Young's modulus E was extracted from the linear part of the stress–strain curve, using the relation: $E = (F_\perp/A)/(\Delta L/L)$ were A is the cross-section area, L is the initial length and ΔL is the change in length under load.

Thin PDMS films were produced on glass substrates covered or not with colloids. This was achieved by spin-coating (speed = 3000 rpm, acceleration = 16,000 rpm/s, time = 60 s) a 70 μL drop of base and curing agent diluted in hexane. For each sample, the base:hexane:curing agent weight ratio is specified (for example, 1:10:0.1). The PDMS films were then cured for 1 h at 95 °C.

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