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Cyto-mechanoresponsive Polyelectrolyte Multilayer Films

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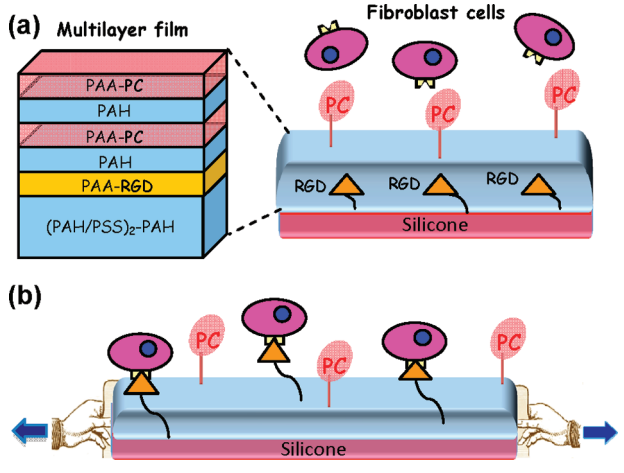
Supporting Information

ABSTRACT: Cell adhesion processes take place through mechanotransduction mechanisms where stretching of proteins results in biological responses. In this work, we present the first cyto-mechanoresponsive surface that mimics such behavior by becoming cell-adhesive through exhibition of arginine−glycine−aspartic acid (RGD) adhesion peptides under stretching. This mechanoresponsive surface is based on polyelectrolyte multilayer films built on a silicone sheet and where RGD-grafted polyelectrolytes are embedded under antifouling phosphorylcholine-grafted polyelectrolytes. The stretching of this film induces an increase in fibroblast cell viability and adhesion.

The last years have seen the emergence of new domains in materials and surface science. The area of external-stimuli-responsive systems is surely among the most active. Almost any conceivable stimulus has been explored, including pH, ionic strength, electrical potential, light, and magnetic field to cite only a few.[1] Among all of the applied stimuli, mechanical stretching has certainly been less investigated even though it is one of the most widely used in nature. Cell adhesion, tissue growth, and response of organisms to their environment all rely on their response toward externally applied mechanical forces.[2] These forces are then transduced into chemical responses through mechanotransductive processes.[3] Materials or surfaces responding to a mechanical stress are called mechanoresponsive systems. Most of the mechanoresponsive materials developed to date have been designed to change color upon stretching.[4,5] Our group recently introduced the first example of films that respond enzymatically to stretching by using polyelectrolyte multilayers.[6] Two types of polyelectrolyte multilayers, obtained by the alternate deposition of polyanions and polycations, were built on silicone sheets to design such a mechanoresponsive film. A first exponentially growing film was loaded with enzymes that keep their enzymatic activity and then capped by a linearly growing film acting as a barrier for enzyme substrates. Stretching this architecture exposed the enzymes to the solution containing its substrate, allowing the reaction to take place.

Here we present the first cyto-mechanoresponsive surface that responds to stretching by inducing cell adhesion through specific interactions. We used the versatility of polyelectrolyte multilayers to design the most appropriate film architectures.[7,8] Polyelectrolyte multilayers can be deposited on almost any kind of substrate and in particular on bare silicone.[9,10] Our idea was to embed poly(acrylic acid) (PAA)-bearing arginine−glycine−aspartic acid (RGD) adhesion peptides (PAA-RGD, grafting ratio 5%) under a multilayer resistant to cell adhesion and then to expose the RGD peptides by stretching the film (Scheme 1).

Scheme 1. Schematic Representation of the Strategy Used to Design a Cyto-mechanoresponsive Multilayer Film: (a) At Rest, RGD Peptides Are Buried inside the Film; (b) Under Stretching RGD Peptides Are Accessible to Cells



In a previous study, we showed that silicone sheets can be rendered non-cell-adherent at rest as under stretching by

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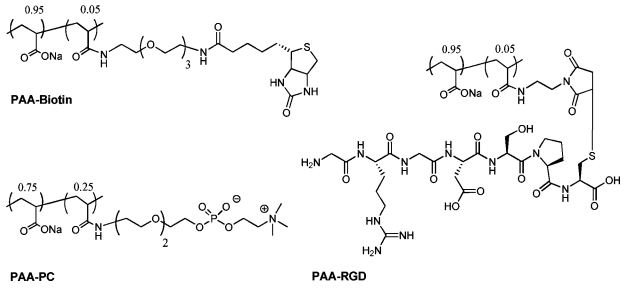
depositing a PAA-PC/PAH/PAA-PC multilayer on a precursor $(PAH/PSS)_2/PAH$ film[10,11] [PAH, poly(allylamine hydrochloride); PSS, poly(styrene sulfonate); PAA-PC, PAA modified with phosphorylcholine (PC) moieties, grafting ratio 25%]. PC groups cover the outer membrane of cells and are known to render surfaces antifouling when grafted at high enough densities on surfaces.[12] Chemical structures of modified PAAs are drawn in Scheme 2. In this study, a $(PAH/PSS)_2/PAH$ precursor film was built on silicone sheets before adsorption of PAA-RGD and capping of the architecture with several PAH/PAA-PC bilayers. Polymer synthesis and all experimental details are given in sections 1, 2, and 3 in the Supporting Information (SI). The stretching ratio (α) is defined as the ratio of the lengths of substrate after and before stretching.

Scheme 2. Molecular Structures of the Functionalized Polymers PAA-Biotin, PAA-PC, and PAA−RGD



We first followed the buildup of a poly(ethylene imine)/$(PSS/PAH)_3$/PAA-RGD/$(PAH/PAA-PC)_6$ multilayer using a quartz crystal microbalance (QCM) (Figure S1 in the SI). A regular mass increase was observed up to the third (PAH/PAA-PC) bilayer.

The viability and cellular adhesion of primary gingival fibroblasts were then quantified by AlamarBlue assay and immunolabeling for different multilayer architectures deposited on silicone substrates. We first compared, with respect to cell adhesion, the following multilayer films in the nonstretched and stretched states at α =1.5: $(PAH/PSS)_2/PAH$, $(PAH/PSS)_2/PAH/PAA\text{-}RGD$, and $(PAH/PSS)_2/(PAH/PAA\text{-}PC)_2$ (Figures S2 and S3). Cells were always deposited after stretching of the film. RGD-functionalized films[13] and PAH/PSS multilayers ending with PAH[14] are known for their strong cell adhesion and proliferation tendencies. As expected, strong adhesion and proliferation were observed on multilayers ending with PAA-RGD or PAH and very weak ones on films ending with two PAH/PAA-PC bilayers. Stretching PAA-PC- and PAA-RGD-ended films at α = 1.5 did not modify the cell adhesion and proliferation properties (Figure 1). Next, we determined the minimum number of (PAH/PAA-PC) bilayers required to cover the PAA-RGD layer to obtain a non-cell-adherent film in the nonstretched state. While with one bilayer the cell adhesion and proliferation were only marginally modified, they were strongly reduced with two or more (PAH/PAA-PC) bilayers covering the PAA-RGD (Figure S4).

We then investigated the stretching effect on $(PAH/PSS)_2/PAH/PAA\text{-}RGD/(PAH/PAA\text{-}PC)_n$ films (denoted as RGD-PCn) with n up to 6 (Figure S5). It appears that under stretching (α = 1.5), cell adhesion takes place for n = 2. On the contrary, RGD-PCn films remain antifouling under stretching for larger values of n. The RGD-PC2 film appears to be the optimal configuration to hinder cell adhesion at rest and allow cell adhesion under stretching. Thereafter, the experiments were performed with n = 2.

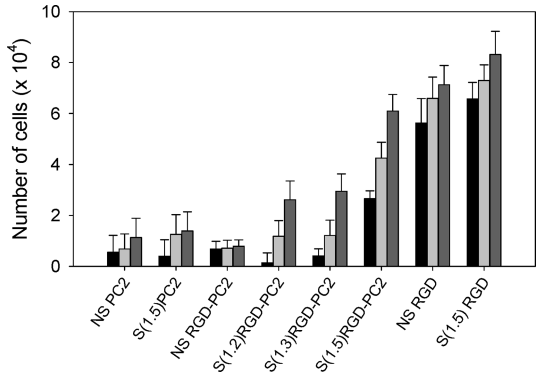


Figure 1. Viability of fibroblast cells as determined by the Alamar Blue test after 1 day (black bars), 2 days (gray bars), and 7 days (dark-gray bars) of culture on different multilayer films built on a silicone basin: a $(PAH/PSS)_2/(PAH/PAA\text{-}PC)_2$ film named PC2, a $(PAH/PSS)_2/PAH/PAA\text{-}RGD/(PAH/PAA\text{-}PC)_2$ film named RGD-PC2, and a $(PAH/PSS)_2/PAH/PAA\text{-}RGD$ film named RGD in either the nonstretched state (NS) or the stretched state at a particular α ratio [S(α)]. The reported numbers of cells correspond to means ± standard deviations from three experiments.

It is known that an increase in RGD density on a surface induces better cell adhesion and spreading.[15] We thus determined the number of adherent cells on RGD-PC2 films stretched at α = 1.2, 1.3, and 1.5 after 1, 2, and 7 days of cell culture (Figure 1). At α = 1.2 and 1.3, the increase in the number of adherent cells was minor and took place only after 7 days of culture. For a film stretched at α = 1.5, the cell viability increased significantly whatever the number of days of culture. In the absence of the PAA-RGD layer [i.e., for the $(PAH/PSS)_2/(PAH/PAA\text{-}PC)_2$ film], no increase of the cell number was observed for a film stretched at α = 1.5 relative to the nonstretched one (Figure 1). This clearly indicates that the increase in the number of adherent cells at α = 1.5 was not due to nonspecific interactions but must be attributed to the RGD groups that become accessible to the cells under stretching. Cell adhesion remained smaller for the stretched $(PAH/PSS)_2/PAH/PAA\text{-}RGD/(PAH/PAA\text{-}PC)_2$ film relative to the $(PAH/PSS)_2/PAH/PAA\text{-}RGD$ film (Figure S1), indicating that even for α = 1.5 all of the RGD peptides are not exposed.

Immunostaining allowed checking of the fibroblast morphology. When cells were cultured for 1 week on a nonstretched RGD-PC2 film, only a few cells remained adherent (Figure 2a).

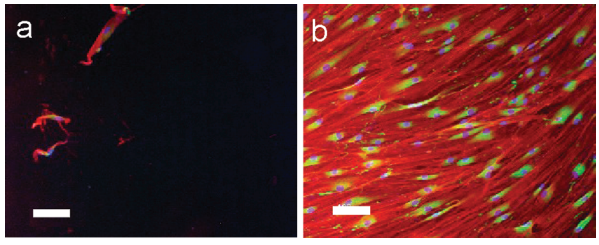


Figure 2. Typical fluorescence microscopy images of fibroblast cells after 1 week of culture on RGD-PC2 built on a silicone basin in (a) the nonstretched (NS) state and (b) a stretched state with α = 1.5 ratio [denoted S(1.5)]. Immunochemical detection of actin fibers of the cytoskeleton by phalloidin (red labeling), collagen type I compounds of the extracellular matrix by anticollagen I (green labeling), and nuclei by DAPI (blue labeling). Scale bars correspond to 100 μm.

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However, when cells were cultured on the same type of film, RGD-PC2, in a stretched state at α = 1.5, a dramatic proliferation of adherent cells was observed. Cytoskeleton staining showed the presence of actin fibers lying parallel to each other (Figure 2b), a signature of cell spreading and organization. Collagen type I synthesis by cultivated fibroblast cells were investigated as well. Adherent cells revealed the production of collagen type I, the early component of the extracellular matrix produced by fibroblasts, a signature of their good secretory activity.

Next, we verified by atomic force microscopy that the films remained homogeneous over the whole surface under stretching up to α = 1.5. No cracks appeared on the surface, indicating that the silicone substrates remained totally covered by the multilayers under stretching (Figure S6).

To confirm that film stretching renders the embedded RGD sites accessible to cells, RGD peptides were replaced by biotin groups to study their accessibility to fluorescein isothiocyanate (FITC)-labeled streptavidin present in solution. We used biotin because it binds to streptavidin, a 53 kDa protein, through a noncovalent bond known to be the strongest measured in biological systems,[16] making it easier to quantify. In the nonstretched state, the $(PAH/PSS)_2/PAH/PAA\text{-}Biotin/(PAH/PAA\text{-}PC)_2$ film was nonadsorbent to streptavidin, as no fluorescence was observed on the surface after contact with the streptavidin solution followed by rinsing (Figure 3, inset labeled α = 1.0).

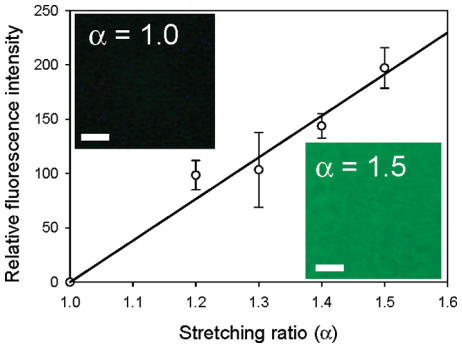

Figure 3. Fluorescence intensity measured from FITC-streptavidin adsorbed on a $(PAH/PSS)_2/PAH/PAA\text{-}Biotin/(PAH/PAA\text{-}PC)_2$ multilayer film built on a silicone sheet at different stretching ratios α. The relative fluorescence intensity was measured using Image J software. The data correspond to means ± standard deviations from three experiments. A strong correlation between the amount of FITC-streptavidin adsorbed and α (R = 0.980) was obtained. The insets represent typical images of the film brought in contact with FITC-streptavidin in the nonstretched (α = 1.0) and stretched (α = 1.5) states. The scale bars represent 20 μm.

Increasing α from 1.0 to 1.5 led to a roughly linear increase in the fluorescence with α when the film was in contact with FITC-streptavidin (Figure 3 and its inset labeled α = 1.5). This indicates that an increasing number of biotin groups became available on the top of the film. In a control experiment with no PAA-Biotin embedded [i.e., a $(PAH/PSS)_2/PAH/PAA/(PAH/PAA\text{-}PC)_2$ film], no fluorescence was observed after contact with FITC-streptavidin in either the stretched or nonstretched state (data not shown). The fluorescence was thus due to specific interactions between biotin and streptavidin.

These results can be interpreted as follows: in the nonstretched state, the ligands (RGD or biotin) embedded under two or more PAH/PAA-PC bilayers are not accessible to their receptors. When the film is stretched, resulting in a thinning of the capping layer, an increasing number of ligands become accessible as α increases. However, a critical RGD density must be reached for fibroblasts to adhere correctly. This critical density seems to be obtained at α = 1.5 (Figure 1). When the ligands are embedded under four or more PAH/PAA-PC bilayers, exposure becomes impossible even at α = 1.5 and cells thus hardly adhere (Figure S5).

Finally, we also addressed the reversibility of the system with respect to stretching. We stretched a $(PAH/PSS)_2/PAH/PAA\text{-}RGD/(PAH/PAA\text{-}PC)_2$ film at α = 1.5 for 30 min before returning it to the nonstretched state during 1 h. We then seeded the surface with cells in this nonstretched state (Figure S7). Only partial recovery of the nonadherent effect was observed. This can be explained by film restructuring consecutive to the stretching/relaxation steps. Thus, only a partial remasking of the RGD peptides takes place, leading to a slight decrease in cell adhesion.

To summarize, we have presented here a new type of mechanotransductive film that becomes cell-adherent under stretching by rendering RGD ligands accessible to cells. This mechanotransductive film was obtained using multilayer technology with polyelectrolytes bearing RGD ligands embedded under nonadhesive polyelectrolytes bearing phosphorylcholine. The effect is partially reversible upon stretching/relaxation. Up to now, only a few synthetic mechanotransductive materials and films have been reported, most leading to a change in color when stretched. This change of color can be due to a chemical reaction that takes place with the stretched molecules.[5] None of these processes induced by stretching are reversible, even partially. For films becoming enzymatically active under stretching, the process is reversible to some extent. Our next goal is to design fully reversible cyto-mechanoresponsive materials that induce changes in behavior of already adherent cells. Through gradual changes in the density of adhesion ligands, the fate of adherent cells could be modified simply by stretching, allowing one to envision applications in tissue engineering.

■ ASSOCIATED CONTENT

Supporting Information

Polymer modification; multilayer buildup and characterization; cellular viability and adhesion method; buildup of $(PAH/PSS)_2/PAH/PAA\text{-}RGD/(PAH/PAA\text{-}PC)_6$ films monitored by QCM; polydimethylsiloxane characterization; and fibroblast viability and adhesion on different stretched and nonstretched multilayers. This material is available free of charge via the Internet at http://pubs.acs.org.

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