Surface Modification using Elastin-Like Polypeptide and Polyethyleneimine for Three-Dimensional Cell Cultures

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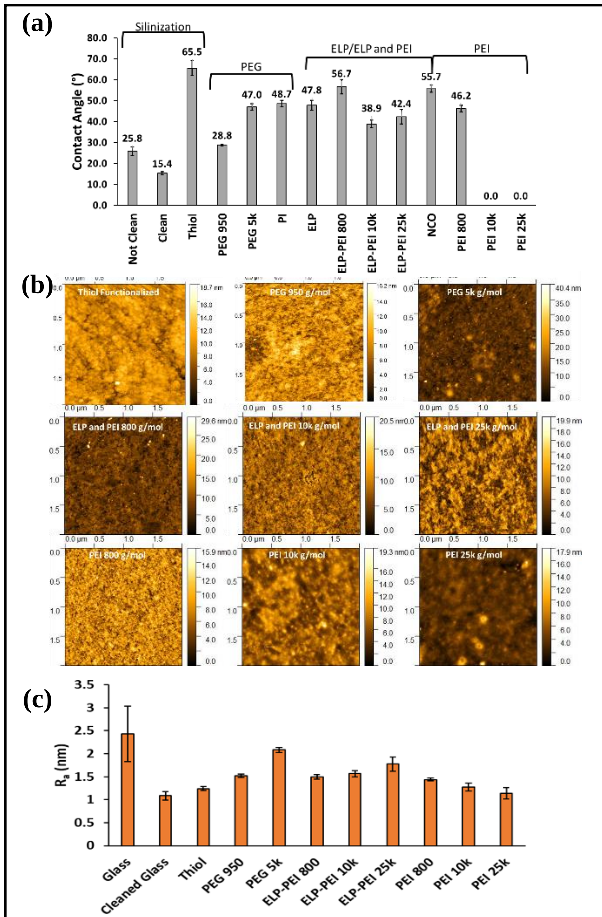

Figure 1. (a) Contact angles, (b) AFM images, and (b) surface roughness of PEG, ELP. PEI-modified surfaces.

Statement of Purpose: Elastin-like polypeptide-poly ethyleneimine (ELP-PEI) copolymer coatings have been shown to form three-dimensional (3D) spheroids of multiple cell types *in vitro*.[1,2] This arrangement more closely mimics the cells' natural *in vivo* orientation than traditional 2D monolayer cell culture. This 3D spheroid model is currently limited by the non-uniformity in both ELP-PEI coating thickness and the distribution of the PEI on the surface, which can lead to an erratic scattering of spheroids across the coated surface. This work uses an ultraviolet (UV) induced, "grafting-to" approach to graft polyethylene glycol (PEG) to prevent general cell attachment and controllably influence the cell attachment by backfilling the surface with ELP and PEI using isocyanate chemistry.

Methods: *Surface Preparation/ PEG Functionalization:* Silanization of 1 cm^2 borosilicate glass microscope slides was performed similarly to a vapor deposition method outlined by Khire et al.[3] The samples were placed in a solution of PEG methylether acrylate (950 or 5,000 g/mol, Sigma Aldrich) and photo-initiator dissolved in CH_2Cl_2 and exposed to UV light (14 mW/cm^2) for 8 min.

ELP/PEI Surface Attachment: The thiol-functionalized slides were exposed to an excess of Hexamethylene Diisocyanate (NCO) dissolved in anhydrous DMF and held in an N_2 atmosphere for 4 hours. The samples were washed with DMF and placed into a 10 mg/mL solution of ELP (Valine-Proline-Glycine-Valine-Glycine)$_{40}$ (17,000 g/mol) and/or PEI (800, 10,000, 25,000 g/mol) dissolved in DMF for 12 hours.

Contact-Angle Goniometry: A Rame-Hart Goniometer was used to probe the physical properties of the different surfaces using a 5-µL drop of ultra-pure water.

Atomic Force Microscopy (AFM): A Bruker Bioscope AFM was used to capture images of the modified glass surfaces to determine the morphology of the surface and average roughness (R_a). Gwyddion software version 2.64 as used for post-processing of the images.

Results: Water contact angle demonstrated the successful silanization of the glass wafer. Before silanization, the contact angle of the cleaned glass was 15.4 ± 0.7, upon successful vapor deposition of the silane the contact angle increased to 69.5 ± 3.5 (Fig. 1a). AFM showed a small increase in the mean surface roughness from the cleaned glass (1.12 ± 0.09 nm) to 1.19 ± 0.05 nm for the silanized surface (Fig. 1c). This non-significant roughness increase indicates that the silane was deposited onto the surface with minimal multilayering. Upon reacting with the PEG, the water contact angle decreased, the roughness increased, and the surface morphology changed from a pronounced grain structure to that of less visible grains (Fig. 1). The less drastic decrease in water contact angle for the 5,000 g/mol PEG (Fig. 1a) may be due to a less dense packing of the brushes on the surface, leading to exposure of the more hydrophobic alkyl silane. The mercapto-silane wafers were reacted to a diisocyanate to create an NCO functionality on the surface by which to react the terminal amine group of the ELP and PEI molecules. The NCO functionalized surfaces showed a water contact angle of 55.7 ± 1.7 which decreased upon its reaction to ELP to 47.8 ± 2.3. When PEI was incorporated into the reaction along with ELP, the surface roughness remained unchanged (Fig. 1c) and the water contact angle decreased only slightly to 42.4 ± 3.4 (Fig. 1a), indicating that the lower molecular weight PEI was unable to hide the alkyl chains on the surface. This hypothesis is supported when only PEI is reacted onto the surface, the contact angle for the PEI800 remained high at 46.2 ± 1.6 compared to the larger molecular weight PEIs that allowed complete wetting (Fig, 1a).

Conclusions: Although some optimization is needed to increase the surface density of the polymers; ELP, PEI, and ELP-PEI were successfully grafted giving a new surface modification method for cell cultures studies.

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References:
1. Weeks et al., *ACS Biomat Sci & Eng* 2016;2:2196.
2. Turner et al., *J Biomed Mater Res* 2014;102A:85.
3. Khire et al, *Macromolecules* 2007,40, 5669-5677.