**Decreased lung carcinoma cell density on select polymer nanometer surface features for lung replacement therapies**

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**Statement of Purpose:** The interaction between cells and extracellular matrix (ECM) proteins is complicated, drawing much attention for biomaterial interfacial research since understanding this interaction will significantly contribute to the design of better implants. Different implant material properties, including surface chemistry, surface texture and surface energy have been investigated to date to create better implantable materials. Specifically, recent research has demonstrated that implant nanometer morphology had greatly influenced cell attachment, proliferation and differentiation. Various different micro- and nanostructured patterns on substrate surface have been created to investigate the cell responses. However, the underlying mechanism of cell response to micro- and nanometer patterns is still poorly understood. In this study, uniform nano-spherical surfaces were created on poly-lactic-co-glycolic acid (PLGA) films by using polystyrene (PS) beads self-assembled with different diameters to evaluate lung carcinoma epithelial cell responses on such surfaces. Lung cancer cells were chosen since they are responsible for about 1.3 million deaths worldwide annually, making lung cancer the leading cause of cancer death. Cell attachment and short-term cell density assays were examined on the novel nano-rough PLGA surfaces.

**Methods:** To create nanometer surface features on PLGA, different size (specifically, 190 nm, 300 nm, 400 nm, and 530 nm diameter) polystyrene beads were placed onto glass coverslips to create monolayers of highly ordered arrays. Then, poly(dimethylsiloxane) (PDMS) molds were prepared by placing PDMS onto these various polystyrene monolayers. The resulting PDMS molds were used as templates to cast PLGA films with various nanometer surface features. Finally, soluble PLGA was poured over the PDMS molds which were then allowed to evaporate at room temperature. As a result, the surface of the resulting PLGA films replicated the initial polystyrene nanobead topographical features. A solution evaporation method was also used to control PLGA surface roughness by using PLGA concentrations of 0.5 g PLGA : 4 mL chloroform and 0.5 g PLGA : 8 mL chloroform, separately. Atomic force microscopy (AFM) images, electron spectroscopy for chemical analysis (ESCA) and contact angles were used to characterize the surfaces. Lung carcinoma cells (A549; ATCC) were seeded at a density of either 3,500 cells/cm$^2$ or 7,000 cells/cm$^2$ and cultured in F-12K medium with 10% fetal bovine serum (FBS) at 5% $CO_2$ and 37$^o$C for 4hrs. After 4hrs, cells were stained and counted under a fluorescence microscope. Also, lung carcinoma cells were seeded at a density of 50,000 cells/cm$^2$ and cultured in F-12K medium with 10% fetal bovine serum (FBS) at 5% $CO_2$ and 37$^o$C for 3 days. After 3 days, cells were typsinized and counted. All experiments were run in duplicate and repeated at least three separate times. Results were analyzed for statistical significance using Student T-tests.

**Results:** AFM images and root mean square roughness (RMS) values provided evidence that the intended spherical surface nano-topographies on PLGA with RMS values of 2.23, 5.03, 5.42 and 36.90 nm were formed for the PLGA surfaces which employed 190, 300, 400, and 530 nm beads. The solution evaporation method was also used to control PLGA surfaces with RMS values of 0.62 and 2.23 nm by using PLGA concentrations of 0.5 g PLGA : 4 mL chloroform and 0.5 g PLGA : 8 mL chloroform, respectively. ESCA results showed the same chemistry between all PLGA surfaces created by the two different methods (that is, using different concentrations of PLGA in chloroform or using PS beads). Cell adhesion results showed the least number of lung carcinoma epithelial cells adherent to the PLGA surfaces with RMS values of 0.62, 2.23 and 5.42 nm compared to nano-smooth (currently-used) PLGA (Figure 1). Three day cell proliferation results on the different nano-rough PLGA surfaces revealed similar trends as the adhesion results. Specifically, PLGA surfaces with an RMS value of 0.62 nm had much lower cell density. Interestingly, the 0.62 nm RMS PLGA was one of the most hydrophobic PLGA surfaces created in this study which may have influenced initial protein adsorption events to decrease cell density.

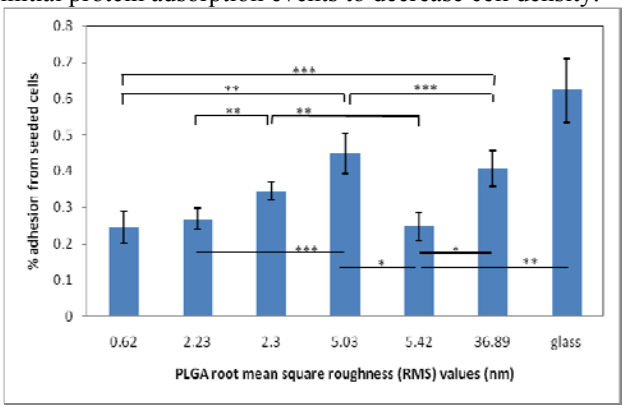


Figure 1. Lung cancer epithelial cell adhesion results on the various PLGA samples and borosilicate glass. Data expressed as mean ± standard deviation of the mean. *p < 0.01, **p < 0.05 and ***p < 0.1.

**Conclusions:** In this research, a simple method was used to create nanorough PLGA surface features with highly ordered nanospherical structures. The results from cell adhesion and cell density assays after three days showed that the PLGA surfaces with RMS values of 0.62 and 5.42 nm can inhibit cell density. In this manner, PLGA with nanometer surface features may inhibit lung cancer cell density which may provide for an important biomaterial for the treatment of lung cancer for a wide range of applications (from drug delivery to regenerative medicine).