Decreased VEGF synthesis by lung carcinoma cells on polymer nanometer surface features

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Statement of Purpose: Poly (lactic-co-glycolic acid) (PLGA) has been widely investigated as a biomaterial from drug delivery to regenerative medicine due to its desirable biocompatibility and biodegradability properties. In addition, many studies have shown that cells (such as bladder smooth muscle cells, chondrocytes and osteoblasts) respond differently to nano-structured PLGA surfaces compared to nano-smooth surfaces. As early as 1962, Rosenberg claimed that nanometer sized features may influence cell functions [1]. Clearly, there is now a plethora of studies to show that nanoroughness can regulate cell functions (including adhesion, proliferation and differentiation), probably due to the correlation between the surface topography and the adsorption of endogenous proteins that regulate cell behavior. However, despite the recognition of the importance of substratum nanotopography on promoting tissue growth, relatively little is known concerning the effects of nanometer topographical features on cancer cell behaviors. Thus, in this study, lung epithelial carcinoma cell functions (including adhesion, proliferation, apoptosis and vascular endothelial growth factor (VEGF) synthesis) on PLGA films with various nanotopographies (but of similar chemistry) were systematically investigated. It was an important objective of this study to determine what size of nanofeatured polymer surface decreases cancer cell responses.

Methods: To create nano features on PLGA surfaces, different size polystyrene (PS) beads (23 nm, 300 nm and 400 nm) were used to cast poly (dimethylsiloxane) (PDMS) molds. The molds were further used as templates to create nano-featured PLGA films. A solution evaporation method was also used to create nano-smooth surfaces as controls. All PLGA films were characterized by atomic force microscopy (AFM) and electron spectroscopy for chemical analysis (ESCA).

Lung carcinoma cells (A549; ATCC) were seeded at a density of either 3,500 cell/cm^2 or 1,000 cell/cm^2 and cultured in F-12K medium with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin (P/S) at 5% CO_2 and 37^{o}C. After 4 hrs, 1 day, 3 days, and 5 days, cells were stained and counted under a fluorescence microscope. Lung carcinoma cells on PLGA films after culturing for 12 hrs at a seeding density of 5,000 cell/cm^2 were stained with fluorescent phalloidin conjugates (Alexa Fluor® 555 phalloidin, Invitrogen) and cell morphology observed by fluorescence microscope. Approximately 5 × 10^5 lung carcinoma cells were seeded on PLGA films in 24 well plates with 1 ml media and cultured in a similar manner to that described above. After 1, 3 and 5 days, cell apoptosis and VEGF synthesis were evaluated according to standard protocols.

Results: AFM images demonstrated different nanometer roughness, which provided evidence that the intended spherical surface topography on the PLGA surfaces were created using the method described above. The ESCA spectra of nano-smooth PLGA (by solvent evaporation) and nano surface featured PLGA (by PS beads) showed the same chemistry. Thus, the only difference between all the PLGA films was the surface topography. Results showed that after one and three days, the 400 nm surface featured PLGA had less cancer cell density among all the substrates, while the 300 nm surface featured PLGA showed the highest cell density. As for cell morphology, the morphology of phalloidin-stained F-actin networks in cells on the 300 nm surface featured PLGA exhibited more and thicker contractile filaments and more cell-cell connections than on any other sample. For three and five day cell apoptosis results, the 23 nm surface featured PLGA showed significantly higher apoptotic cell percentage compared to any other PLGA. Consistent with this result, cancer cells on the 23 nm surface featured PLGA secreted significantly less VEGF than on any other sample, suggesting decreased lung carcinoma cell function on such nano-featured PLGA substrates (Fig. 1).

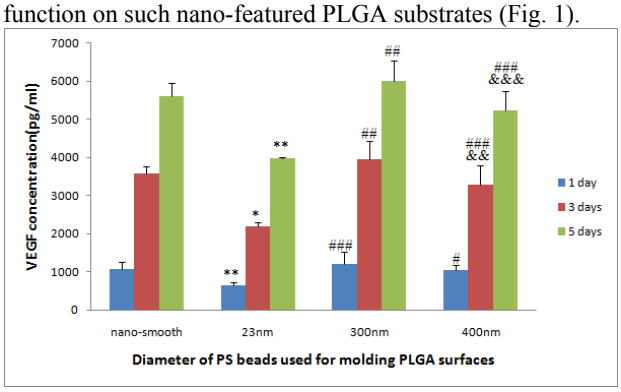

Fig. 1. VEGF secreted by lung carcinoma cells cultured on PLGA films after one, three, and five days. Data = mean ± SD, N=3. *$^{(\#,\ \&)}$p < 0.01, **$^{(\#\#,\ \&\&)}$p < 0.05, and ***$^{(\#\#\#,\ \&\&\&)}$p < 0.1. *: compared to nano-smooth PLGA at the same time; #: compared to 23 nm PLGA at the same time; &: compared to 300 nm PLGA at the same time.

Conclusions: A simple and effective method was used here to create various PLGA nanotopographies with the same surface chemistry. The effect of nanotopography on lung epithelial carcinoma cell functions for up to 5 days was systematically investigated here. Results indicated that the cells adhered and spread less on the nano-smooth and 400 nm surface featured PLGA, while the 23 nm surface featured PLGA increased apoptotic cell percentages and significantly decreased VEGF synthesis. The responses of lung carcinoma cells on nanotopographies indicated that cancer cell functions (like adhesion, proliferation, apoptosis and VEGF synthesis) can be inhibited by different nanotopographies. Thus, these results provided important information for the use of nanotopographies in numerous anti-cancer implants.

References: [1] Rosenberg MD. P Natl Acad Sci USA. 1962;48:1342-1349.

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