Internal Stress in Biomimetic Coatings due to Cell-Material Interactions

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Statement of Purpose: In the emerging area of biomimetic surfaces, exploring the cell-material interface is of great interest and many studies have revealed that the chemical and physical properties of biomimetic surface features have a great impact on cell responses. Appropriate design and fabrication of biomimetic surfaces can promote cell functions and tissue regeneration, even resembling the performance of native tissues. However, the roles of the biomimetic surface features in modulating cell or tissue responses and how the material and topological implications are recognized by the biological systems have not been fundamentally understood to date. The purpose of the present work was to establish a new approach to answer the above questions by investigating the internal stress generated in the biomimetic coating materials during the cell-material interactions. It hopes that characterizing the stress evolution due to cell activity on biomimetic surfaces would provide important information to understand the role the biomimetic surfaces play in mediating cell functions.

Methods: 50 nm nanostructured titanium films (N-Ti) that mimic the nanoscale hierarchical features of many biological systems (e.g., bone, blood vessel, etc.) were deposited on double-polished quartz (250 μm in thickness) by e-beam evaporation. Two types of cancer cells, lung epithelial carcinoma cell (LECC) and breast epithelial adenocarcinoma cell (BEAC), were seeded on the N-Ti and cultured ATCC-formulated F-12K medium and Dulbecco's Modified Eagle's Medium (DMEM), respectively, with 10% fetal bovine serum (FBS, Hyclone) and 1% penicillin/streptomycin (P/S, Hyclone). The samples were then incubated in a sterile, humidified environment of 95% air, 5% CO_2, and 37 ^oC. After 24 and 72 hrs, the N-Ti samples attached with cells were removed from the incubator for stress measurement.

Stress in the N-Ti films was measured with a multi-beam optical stress sensor (MOSS), showing in Figure 1. The general application of this technique to measure internal stresses in thin films is described elsewhere [1]. Briefly, the internal stress generated in a thin film produces curvature changes (Δκ) in the thin film-substrate system (in this case, N-Ti was the thin film and quartz was the substrate). The curvature measurements can be related to the average stress across the thickness of film ($<\sigma_f>$) with Stoney's equation:

$$\langle \sigma_f \rangle h_f = \int_0^{h_f} \sigma_f \, dh = \frac{M_s h_s^2}{6} \Delta\kappa$$

where M_s is the biaxial modulus of the substrate (87.95 GPa for quartz), h_f and h_s are the thickness of the thin film (50 nm) and quartz substrate (250 μm), respectively. By convention, negative curvature values indicate compressive average stress while positive curvature values indicate tensile stress. For each cell type and time point, the measurement was repeated on five samples.

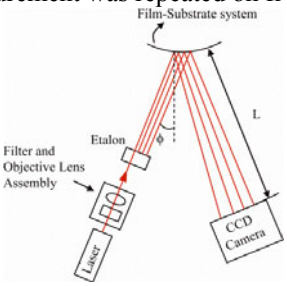

Figure 1. Schematic depicting the multi-beam optical stress sensor (MOSS).

After stress measurement, cell morphology on N-Ti films was observed by fluorescence microscopy. The adherent cells on the sample were washed with phosphate buffer solution (PBS) and fixed with 4% formaldehyde for 10 mins. After fixation, the cells were stained with rhodamine phalloidin conjugates (Alexa Fluor® 555 phalloidin, Invitrogen) following the instructions provided by the manufacturer. The cell nuclei were further stained with DAPI.

Results: The measurements of the curvature change in N-Ti indicated that there were large compressive stresses generated in the BEAC attached titanium films after 24 and 72 hrs, and the increase in the magnitudes of the compressive stress was observed between 24 and 72 hrs. In contrast, only small stresses were seen in the LECC attached titanium films and the stress levels between 24 and 72 hrs remained similar. These results indicated that the biomimetic N-Ti films underwent large compression when BEAC adhered to its surface while LECC exerted almost no mechanical deformation to N-Ti.

The fluorescence microscopy examination of cell morphology also gave cell type-dependent results on N-Ti films. After 24 and 72 hrs, BEAC were more spherical and spread less on the N-Ti compared to LECC. Results of cell density and coverage area by the spreading cells also revealed that BEAC had less cell-cell interactions than LECC.

The stress and microscopy results indicated that BEAC revealed strong mechanical interactions with the biomimetic N-Ti films while had a low extent of cell-cell interactions. In contrast, LECC exhibited strong cell-cell interactions while small mechanical interactions with N-Ti.

Conclusions: For the first time, the internal stress in nanostructured biomimetic coatings due to cell-materials interactions was measured. The results showed cancer cells could exert different amount of forces to the materials coatings depending on the cell type.

References:

[1] Chason E. Surf Eng 2003; 19: 387-391.

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