

Towards Inert Implant Metal Oxide Surfaces

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Statement of Purpose: The purpose of this study is to design and synthesize monolayers which will render metal implant surfaces, specifically SS316L and nitinol, inert to non-specific adsorption of proteins and cells.

Methods: SS316L and nitinol foils were polished and cleaned with organic solvents. Monolayers were deposited from solution using organic acids (pKa 4-10). Monolayer formation was analyzed by diffuse reflectance infrared spectroscopy, x-ray photoelectron spectroscopy and MALDI-TOF MS. Coverage of the surface by the monolayer was monitored by contact angle goniometry and atomic force microscopy.

Adhesion of 3T3 fibroblasts was tested after exposure to modified substrates for twenty-four hours. Substrates were rinsed. Cell adhesion was quantified by a Live/Dead viability assay. Four independent experiments were conducted and the results evaluated using ANOVA analysis.

Results / Discussion: Surface preparation was mild and left the native oxide surface intact, as evidenced by XPS. Initial scans showed that the surface content was very close to that of the bulk material. Monolayers of long alkyl chain organic acids (phosphonic, carboxylic and hydroxamic) were then prepared on the native oxide of stainless steel 316L and nitinol. Diffuse reflectance infrared spectroscopy results support ordered monolayer formation, with peaks at $<2918\text{ cm}^{-1}$ (CH_2 stretch) for each substrate. Peaks in the fingerprint region associated with the acidic head groups are consistent with covalent binding to the surface (Figure 1). Films were resistant to removal by chemical or mechanical testing. Contact angle measurement and AFM imaging was consistent with complete, hydrophobic coverage of the surface.

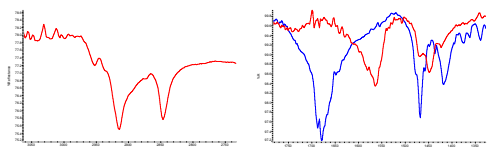


Figure 1. Example supporting data: Octadecylcarboxylic acid on SS316L: Left. IR spectra of C-H region 3200 to 2800 cm^{-1} . Peaks present at 2914 cm^{-1} and 2849 cm^{-1} are consistent with the formation of an all-trans monolayer. Right. IR spectra of $1800\text{ to }1300\text{ cm}^{-1}$. Before deposition there are distinct peaks for C-O and C=O at 1473 and 1710 cm^{-1} . After deposition, (red spectra) there is no peak at 1700 for the C=O stretch but there is a peak at 1555 cm^{-1} indicating the presence of C-O groups as single bonds or with a bond order of 1.5 due to surface interactions. X-axis: wavenumbers (cm^{-1}) Y-axis: % Reflectance

This method can be used to form monolayers that present functional groups ($-\text{CH}_3$, $-\text{OH}$, $-\text{NH}_2$, $-\text{COOH}$) at the interface, as evidenced by infrared spectroscopy and contact angle changes. Contact angles of the hydrophilic interfaces were less than the those of the control substrate and the hydrophobic interfaces were $>108^\circ$.

Modified and control (unmodified) substrates were placed in wells (10,000 cells/well) with 3T3 fibroblasts for twenty-four hours. Live/Dead viability assays and cell counting experiments were consistent with hydrophobic surfaces being more resistant to non-specific adsorption of cells (Figure 2).

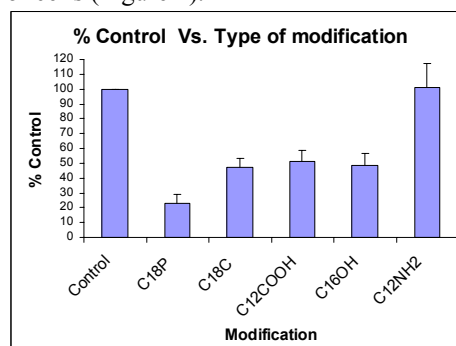


Figure 2: Comparison of the number of 3T3 Swiss fibroblast cells attached on modified stainless steel 316L substrates. The control and amine-terminated surfaces show no statistically significant differences in non-specific cell attachment. Methyl-terminated phosphonic acid monolayers show the greatest reduction in non-specific adhesion. Shown are the means and standard deviations of four independent experiments.

Conclusions: Nickel-containing alloy oxides, can be modified with self-assembled monolayers under mild, solution deposition conditions. These monolayers are strongly-adhered and mechanically stable under organic and biological conditions. This study is important because previous monolayer formation on stainless steel, and other nickel containing surfaces has required reduction of the metal oxide surface to metal by electrochemical methods. This method allows for covalent attachment of monolayers to the native oxide surface. Further, the tail groups of these monolayers can be used to control the surface properties such as hydrophobicity. Finally, methyl-terminated monolayers on metal alloy oxide surfaces reduce non-specific cellular adhesion more than hydrophilic surfaces.