TOWARDS BIOMIMETIC INTERFACES: NANO-SCALE MODIFICATION OF METAL SURFACES

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INTRODUCTION

The fate of a biomaterial *in vivo* is determined by the biological response it elicits. This biological response is primarily governed by the *implant-tissue interface*, which in turn is dictated to a significant extent by the chemical makeup and topology of the implant surface. When the implant surface lacks the appropriate chemistry and topology, undesirable clinical outcomes can result e.g., osteolysis, due to poor integration of the implant with the surrounding bone, which is a significant mode of failure for metallic orthopaedic implants [1]. Due to our improved understanding of cellular processes, peptides, proteins and genes may be used to manipulate cell migration, proliferation and differentiation. Biocompatibility and performance of an implant can be significantly improved by localization of such biomolecules on the surface. However, such modifications require surface chemical functionality in the form of a reactive functional group or charge. Typical approaches to modify an implant surface include reactive plasma treatment [2], hydrolysis [3], surface segregation of functionalized polymers from blends [4], and coatings [5]. Coatings are highly preferred due to their ease of application and improved control over surface chemistry. Since clustering of receptors on a cell surface in response to an external biomolecular signal is a key event in the modulation of cellular processes; presentation of biomolecules is a size scale that is similar to these receptor clusters, which span typically a few 100 nanometer, is key. Therefore, surfaces that are well defined at a nanometer-sub micron level are extremely important. Current coating methodologies in general offer poor control over topography and chemical composition of a surface at the nano-scale level.

We hypothesized that a nano-scale modification of a surface with respect to topology and chemistry can be achieved using pre-functionalized silica nano-particles (colloids). The strength of this new paradigm lies in the ability to present a range of chemical functionality, by leveraging silica chemistry; in a spatially well-defined manner; and in being able to modify a broad class of materials from aqueous solutions. In this study we have investigated the surface modification of type 316L stainless steel and titanium, two commonly used metals in orthopaedics and interventional cardiology, using amine-modified silica colloids.

MATERIALS AND METHODS

Materials

Foils (0.05 mm thick) of type 316-L stainless steel and titanium (99.99%) were obtained from Goodfellow Corporation (UK). Chemicals for the quantification of amine groups: sulfo-SDTB, dimethylformamide and perchloric acid were purchased from Pierce and Aldirch. All cell culture supplies were obtained from Gibco-BRL.

Preparation of Functionalized Silica Colloids

Silica nano-particles were prepared via the Stober process using tetraethylorthosilicate as the silica precursor using ammonia and alcohol as catalysts as described elsewhere [6]. Functionalization of the colloidal silica surface with primary amines ($Si\text{-}O\text{-}R\text{-}NH_2$) was achieved by condensation with aminopropyltriethoxysilane (APS).

Coating of Metal Samples

Metal substrates were cut into rectangle pieces (0.7mm x 10mm), ultrasonically cleaned in hexane and acetone for 5 minutes each, and subsequently washed in ethanol, followed by rinsing in deionized water and then dried in a 60º C oven for 24 hours prior to use. Modification of metal substrates with silica colloids was carried out by spin coating. In brief, the sample substrate was mounted on the stage and held in place by light vacuum and an ethanol solution of the colloids (50 uL, 1 wt%) was dropped on the rotating substrate (2000 rpm). After a 20-second drying period subsequent layers were deposited at 1-2 minute intervals for a total of 10 depositions.

Characterization of Colloid Modified Metal Substrates

The modified metal surfaces were characterized using Scanning Electron Microscopy (SEM), Energy Dispersive X-ray Analysis (EDAX), Rutherford Back Scattering (RBS), X-ray Photoelectron Spectroscopy (XPS), and Atomic Force Microscopy (AFM, tapping mode image). The concentration of amine groups on the surface was determined by a quantitative assay as described in the literature [7,8]. The assay involves first reacting the amine group with sulfo-SDTB and then hydrolyzing the adduct with perchloric acid to liberate at colored cation that is assayed spectrophotometrically at 498 nm.

Cell Proliferation Studies

Cell proliferation studies were carried out using MC3T3-E1 osteoblast-like cells (ATCC). Cells were cultured in alpha-MEM supplemented with 10% FBS and 1% pen-strep. All cell culture studies were carried out at 37º C in 5% CO_2 in a humidified incubator. Metal substrates were mounted in 4-chambered glass slides using sterile vacuum grease (Dow Corning) and then surrounded with low-melting agarose (1% solution) to prevent cell attachment to the glass surface. Cells were seeded at 10,000 cells/well (1 ml). After 4 days the media was aspirated and the cells were trypsinized and then manually counted using a hemacytometer. Tissue culture polystyrene (TCPS) was used as a positive control. Samples for SEM analysis were prepared by first fixing with 1% glutaraldehyde for 5 minutes followed by dehydration in an ethanol series (50, 60, 80, 90 %) 5 minutes each.

RESULTS

The silica colloids were obtained as a mono-dispersed population with a mean diameter of 58 nm as ascertained by dynamic laser light scattering. The attainment of a colloid surface rich in amines upon derivatization with APS was verified by a salisaldehyde based calorimetric assay at 404 nm. The derivatization step did not significantly change the mean diameter of the silica colloids.

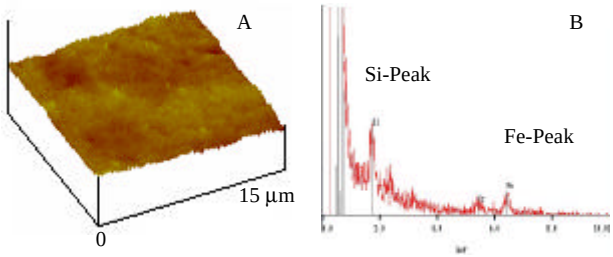

Figure 1. Type 316-L stainless steel surface modified with silica nano particle (A) AFM image showing the presence of stacks of nano particles and (B) EDAX spectrum of the surface confirming the presence of Silica.

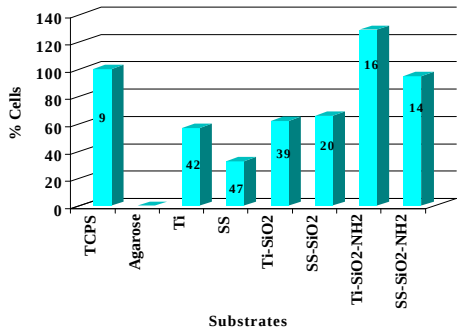

Figure 2: MC3T3-E1 proliferation on metal substrates normalized to TCPS (n =7). Number on the bar is % SD.

The presence of silica colloids (nanoparticles) on the metal surface was confirmed by AFM and EDAX analysis (Figures 1a and 1b respectively). RBS analysis revealed that the thickness of the silica-nanoparticle layer was reproducible and ranged from 380-420 nm after 10 depositions depending on the metal substrate. Using the SDTB assay it was determined that the spatial concentration of amine was quite high at 0.6 $NH_2/Å^2$.

Cell proliferation studies carried out with MC3T3-E1 cells showed that the silica modified surfaces possessed excellent cytocompatibility (Figure 2). It was observed that the cell proliferation on metal substrates modified with plain colloidal SiO_2 was comparable to unmodified metal substrates, suggesting that the presence of nano - particulate surface structure did not negatively impact cell behavior. However, upon the introduction of amine rich surface cell proliferation was dramatically enhanced and was comparable to TCPS (Figure 2). The trend observed in the cell proliferation studies, i.e., unmodified metal metal-SiO_2<metal-SiO_2-NH_2 was borne out by SEM as well (Figure 3)

DISCUSSION

We have developed a novel means of modifying metal surfaces using silica nanoparticles that are structurally and chemically well defined *a priori*. This novel surface modification approach allows for a high-degree of control with respect to important surface properties such as coating thickness, topography, roughness, and nature and density of chemical functionality. Cytocompatibility and cell proliferation studies using MC3T3-E1 cells have shown that the modification does not have adverse consequences on cell attachment and proliferation. It appears that the presence of a nano-structure may improve cellular interactions (Figures 2 & 3). The introduction of an amine rich surface appears to increase cell spreading as assessed by SEM analysis.

Figure 3: SEM of type 316-L stainless steel substrates seeded with MC3T3-E1 cells. (a) Unmodified surface, (b) SiO_2-modified, and (c) SiO_2-NH_2 modified.

We believe that these functionalizable coatings have three important characteristics for the generation of a bio-mimetic interface, which are:
1.Nano-scale control over presentation of chemical moieties,
2. Ability to vary local and global density of ligands, and
3. Aqueous phase processing.
Currently, we are exploring the tethering and presentation of growth factors and viral vectors to influence cell function.

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