Coating of Titanium with Electrically Polarized Hydroxyapatite Modulates Mesenchymal Stem Cell Adhesion

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Disclosures:

Introduction: While orthopedic implants are generally successful, they are not without debilitating complications due to loosening. Success of any orthopedic implant relies on effective integration between the surface of the implant and bone, with no fibrous tissue interface. Titanium (Ti) and its alloys are among the most successful implantable materials used in orthopedics. The combination of mechanical and corrosion resistance properties as well biocompatibility make Ti alloys good candidates as a biomaterial for bone implants and fixation devices. It’s been shown that improvements in implant surface topography enhance bone adherence. To improve osseointegration, Ti surfaces can be coated with hydroxyapatite (Ca$_{10}$(PO$_4$)$_6$(OH)$_2$ or HAP).

However, the ability of synthetic HAP surfaces to promote osseointegration remains limited, prolonging patient recovery after surgery and increasing the likelihood of device failure. We have developed a novel HAP coating technique with the ability to promote electrical polarization and strong charge storage. We hypothesize that polarized HAP coating on Ti can promote attachment of osteogenic cells and influence their morphology and focal adhesions, which may affect osseointegration.

Methods: Preparation of HAP substrates: Grade 2 Ti sheets (0.25mm thick) were used to prepare 9.5mm diameter discs. Discs were washed with alconox twice and rinsed four times in distilled water. HAP was deposited onto samples using a published electrochemical-hydrothermal synthesis method. Five different surfaces were examined in this study: Ti, positive polarized HAP, negative polarized HAP, heated HAP and as-synthesized HAP. Discs were sterilized by overnight irradiation with UV light prior to cell culture experiments.

Surface Characterization: Surface topography of the samples was determined using atomic force microscopy (AFM), scanning electron microscopy (SEM) and contact angle measurements. The surface root mean squared roughness (rms) of the prepared samples was estimated using a NT-MDT AFM/Scanning Probe Microscope system at ambient temperature and humidity. Scans of size 2µm × 2µm were made in non-contact mode using standard tips (10nm radius of curvature, 125µm cantilever length, 45µm cantilever width, 10µm cantilever thickness, 300Hz resonance frequency, and 40 N/m force constant) at a scan resolution of 512 × 512 pixels. The rms values were calculated using software provided by the manufacturer and determined from the average of six random fields per sample. The gross surface morphology of the prepared samples was examined using a Zeiss Auriga SEM at 5 keV and 10µm aperture. Water and growth media contact angle measurements were directly read on a NRL Contact Angle Goniometer by manufacturer Ramé-hart. Measurements for the contact angle were repeated three times and determined by the mean value of three samples.

Cell culture: Human bone marrow-derived MSCs were obtained from Lonza (Berkshire, UK) and cultured in vitro according to the manufacturer’s protocol. Ti and HAP-coated Ti discs (n=3/group) were placed into 48-well plates and glass bottom multi-well dishes were used as a control. The samples were seeded with MSCs at a low seeding density of 5000 cells/cm$^2$ to avoid artifacts from cell aggregation in cell (nuclear) and vinculin quantification.

Immunofluorescence staining: After a 48 h incubation period, cells were washed in DPBS, fixed in 4% PFA, and then sequentially stained with mouse monoclonal primary antibodies against vinculin, F-actin, and nuclear To-Pro3. Cells were examined on a laser scanning confocal microscope (FV1000 Olympus) with a Zeiss 4× dry, 10× dry, and 40× oil objective lens using tiling and stacking modes for nucleus and vinculin quantification, respectively. Samples were then processed for SEM to further characterize the morphology of the cells.

Statistics: Quantitative cell attachment density and vinculin distribution per cell were contrasted among the experimental groups using ANOVA with Tukey's post hoc test (GraphPad Prism, $\alpha=0.05$).

Results: SEM imaging gave insight into the different treatments of the hydroxyapatite structure with respect to porosity, density and crystal size (Fig 1).
Differences were observed between the surfaces and confirmed with both AFM (Fig 2A) and contact angle measurements (Fig 2B). In general HAP coating, regardless of polarization and charge, resulted in more wettable, hydrophilic surfaces compared to bare Ti.

Forty-eight hours after seeding MSCs on the different surfaces, immunostaining revealed cells containing many thick stress fibers in a parallel arrangement. Vinculin-labeled focal contacts formed short and dense patches, evenly distributed on the membrane surface, regardless of the surface coating. These focal points appeared more frequent on the glass, Ti, negative polarized HAP and heated HAP surfaces (Fig 3).

An intersurface comparison of cell (nuclear) density and focal adhesion showed that, although lower than the glass control, negative polarized HAP maintains the same quantity of cells compared to Ti, while as-synthesized HAP has a significant reduction in attached cells (Fig 4A). The quality of cellular attachment, quantified by the number of focal adhesions per cell, tends to be improved with negative polarized and heated HAP compared to bare Ti (Fig. 4B). Confocal observations showed that glass, Ti, negative polarized HAP and heated HAP surfaces favored rapid cell attachment and denser focal adhesion points, while the positive HAP surface had limited cellular response. These observations were also confirmed with SEM imaging.

**Discussion:** This study examined the effect of different surface topographies and polarization on the attachment of mesenchymal stem cells and the expression of focal adhesion. With this preliminary data, we hypothesize that the electrostatic charge on the surface of polarized HAP has the potential to promote bone cell attachment that can lead to enhanced osseointegration. The mechanism is as of yet unknown. Future studies will focus on gene expression analysis, and testing the efficacy of polarized HAP coating on osseointegration in an *in vivo* model.
Significance: Examining the effect of a novel HAP coating technique with the ability to promote electrical polarization and strong charge storage on the attachment of mesenchymal stem cells and the expression of focal adhesion will enable us to improve osseointegration and decreasing the likelihood of implant failure after surgery.

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