Influence of Surface Chemistry of Microtextured Titanium on the Osteoblast Cell Response

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Statement of Purpose: Titanium (Ti) surfaces with micron and sub-micron scale roughness have shown increasing osteoblast differentiation *in vitro* and bone-to-implant contact *in vivo*. Enhanced surface wettability shortens wound healing time and increases tissue integration of titanium implants. Hydrophilic surfaces promote rapid adsorption of tissue fluids, improving clot formation and reducing the gap between peri-implant tissue and the biomaterial surface. However, lower surface wettability due to increased surface roughness can delay initial interactions with the physiological environment. Coating surfaces with polyelectrolytes is a versatile approach to modify surface properties without changing the underlying geometry of the surface. The aims of this study were to modify surface hydrophilicity of microstructured Ti surfaces through polyelectrolyte coating without changing surface microstructure and to investigate whether these chemical modifications alter osteoblast maturation.

Methods: Three polyelectrolytes, chitosan (CHI), poly(L-glutamic acid) (PGA), and poly(L-lysine) (PLL), were used to coat titanium surfaces with micron and submicron scale roughness (PT, Ra = 0.3 (±0.02) µm and SLA, Ra =2.5 (±0.08) µm). Surface characterization was performed with X-ray photoelectron spectroscopy (XPS), scanning electron microscopy (SEM), contact mode profilometry, and contact angle measurements. Following surface characterization, human MG63 osteoblast-like cells were cultured on the chemically modified surfaces. At confluence, cells were counted, alkaline phosphatase (ALP) specific activity measured in the cell lysates, and osteocalcin (OCN) and osteoprotegerin (OPG) analyzed in the conditioned media. For each surface analysis experiment, there were n=2 samples. For each cell study, there were n=6 independent cultures per variable. Statistical significance was determined using ANOVA followed by Bonferroni's modification of Student's t-test.

Results: Surface characterization results indicate that CHI, PGA, or PLL were formed in a uniform thin layer on the PT and SLA surface with increasing surface wettability without modifying surface roughness. The number of cells on PGA or PLL coated PT and SLA surfaces and on PT-CHI coated Ti was not different compared to PT and SLA control surfaces whereas cell number was increased the SLA-CHI surface. Cells on the PT-PGA surface had lower ALP than cells on PT, PT-CHI, and PT-PLL. In contrast, cells on SLA-CHI had higher ALP than on SLA, SAL-PGA, and SLA-PLL (Figure 1A). Cells on SLA and polyelectrolyte coated surfaces secreted more OCN than on PT surfaces (Figure 1B). Cells on PT-PGA surfaces secreted less OCN than on PT. However, cells grown on PT-PLL exhibited enhanced OCN compared to PT-PGA. There was no difference in OCN levels between cells on PT and PT-CHI. Cells on SLA produced more OCN than cells grown on polyelectrolyte coated SLA surfaces. Osteoblasts growth on SLA-PGA and SLA-PLL had lower OCN levels than when grown on SLA.

Cell number, ALP, and OCN differed on PT and SLA surfaces coated with CHI or PLL. Both CHI and PLL are positively charged polyelectrolytes. However, CHI has hydroxide groups (-OH) not present in PLL. The difference in cell response suggests that the surface chemistry influenced osteoblast differentiation in the early and late stages.

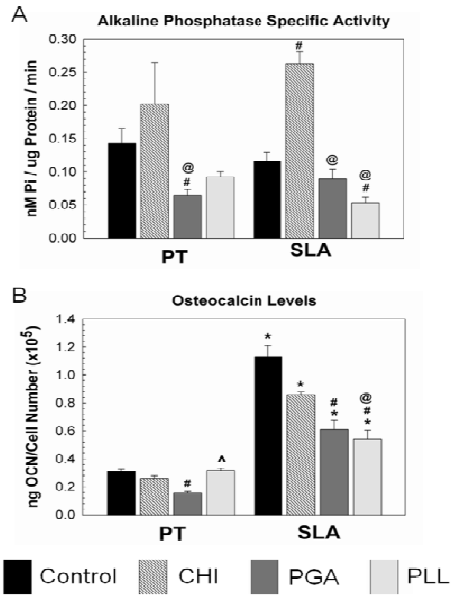

Figure 1. Influence of the polyelectrolyte coating on Titanium surfaces (A) Alkaline phosphatase specific acitivity (B) osteocalcin levels. * $p<0.05$, PT vs. SLA; # $p<0.05$, vs. Control; @ $p<0.05$, vs. CHI; ^ $p<0.05$, vs. PGA

Conclusions: Chemical modification of Ti by increasing its surface wettability without altering its surface roughness affects MG63 cell differentiation. In addition, the results show that there is a correlation between surface chemistry and surface microstructure, which modulates cell response.

This work was supported by NIH AR-052102, and a PSE Graduate Fellowship (to JHP) from the IPST at the Georgia Tech. Ti substrates were provided by Institut Straumann AG.