Simple Application of Fibronectin-Mimetic Coating Enhances Implant Osseointegration

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Statement of Purpose: Emerging biomimetic orthopaedic implant surface strategies have focused on the covalent tethering of biological motifs, including extracellular matrix sequences and growth factors, on biomaterial supports to promote bone cell ingrowth. These bio-inspired approaches often involve labor-intensive and clinically unattractive multi-step biofunctionalization procedures. In contrast, we recently described a simple, one-step coating procedure that relies on the passive adsorption of a synthetic collagen-based peptide onto biomedical grade titanium to promote integrin binding and enhance osseointegration [1]. Integrins are a large family of heterodimeric ($\alpha\beta$) transmembrane receptors that mediate cell-matrix and cell-cell adhesion and regulate osteoblast function and mineralization. Given the importance of integrins in osteogenic pathways, especially $\alpha_5\beta_1$, recent implant surface strategies have focused on utilizing short, bioadhesive motifs, including the arginine-glycine-aspartic acid (RGD) sequence from fibronectin (FN). However, these implant surfaces presenting RGD motifs do not consistently enhance osseointegration and bone formation in animal models [2]. In the present work, we evaluated the ability of different fibronectin-inspired biomolecular coatings to promote *in vitro* osteoblastic differentiation and implant osseointegration in a rat cortical bone model. Notably, these biomolecular coatings relied on simple physisorption of FN-based ligands onto biomedical-grade titanium as a simple, clinically-translatable, implant biofunctionalization strategy.

Methods: Titanium surfaces were coated with serum proteins or equimolar surface densities of either linear RGD peptide, full-length FN (pFN) or a recombinant fragment of FN, termed $FNIII_{7\text{-}10}$, spanning the central cell binding domain. The adhesion and integrin specificity of rat bone marrow stromal cells (rBMSCs) on these surfaces were assessed by an integrin-subunit blocking centrifugation assay, integrin binding analysis, and a FAK activation assay in conjunction with integrin subunit blocking. In addition, gene expression of osteoblastic differentiation markers in addition to mineralization was analyzed. Implant osseointegration of clinical-grade titanium implants, unmodified or dip-coated with equimolar densities of either pFN or $FNIII_{7\text{-}10}$, was evaluated in a rat tibial cortical bone model at 28 days post-implantation by mechanical pull-out testing.

Results: We have previously shown that in vitro surfaces presenting $FNIII_{7\text{-}10}$ promote $\alpha_5\beta_1$-binding specificity [3]. In this study, clinical-grade titanium surfaces presenting $FNIII_{7\text{-}10}$ engaged higher relative levels of $\alpha_5\beta_1$ binding and FAK activation compared to pFN-coated and unmodified titanium surfaces. Adhesion to RGD peptide surfaces was minimal owing to the lack of adsorption of these peptides to titanium. The adhesion and signaling responses of rBMSCs on these surfaces were integrin-specific, as $\alpha_5\beta_1$ blocking significantly reduced FAK activation (2hr) and adhesion (1hr) on only $FNIII_{7\text{-}10}$ surfaces, whereas blocking $\alpha_v\beta_3$ reduced these levels on only pFN and unmodified Ti surfaces. In addition, osteoblastic differentiation, including osteogenic gene expression (7d) and mineralization (14d, 21d), was enhanced on $FNIII_{7\text{-}10}$-coated surfaces compared to unmodified titanium and pFN-presenting surfaces. Importantly, simple dip-coating of $FNIII_{7\text{-}10}$ on clinical grade titanium implants significantly improved bone apposition and functional implant osseointegration (43 N) in a rat tibial bone model compared to pFN-functionalized (25 N, $p<0.05$) and unmodified titanium (15 N, $p<0.001$) *in vivo* as measured by pull-out testing of implants after 28 days post-implantation.

Conclusions: Surfaces coated with a recombinant fragment of FN spanning the central cell binding domain enhanced osteoblastic differentiation and mineralization in bone marrow stromal cells and increased implant osseointegration in a rat cortical bone model compared to passively adsorbed RGD peptides, serum proteins, and full-length FN. Differences in biological responses correlated with integrin binding specificity and signaling among surface coatings. This work validates a simple, clinically-translatable, surface biofunctionalization strategy to enhance biomedical device integration.

References : [1] Reyes et al., *Biomaterials*; 28 :3228-35 (2007); [2] Barber et al., *J Biomed Mater Res A*; 80:306-20 (2007); [3] Petrie et al., *Biomaterials*; 27:5459-70 (2006).