Integrin Specificity Modulates Implant Osseointegration: Functionalization of Stable Poly(OEGMA) Brush-Coated Ti Implants with $FNIII_{7-10}$ and RGD

Timothy A. Petrie[1,3], Jenny E. Raynor[2], Catherine D Reyes[1,3], Kellie L. Burns[1,3], David M. Collard[2], Andres J. Garcia[1,3]
[1]Woodruff School of Mechanical Engineering; [2]School of Chemistry and Biochemistry; [3]Petit Institute for Bioengineering and Bioscience
Georgia Institute of Technology, Atlanta GA 30332

Introduction: Cell adhesion is primarily mediated by cell-surface integrin receptors. Integrins activate signaling pathways regulating cellular activities, and recent studies have shown that integrin binding specificity can direct specific cell fates including proliferation and differentiation [1]. We have recently shown that a recombinant fragment of FN ($FNIII_{7-10}$) incorporating both the RGD and PHSRN synergy sites displays $\alpha_5\beta_1$ binding specificity, while RGD peptides bound to $\alpha_V\beta_3$ [2]. These differences in integrin binding resulted in significant differences in cell adhesion and proliferation. Using a recently developed dense poly(oligoethylene glycol methacrylate) (poly(OEGMA)) brush system [3] on Ti that affords nonfouling properties, yet can be easily tethered with peptide ligands, we examined the importance of integrin specificity in vivo in the context of implant osseointegration. Equimolar densities of RGD and $FNIII_{7-10}$ were tethered to the poly(OEGMA) brushes on Ti implants and evaluated for functional osseointegration in a proximal tibia implantation model in rats.

Materials and Methods: Recombinant $FNIII_{7-10}$ was expressed in E.coli and purified [2]. $FNIII_{7-10}$ and linear GRGDSP peptide (Bachem) were tethered onto poly(OEGMA) brushes on Ti substrates via urethane linkages (Fig.1) [3]. Tethered density profiles were obtained as a function of ligand solution concentration from SPR analyses. In vitro bioresistance of the poly(OEGMA) brushes was verified by short and long-term cell adhesion experiments. Implants containing equimolar surface densities of tethered $FNIII_{7-10}$ and RGD, as well as unfunctionalized poly(OEGMA) brushes, were press fit into defects in rat tibiae, and implant osseointegration was assessed at 28 days post-implantation by mechanical pull-out testing at a fixed displacement rate of 0.01 mm/sec.

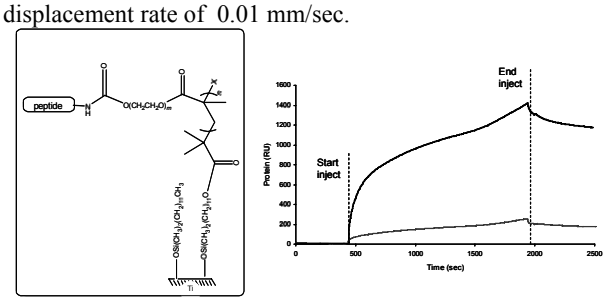

Fig.1: A) Schematic of modified Ti surfaces with a dense, polymer brushes of poly(OEGMA) using a 'grafting-from' approach. Conversion of OH-end groups to 4-nitrophenyl carbonate groups allows for functionalization of brushes with amine groups of bioactive peptides or proteins; B) Quantification of tethered RGD-peptide via SPR displaying 10-fold higher levels of peptide over unmodified brushes.

Results and Discussion:

Fig 1A presents a schematic of the poly(OEGMA) brushes used in this study. This novel brush surface system allows precise control of tethered peptide density in a nonfouling background, yet remains stable in cell culture media much longer than tri(ethylene glycol)-terminated alkanethiol SAMs [3]. Cells did not adhere to unfunctionalized brushes incubated in media for over 50 days. Controlled densities of tethered $FNIII_{7-10}$ and RGD were attained by varying the solution concentration. As shown by **Fig 1B**, functionalized brushes displayed surface peptide density 10 times greater than on unfunctionalized brushes, demonstrating effective surface tethering.

Pull-out tests of implants after 28 days in a rat tibial bone model revealed significantly higher mechanical fixation for $FNIII_{7-10}$-tethered implants than either RGD or unfunctionalized brushes, demonstrating enhanced mechanical osseointegration of the $FNIII_{7-10}$ surfaces over RGD (**Fig. 2**). In addition, implants with unmodified poly(OEGMA) brushes displayed minimal mechanical anchorage, suggesting effective bioresistance of this brush system in vivo. Taken together, these results form the first study indicating that integrin specificity may have a significant effect on downstream cell function in vivo and, indeed, on the ultimate bone-healing function of implants.

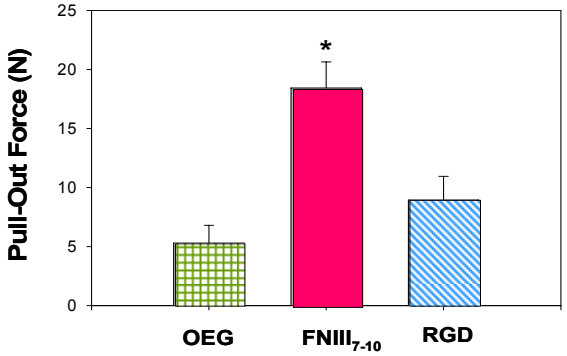

Fig.2: Mechanical osseointegration of implants via pull-out testing. Tethered surface density for each peptide surface was 0.9 pmol/cm^2 (SPR). $FNIII_{7-10}$ > RGD, $p<0.008$; $FNIII_{7-10}$ > OEG, $p<0.001$; n=5.

Conclusions: The increased osseointegration of $\alpha_5\beta_1$–specific $FNIII_{7-10}$-tethered brush surfaces compared to RGD-tethered surfaces points to a significant role of integrin specificity in vivo in modulating bone formation. These results are consistent with in vitro analyses demonstrating enhanced osteoblastic differentiation via regulation of integrin binding specificity [1]. Current analyses focus on evaluating histomorphometic data and further in vitro work. In addition, a concurrent study is utilizing this poly(OEGMA) brush system to investigate the effect of varying tethered $FNIII_{7-10}$ density on functional outcomes in vivo in this bone model.

References : [1] Keselowsky et al. *PNAS*, 102:5953-5957 (2005); [2] Petrie et al. *Biomaterials,* 27(31):5459-70 (2006); [3] Raynor et al. *Advanced Materials*. (submitted).

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