Peptide Functionalized Titanium Substrates Differentially Modulate Osteoblast Behavior

B.F. Bell[1], M. Schuler[2], M. Chervonski[1], S. Tosatti[2], M. Textor[2], Z. Schwartz[1], and B.D. Boyan[1]
[1]Georgia Institute of Technology, Institute of Bioengineering and Bioscience, Atlanta, USA
[2]ETH-Zurich, Laboratory for Surface Science and Technology, Zurich, Switzerland

Statement of Purpose: The extent of osteointegration of orthopaedic implants depends on their surface characteristics, including microarchitecture and chemistry, and resulting surface energy. This has led to the development of engineered ligands that bind integrins or other receptors and elicit specific responses from cells. In the present study, we have coated sandblasted, acid etched (SLA) titanium surfaces with poly-L-lysine grafted polyethylene glycol (PLL-*g*-PEG) functionalized with peptides containing the integrin binding domains RGD and KRSR. PLL-*g*-PEG is a copolymer that forms a comb-like monolayer on titanium surfaces and acts to limit non-specific protein adsorption. RGD is a critical domain for cell adhesion and is found in proteins such as fibronectin or vitronectin, which are well know to bind $\alpha_5\beta_1$. Previous studies by Tosatti et al [1] demonstrated that PLL-*g*-PEG functionalized with RGD increased proliferation, but decreased differentiation versus PLL-*g*-PEG controls. KRSR was designed based on its basic-basic-nonbasic-basic (BBXB) amino acid charge structure that was proposed by Cardin et al to bind heparin sulfate and thus mediate osteoblast adhesion [2]. Dee et al showed KRSR selectively increased osteoblast adhesion [3] compared to other similar ligands, though the effects of KRSR on osteoblast differentiation are currently unknown.

The present study was based on the hypothesis that RGD-KRSR functionalized PLL-*g*-PEG surfaces can increase osteoblast differentiation while maintaining high levels of proliferation compared to PLL-g-PEG surfaces. This study is clinically relevant since the RGD-KRSR functionalized PLL-*g*-PEG can be easily dip-coated directly onto material surfaces for the purpose of increasing osteoblastic differentiation and improving osteointegration of orthopaedic implants.

Methods: PLL-g-PEG copolymers were synthesized and covalently linked to the RGD and KRSR peptides using methods described by Tosatti et al [1]. 15mm diameter titanium SLA (Ra = 4.0 ± 0.1 μm) disks (Institut Straumann AG, Basel, Switzerland) were dip-coated with PLL-*g*-PEG solutions containing varying concentrations of peptide. KRSR concentrations of 0, 5, 10, 15, and 20 pmol/cm^2 were varied with 0, 0.05, or 1.26 pmol/cm^2 of RGD. Tissue culture polystyrene (plastic), SLA, and PLL-*g*-PEG coated SLA surfaces, as well as surfaces coated with PLL-*g*-PEG functionalized with a KSSR peptide were used as controls. KSSR was selected as a control because it presents a BXXB chemistry in contrast to the BBXB chemistry of KRSR. Osteoblast-like MG63 cells were cultured in DMEM containing 10% FBS and 1% antibiotics. At confluence, cell number and cellular alkaline phosphatase specific activity were measured and media levels of osteocalcin, active and latent TGF-β1, and PGE_2 were assessed using commercially available assays. Each variable was tested using six independent cultures and data were analyzed by ANOVA using Bonferroni's modification of Student's t-test ($p<0.05$). Experiments were repeated twice to ensure validity.

Results: Cell number decreased on SLA and PLL-*g*-PEG surfaces compared to plastic controls, while alkaline phosphate activity and levels of osteocalcin, active and latent TGF-β1, and PGE_2 were increased, confirming previous observations [1]. Addition of 1.26 pmol/cm^2 RGD to SLA-PLL-*g*-PEG increased cell number and decreased all markers for osteoblastic differentiation. KSSR decreased cell number; cell number on surfaces coated with KSSR together with the highest concentration of RGD was comparable to cultures grown on PLL-*g*-PEG alone (Figures 1A, 1B). Osteocalcin was increased in cultures grown on disks coated with KSSR and this effect was enhanced by low RGD; however, the KRSR surfaces had no effect on osteocalcin or other osteoblastic markers (Figures 1C, 1D). KSSR also increased levels of PGE_2 and TGF-β1, although alkaline phosphatase activity was not affected.

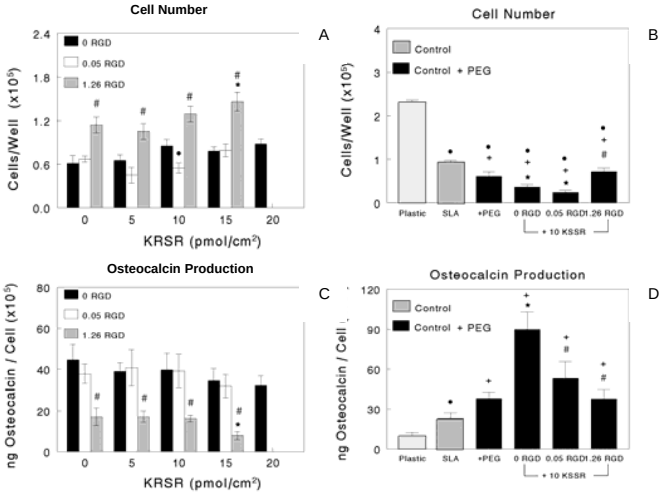

Fig 1. Effect of peptide functionalized substrates on MG63 cell number and osteoblast differentiation. Data are means ± SEM for N=6 independent cultures. $P<0.05$. Panels A and C: *v. 0 KRSR at each concentration of RGD; #v. 0 RGD at each concentration of KRSR; ●v. 0.05 RGD. Panels B and D: ●v. plastic; +v. SLA; *v. PEG; #v. 0 RGD.

Discussion: PLL-*g*-PEG increased markers for osteoblastic differentiation, while adding RGD partially restored the phenotype observed on uncoated SLA surfaces, confirming our previous observations [1]. KRSR enhanced osteoblast adhesion when used to coat Ti substrates directly [2], but it had only minor effects when used with PLL-g-PEG. Moreover, KRSR did not promote increased expression of an osteoblastic phenotype. In contrast, KSSR decreased osteoblast number and dose-dependently stimulated osteoblast differentiation. These effects of KSSR were unanticipated and support the hypothesis that different amino acid motifs regulate adhesion and differentiation. The specific effects of KSSR on osteoblasts are not known and are under investigation.

References:

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