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Short Communication

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Bio-inspired hard-to-soft interface for implant integration to bone

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Abstract

Accomplishing full, functional integration at the host-to-biomaterial interface has been a critical roadblock in engineering implants with performance similar to biological materials. Molecular recognition-based self-assembly, coupled with biochemical signaling, may lead to controllable and predictable cellular differentiation at the implant interface. Here, we engineer a bio-inspired interface built upon a chimeric peptide. Binding to the biomaterial interface is achieved using a molecular recognition domain specific for the titanium/titanium alloy implant surface and a biochemical signal guiding stem cells to differentiate by activating the Wnt signaling pathway for bone formation. During a critical period of host cell growth and determination, the bioactive implant interface signals mouse, as well as human, stem cells to differentiate along osteogenic lineages. The Wnt-induced cells show enhanced mineral deposition in an extracellular matrix of their creation and an enhanced gene expression profile consistent with osteogenesis, thereby providing a bone-to-implant interface that promotes bone regeneration.

Key words: Biomaterial interface; Chimeric peptide; Wnt signaling; Osteogenesis; Bone regeneration

Background

Musculoskeletal disease is the second largest cause of disability worldwide,[1] with osteoarthritis, the leading cause of hip and knee joint replacement,[2] affecting over 30 million people in the USA. Nearly 10% of joint implants fail due to osteolysis and bone defects at the interface.[3] Interfacial integrity of the host-implant interface remains a challenge despite significant efforts to improve the currently employed interface fixation approaches.[4-10] Attempts to boost implant performance by providing bio-active ligands requiring passive absorption or chemical-coupling have met with limited success due to their non-biological conditions resulting in lost bio-activity.[6,11,12] Titanium (Ti) and Ti-alloy implants are frequently used for joint replacement and restoration of craniofacial and axial skeletal birth defects, including bone lost to combat injuries. Despite Ti's biocompatibility, host bone integration crucially depends on the bioactivity of the implant to promote osteointegration.

To address these clinical needs, we engineered a bio-inspired interface for the implant surface, built upon molecular recognition and self-assembly, coupled with the ability to direct cell differentiation by inducing osteogenesis.[13-16] A chimeric peptide was constructed from a peptide binding implant (PBI) domain proven effective for specific self-adherence onto an implant surface[17] fused to a bone-inducing protein (BIP) that activates Wnt signaling.[16] Wnt is an important regulatory pathway for osteogenic differentiation of mesenchymal stem cells, inducing an increase in osteoblastic transcription factors and extracellular matrix molecules that promote bone biomineralization.[18,19] This approach has the potential to modify the biological response at the interface by binding to the implant surface while harnessing the therapeutic value of the Wnt signaling pathway to induce bone growth.

Abbreviations: Wnt, int/Wingless family; Ti, titanium; PBI, peptide binding implant; BIP, bone-inducing protein; PBI-BIP, chimeric peptide; ST2, mouse ST2 stromal cells; hBMMSCs, human bone marrow mesenchymal stem cells; qRT-PCR, quantitative reverse transcription polymerase chain reaction; Runx2, Runt-related transcription factor 2; Osx, osterix; Dlx5, distal-less homeobox 5; FITC, fluorescein isothiocyanate; OM, osteogenic media.

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The authors declare that there are no commercial associations, current and within the past five years, that might pose a potential, perceived or real conflict of interest.

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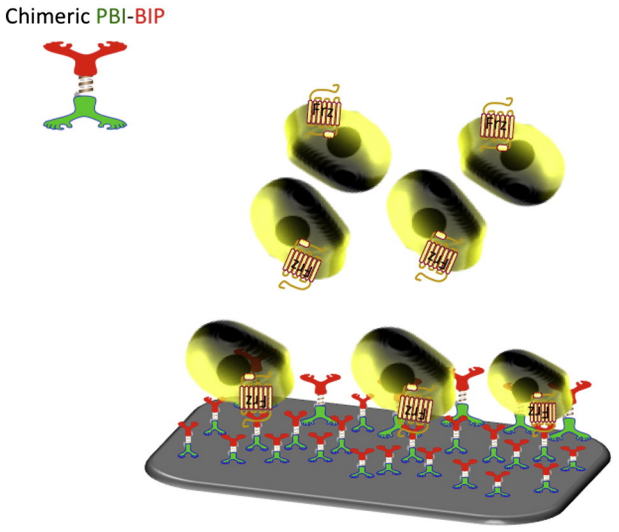

Figure 1. Bio-inspired interface for controlling the interfacial host cell response. The implant surface is modified by a self-organized chimeric peptide that activates the canonical Wnt/β-catenin signaling pathway of stem cells through the Frizzled receptor leading to regulated gene transcription.

Methods

The chimeric peptide (PBI-BIP) was synthesized with a 3-mer linker of aminocaproic acid using solid-state chemistry and its identity was confirmed by mass-spectrometry.[17] Peptide binding was characterized using fluorescent microscopy and quartz-crystal-microbalance spectrometry. PBI-BIP (0.1 mg/ml; 12 μM) in PBS (pH 7.4) was absorbed onto discs of implant material (diameter, 10 mm; thickness, 0.5 mm) by incubation in 48-well plate (total volume: 200 μl) at 37 °C under constant agitation for 4 hours. The disc was eluted with PBS (pH 7.4) over 28 days at 37 °C under constant agitation and released peptides were measured using a NanoDrop spectrophotometer.

Mouse ST2 stromal cells or human bone marrow mesenchymal stem cells (hBMMSCs, Lonza) were cultured on the implant disc for two weeks (ST2) or four weeks (hBMMSCs). Alizarin red S staining was used to quantitate calcium deposited into the extracellular matrix, or the cells were collected and marker gene expression levels quantitated by RNA recovery and conversion to cDNAs followed by real-time PCR using the ΔC_t method.[16,20]

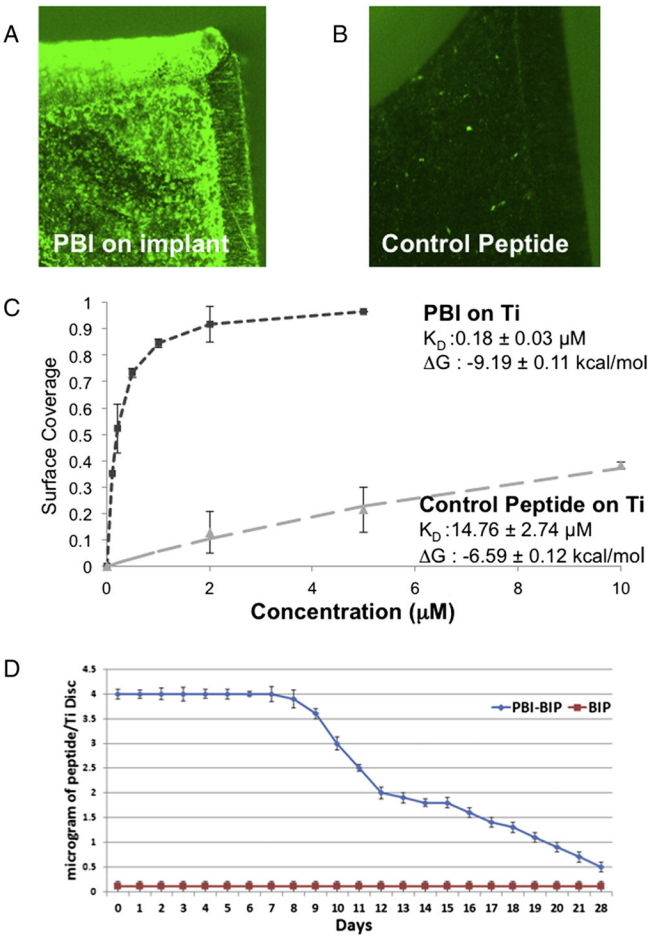

Figure 2. Characterization of PBI-BIP binding. (**A**) Surface coverage of FITC-labeled peptides on implant surfaces using fluorescence microscopy compared to (**B**) control peptides; (**C**) affinity of PBI-BIP to implant surface; (**D**) PBI-BIP bound and released from the implant surface.