Collagen-mimetic Scaffold Coating Enhances Healing of Critically-Sized Bone Defects

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Statement of Purpose: Non-healing bone defects have a significant socioeconomic impact in the U.S., with approximately 600,000 bone grafting procedures performed annually [1]. Emerging strategies focus on delivering osteoinductive growth factors, such as BMPs, or osteogenic cells to heal these defects [2]. However, these approaches are hindered by complex biological interactions, expensive delivery protocols, and potential regulatory concerns [3]. In the present study, we examined the ability of a simple biosynthetic cell- and growth factor-free strategy to heal critically-sized bone defects. The bioadhesive, triple helical collagen-mimetic peptide, GFOGER, binds specifically to the $\alpha_2\beta_1$ integrin to promote osteoblastic differentiation, and when used as an implant coating, GFOGER improves osseointegration of titanium implants [4]. We show that GFOGER-coated poly-caprolactone (PCL) scaffolds promote increased bone formation in critically-sized bone defects compared to uncoated PCL. This simple surface modification imparts biofunctionality to synthetic scaffolds without the use of cells or proteins eliminating regulatory concerns about implanting biologic materials and making this a cost-effective clinically-relevant strategy for bone tissue engineering.

Methods: PCL scaffolds were fabricated by fused deposition modeling as previously described [5]. The peptide GGYGGGPC$(GPP)_5$GFOGER$(GPP)_5$GPC [O=hydroxyproline] was synthesized using step-wise solid phase procedures and characterized by circular dichroism [6]. PCL scaffolds were submerged overnight at 4°C in PBS with or without GFOGER (50 μg/mL). Critically-sized femoral defects were created in 13-week old female Lewis rats by removing 8-mm transectional segments from the diaphyses of both femurs [7]. Defects were stabilized by a modular fixation plate and treated by one of the following 3 conditions: (i) PCL scaffold, (ii) PCL scaffold coated with GFOGER, or (iii) no scaffold (empty defect, negative control). At 4 and 12 weeks, animals were anesthetized and bone volume was quantified by micro-CT. Sanderson's rapid bone stain was used for postmortem histological analysis at 12 weeks.

Results: Sustained inflammatory responses were not observed following surgery and normal ambulation was restored in all rats after one week. MicroCT analysis revealed negligible bone formation in empty defects as expected. Furthermore, significantly more bone formation was observed in defects treated with GFOGER-coated scaffolds compared to uncoated PCL (Figure 1). GFOGER-coated scaffolds also promoted complete bridging of the bony defect after only 4 weeks, while only one PCL-treated defect was fully bridged at 12 weeks. MicroCT data was confirmed by histological analysis.

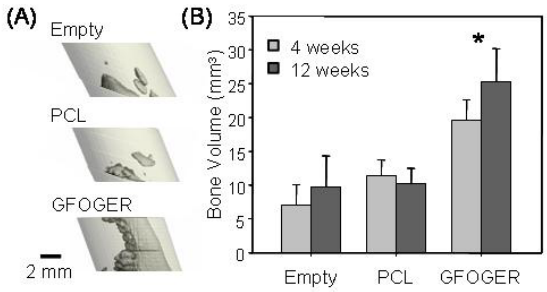

Figure 1. GFOGER-coated scaffolds significantly enhance bone formation in critically-sized defects compared to uncoated scaffolds and empty defect controls. (A) MicroCT images at 12 weeks. (B) Quantified bone volume. * Different from empty defect and uncoated PCL ($p<0.05$).

Conclusions: In the present study, surface modification of PCL scaffolds with the synthetic peptide GFOGER was used to impart biological functionality to a synthetic material for bone tissue engineering. This biomimetic, cell-free technique addresses many of the concerns with traditional protein and cell-based tissue engineering approaches making it a clinically-relevant strategy. Compared to full length proteins, the GFOGER peptide is more stable, cost-effective, and easier to fabricate. Furthermore, GFOGER specifically targets osteogenic signaling pathways, namely the $\alpha_2\beta_1$ integrin, allowing precise control over the response of host cells to orthopaedic implants. We hypothesize that the suboptimal response to uncoated PCL scaffolds results from non-specific protein adsorption and unregulated signaling. Finally, the use of a synthetic peptide eliminates concerns about disease transfer. This biofunctionalization strategy for synthetic bone substitutes provides a simple and elegant approach to bone regeneration that could easily be translated to a clinical setting.

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