Effect of Recombinant Fibronectin Fragment Coating on Osseointegration of Stainless Steel Screws in Healthy and Osteoporotic Rats

Rachit Agarwal[1], Cristina González-García[1, 2], Brennan Torstrick[1], Robert E. Guldberg[1], Manuel Salmeron-Sanchez[2], Andrés J García[1]

[1]Mechanical Engineering, Georgia Institute of Technology, Atlanta, Georgia

[2]Biomedical Engineering Research Division, University of Glasgow, Glasgow, UK

Statement of Purpose: Current surgical treatments for fractures and joint arthroplasties primarily use metal implants for structural support and stability. However, one of the major challenges with metal implants is their failure due to implant micromotion, inflammation and bone resorption and osteolysis due to implant loosening, wear and improper loading[1, 2]. To facilitate strong tissue integration, presentation of extracellular matrix motifs from implant surfaces has been proposed. The objective of this study is to engineer fibronectin mimetic coatings by a simple one-step passive adsorption on clinical grade stainless steel (SS) implants.

Methods: Fibronectin type III fragment 7-10 (FN7-10) was cloned and expressed in E.coli[3]. Human mesenchymal stem cells (hMSCs) were obtained from Lonza, cultured in Lonza MSCM and SingleQuots, and passaged every 3 to 4 days. ALP activity and mineralization (alizarin red staining) was quantified at 9 and 21 days after cell seeding in osteogenic media. Osteoporotic (OVX) rats were generated by bilateral ovariectomy 3 months prior to scaffolds implantation. Osteoporosis was confirmed by bone density measurements using µCT. SS 316 grade M2 screws (McMaster Carr) were incubated in 50 µg/mL FN7-10 for 30 min. Screws were implanted in the tibial metaphysis of osteoporotic and sham rats. Implant pull-out testing was performed after 4 weeks using Instron to measure the force required for implant failure.

Results: Human mesenchymal stem cells (hMSCs) showed enhanced attachment and spreading on FN7-10 coated SS coupons compared to uncoated coupons (Fig. 1 A-D). When cultured in osteogenic differentiation media, hMSCs expressed elevated levels of alkaline phosphatase (ALP) and increased mineral deposition (Fig. 1E-F) on FN7-10 coupons indicating differentiation into osteoblasts. FN7-10 coated screws and uncoated screws were implanted into the tibial metaphysis of each osteoporotic and sham rats. In both, sham and osteoporotic rats, FN7-10 coated screws exhibited significantly higher mechanical fixation at 1 month time point (Fig. 2A-B).

Conclusions: We have shown that fibronectin fragment coatings on SS surfaces promotes osteogenic differentiation of hMSCs. When applied on scaffolds implanted in rat tibial metaphysis, FN7-10 coatings enhanced early implant integration with host bone tissue. Such coatings are easy to apply intra-operatively and can lead to rapid translation in clinics.

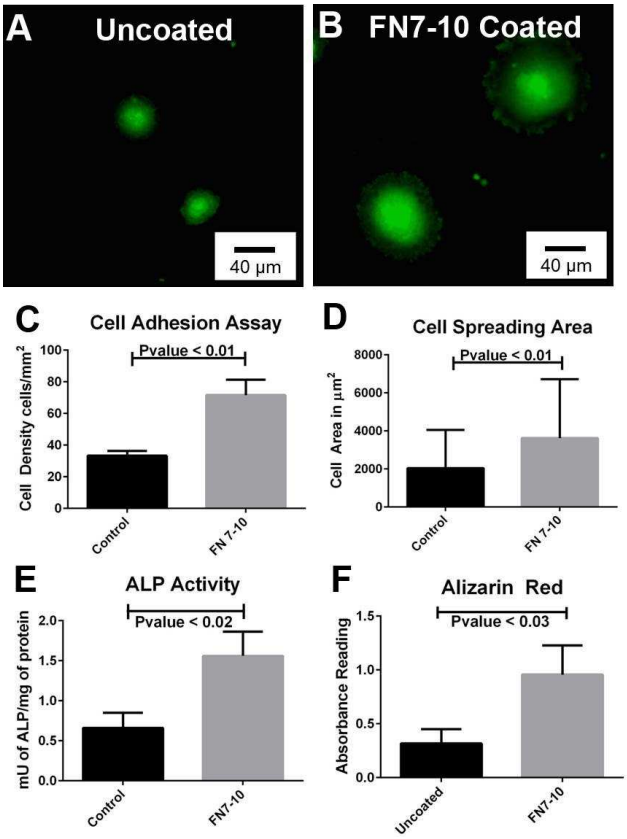

Fig. 1: Calcein staining of hMSCs cultured on A) Uncoated SS coupons and B) FN7-10 coated SS coupons. Quantification of C) cell number and D) cell spreading on SS coupons. E) Alkaline phosphatase activity at 9 days and (F) mineralization at 21 days (Alizarin red staining) for cells cultured on SS coupons

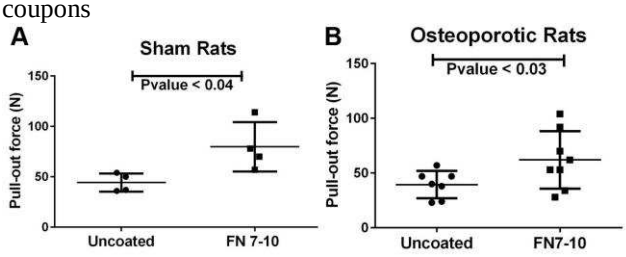

Figure 2: Screw pull-out measurements from A) Sham rats at 1 month and B) osteoporotic rats at 1 month

References:

1. Abu-Amer, Y.; Arthritis Res Ther 2007, 9, 1-7.
2. Bauer, T. W.; Skeletal Radiol 1999, 28, 423-432.
3. Petrie, T. A.; Biomaterials 2006, 27, 5459-70.

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