Dual Biofactor Release from Acellular Hyaluronic Acid Scaffolds for Cartilage Repair in a Pig Model

Anthony Martín, BSE[1,2]
Jay Patel, PhD[1,4]
Michael Eby, MD[1,2]
Mackenzie Sennett, BS[1,4]
Hannah Zlotnick, BSE[1,3]
Kamiel Saleh, BSE[1]
Andrew Chang, BS[2]
Matthew Davidson, PhD[3]
James Carey, MD[1,2]
Jason Burdick, PhD[3]
Robert Mauck, PhD[1,3,4]

1 McKay Orthopedic Research Laboratory
University of Pennsylvania

2 Perelman School of Medicine
University of Pennsylvania

3. Department of Bioengineering
University of Pennsylvania

4 Translational Musculoskeletal Research Center
Philadelphia VA Medical Center

Introduction

Large focal cartilage injuries often progress to osteoarthritis, a costly epidemic affecting over 30% of adults in the United States[1]. To treat these patients, we developed a cell-free hyaluronic acid (HA) scaffold with two embedded signaling proteins designed to enhance cartilage repair: Stromal Cell-Derived Factor-1α (SDF-1α; SDF) chemokine to increase the recruitment of mesenchymal stem cells (MSC)[5] and Transforming Growth Factor-β3 (TGF-β3; TGF) to enhance cartilage regeneration[6]. The objective of this study was to evaluate the effect of SDF and TGF incorporation into electrospun nanofibrous HA scaffolds on cartilage regeneration in a large-animal full-thickness chondral defect model. We hypothesized that SDF and TGF would synergistically improve cartilage defect repair

Materials and Methods

Scaffold Fabrication

Four scaffold groups were tested: 1) Scaffold (w/o biofactor), 2) SDF, 3) TGF, and 4) SDF + TGF. A solution of Methacrylated HA (76kDa, 45% mod, 4% w/v), polyethylene oxide (PEO, 900kDa, 2% w/v), and photoinitiator (Irgacure 2959, 0.05% w/v) in ddH20 was electrospun into nanofibrous scaffolds[6], +/− growth factor. Samples (4.5mm diameter) containing protein had a theoretical maximum of 21.25ng SDF and/or 106.29ng TGF, embedded within the electrospun nanofibers

Scaffold Characterization

Scaffold degradation (uronic acid assay; n = 8), and biofactor release (ELISA assays; n = 5) were measured. To determine *in vitro* bioactivity, scaffolds were co-cultured with bovine MSC pellets (250,000 cells) for 5 weeks. Pellets were analyzed with DMMB (sGAG) and Pico Green (DNA) assays (n = 4), and sectioned for histology (n = 4). Scaffolds were seeded with bovine MSCs (100,000 cells), cultured for 1 week, and imaged on a confocal microscope to measure infiltration (n = 5).

Animal Model

6 male juvenile Yucatan Minipigs underwent bilateral stifle joint surgery[6]. In each knee, 4 full-thickness 4mm trochlear cartilage defects were created, followed by microfracture (MFx). 3 defects per joint were loaded with identical scaffolds to prevent protein cross-contamination, and 1 defect per joint was left as a MFx control (Figure 1A) (n = 3 animals, 3 replicates per knee). Animals were euthanized 12 weeks post-op and underwent second-look arthroscopy (Figure 1B) for ICRS Cartilage Repair Assessment. Defect sites and healthy control regions (Figure 1C) were harvested as osteochondral blocks and mechanically tested using a 2 mm spherical indenter for equilibrium modulus at 30% strain. Samples were then sectioned for evaluation using the ICRS II Histology Scoring system.

Results

Scaffolds degraded uniformly over time with roughly 50% degradation at 5 weeks. ~40%max SDF was released after 7 days incubation and ~45%max TGF was released after 3 days. MSC pellets cultured with scaffolds releasing TGF showed increased proteoglycan and DNA content. MSCs seeded onto scaffolds releasing SDF and/or TGF showed greater infiltration into scaffolds. Second look arthroscopy did not reveal any significant difference between groups. Indentation testing showed the TGF scaffold group had a higher equilibrium modulus than the MFx group (Figure 2). ICRS II Histology

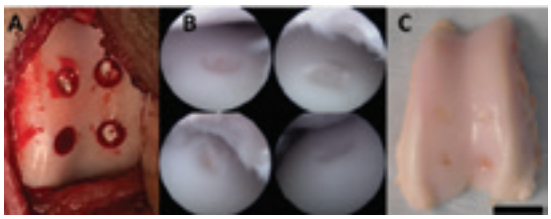

Figure 1. Cartilage defects in a minipig trochlea. **(A)** Scaffolds implanted in 4mm defects at the time of implantation. **(B)** Second-look arthroscopy 12 weeks post-op. **(C)** Gross trochlea harvested 12 weeks post-op, same samples as in B, S+T group (scale bar = 10mm).

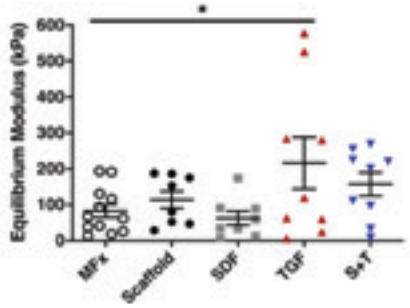

Figure 2. Equilibrium modulus of repair tissue measured via indentation testing at 30% strain.

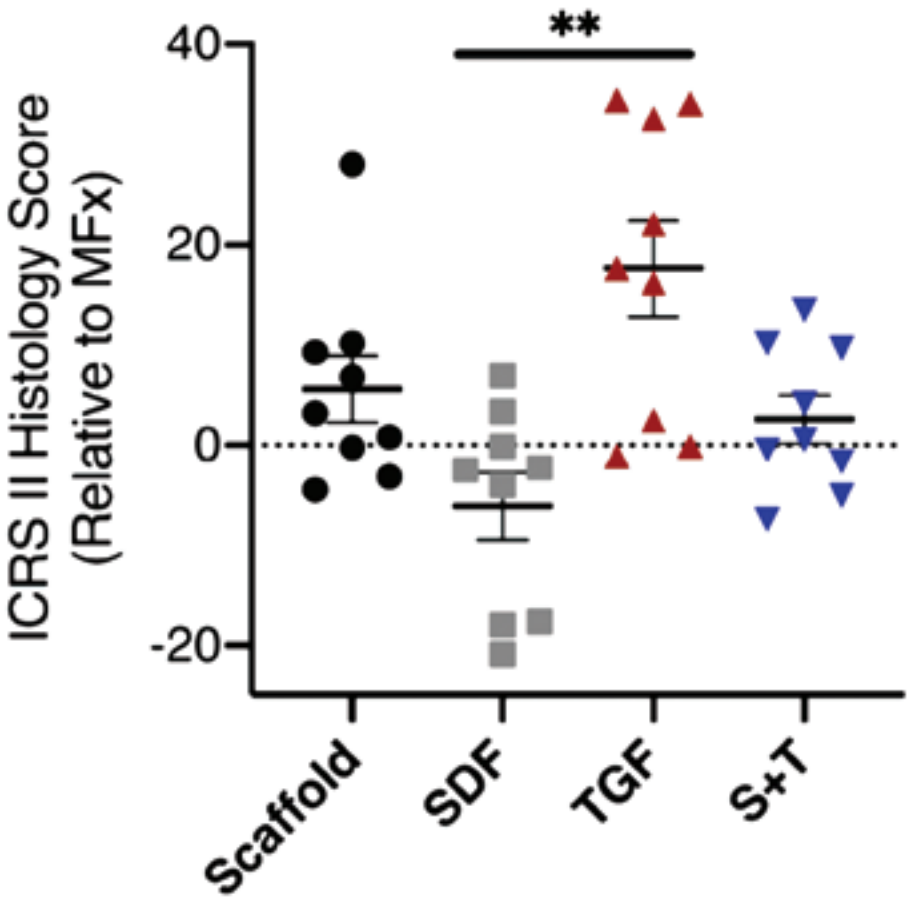

Figure 3. ICRS II Histology scoring by 3 blinded reviewers. * = $p < 0.05$, *** = $p < 0.0005$

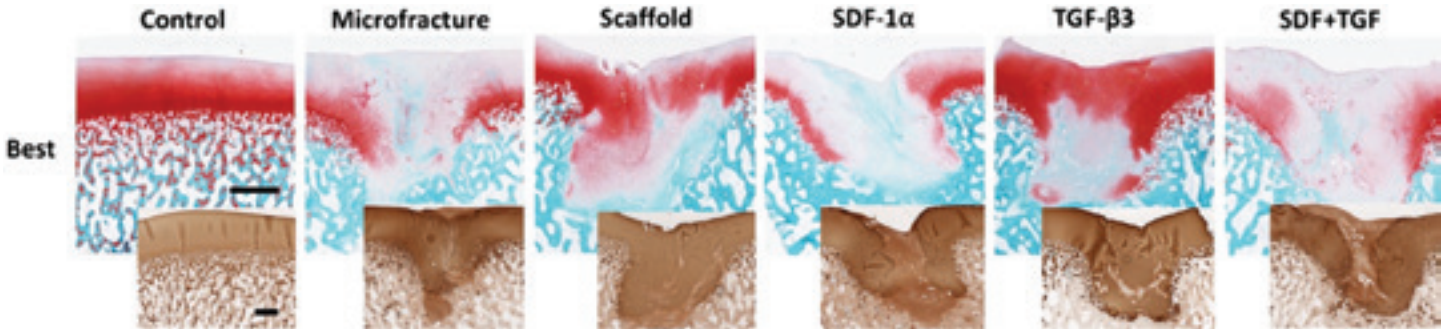

Figure 4. SAfO/FastGreen staining and Type 2 Collagen immunohistochemistry of the best-scoring sections from each group. Scale bar = 2mm

scoring revealed the TGF group outperformed the SDF and S+T groups, but not the drug-free scaffold group (Figure 3). While SDF seemed beneficial *in vitro*, it was detrimental in the large animal model, possibly via the recruitment of inflammatory cells.

Conclusions

This study demonstrates the ability to incorporate more than one biofactor into electrospun HA scaffolds, which could serve as a platform for a variety of regenerative medicine applications. We failed to prove our hypothesis that SDF-1α would act synergistically with TGF-β3 to improve *in vivo* cartilage regeneration.

Acknowledgments

This work was supported by the AOSSM and the NIH NCATS.

References

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