Resorbable Pins Enhance Retention of Nanofibrous Scaffolds in a Porcine Focal Chondral Defect Model

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Introduction

The repair of focal cartilage lesions remains a challenging issue in orthopaedics. Left untreated, large focal lesions may progress to osteoarthritis, ultimately requiring total joint replacement[1]. Current interventions, such as microfracture, result in the formation of fibrocartilage and have poor long-term outcomes[2]. Emerging technologies that involve scaffold placement for cell delivery, such as matrix-induced autologous chondrocyte implantation (MACI), have recently been reported to have superior outcomes[3]. Indeed, the success of early scaffold-based approaches has encouraged considerable innovation in the field of cartilage tissue engineering. As these new scaffolds and constructs emerge, one important consideration is the need for a reliable fixation technique for these various formulations. Previous work by our group evaluated the ability of a subchondral bone anchor (Mitek Microfix, Depuy) to support the retention of a 3D woven poly(ε-caprolactone) (PCL) scaffold in a full-thickness chondral defect in a large animal model[4]. While this fixation method provided adequate retention, it may not be suitable for all scaffold materials and resulted in notable disruption of the subchondral trabecular bone. Thus, the objective of this study was to evaluate additional fixation techniques that could be applied to multiple scaffold compositions in various defect geometries in a large animal model.

Methods

Full-thickness chondral defects were created unilaterally in the trochlear groove of 6 adult male Yucatan mini-pigs following a minimally invasive arthrotomy[5]. In 3 pigs, four 4mm-diameter defects (n=12 defects total) were created, with 10 receiving an electrospun nanofibrous hyaluronic acid (HA) scaffold. Scaffolds were fixed with press-fitting (n=2), fibrin glue (Tisseel, Baxter, n=2), or resorbable pin (Aesculap AG, n=6). In the other 3 pigs, two 8mmx4mm oblong defects were created (n=6 defects total), with five of the defects receiving nanofibrous

PCL scaffolds fixed with two resorbable pins. All defects were subject to bone marrow stimulation via microfracture. The remaining two circular and one oblong defects served as microfracture controls. Animals were allowed to weight-bear immediately after surgery and were euthanized after 2 weeks. Gross evaluation, micro-computed tomography (micro- CT), and histology were used to assess scaffold retention, quality of adjacent and opposing cartilage surfaces, and bone morphometry. Additional biomechanical tests were performed to assess the failure load of the pin in ex vivo osteochondral samples. Pins were inserted into osteochondral explants with a loop of 4-0 vicryl suture around the head. The suture was tensioned at a rate of 0.05mm/s with an Instron mechanical tester until pin failure occurred (n=3). Fisher's exact test was used to compare retention rates between scaffolds fixed with pin to non-pin fixed scaffolds.

Results

Press-fitting and fibrin fixation resulted in no retention of HA scaffolds in the 4mm defects (PF: 0/2, Fib: 0/2). Pin fixation resulted in complete retention of HA scaffolds in the 4mm defects (6/6) and almost complete retention of PCL in the 8mm oblong defects (4/5). The overall retention rate for pin fixed scaffolds was significantly greater than non- pin fixed scaffolds (91% vs. 0%; $p<0.05$). Gross observation of the adjacent cartilage revealed no damage and India ink staining of the patella confirmed that no patterns of abnormal wear had occurred from the head of the pin. (Fig. 1). Micro-CT confirmed gross evaluation of scaffold retention and demonstrated normal trabecular architecture surrounding the pin (Fig. 2). Histological analysis revealed normal safranin O staining of the adjacent cartilage and very little staining of the repair tissue, which was fibrous and hypercellular at this early time point (Fig 3). Ex vivo mechanical testing of the pin showed that the failure strength was 7.4 +/− 1.8N, with pin failure occurring at the mid-substance.

Discussion

Our results conclusively show that bioresorbable polylactide pins provide reliable fixation of multiple scaffold materials in various defect geometries in a mini-pig chondral defect model. All adjacent and opposing cartilage surfaces were healthy and intact, indicating that

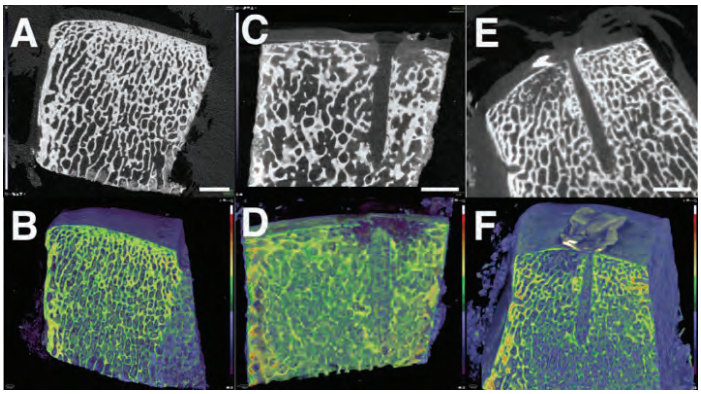

Figure 1. Micro-CT images and heat-mapped volume renderings (warmer colors indicate increased BMD). **(A,B)** Control sample from a non-operative limb showing healthy cartilage and normal trabecular architecture and bone mineral density for an adult mini-pig. **(C)** Image showing intact pin and HA scaffold within a 4mm defect. **(D)** Volume rendering shows normal trabecular architecture and bone mineral density. **(E)** Slice through 8mm oblong defect showing pin and radio-opaque PCL scaffold retained in the same plane as the surrounding.

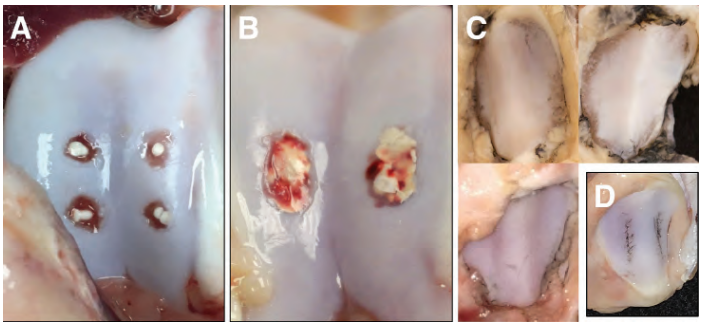

Figure 2. Post-mortem gross images. **(A)** HA scaffolds fixed with single pins in 4mm defects. **(B)** PCL scaffolds fixed with 2 pins in 8mm defects. **(C)** India ink-stained patellae demonstrating a lack of abrasions from pin implantation. **(D)** Control patella stained with India ink after ex vivo abrasion with scalpel (positive control for staining).

these pins did not cause any detrimental mechanical wear during joint motion. Unlike the bone anchor we previously evaluated[4], this pin did not cause major disruption or incur remodeling of the subchondral bone. While histological analysis revealed proteoglycan- deficient, fibrous repair-tissue, we did not expect extensive repair at this early time-point. The ability of this pin to retain nanofibrous cartilage repair

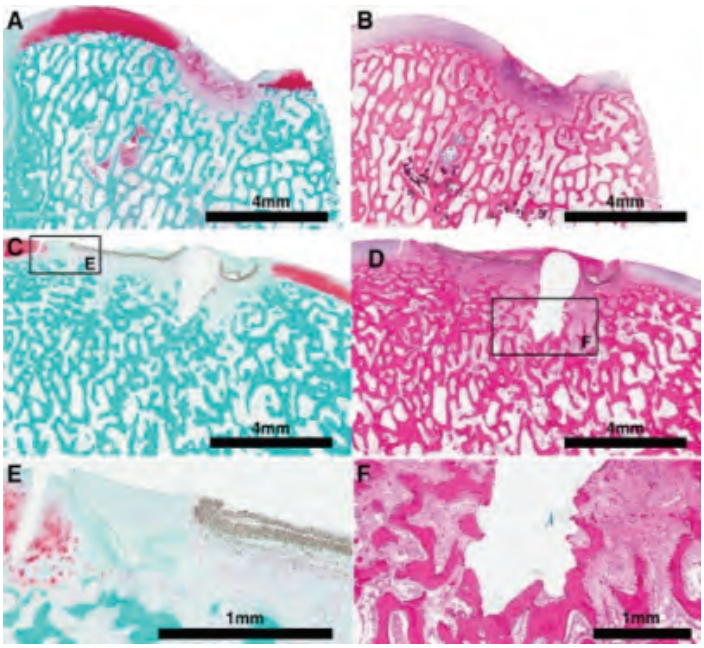

Figure 3. Histology of 4mm and 8mm defects. **(A&B)** SafO/FG and H&E staining of 4mm defect show HA scaffold retained above the subchondral plate. **(C&D)** Pin-fixed PCL scaffold retained above the subchondral plate. **(E)** Edge of scaffold and fibrous tissue at defect border. **(F)** Normal trabecular architecture surrounding site of pin insertion.

scaffolds in a focal chondral defect with no associated damage to surrounding cartilage or disruption to subchondral bone make it a promising fixation technique for long-term cartilage repair studies using advanced scaffold formulations.

Significance

These findings demonstrate a reliable fixation technique that will enable long-term studies of cartilage repair scaffolds in a clinically-relevant large animal model.

References

1. **Messner +** 1996 *Acta Orthop Scand.*
2. **Solheim +** 2014 *Knee Surg Sports Traumatol Arthrosc.*
3. **Saris +** 2014 *Am J Sports Med.*
4. **Friedman +** 2017 *Cartilage.*
5. **Bonadio +** 2017 *J Exp Orthop.*