Optimization of a Novel Scaffold for Cartilage Repair

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Introduction: Articular cartilage defects of the knee are frequently observed. Curl et al. described 53,569 hyaline cartilage lesions in 19,827 patients undergoing knee arthroscopy1. Lesions in articular cartilage can cause considerable musculoskeletal morbidity, with significant economic and social implications. Because of their poor spontaneous repair potential, these lesions present a clinical treatment dilemma, particularly in young and active individuals1. A frequently used method of treatment is Microfracture (MFX); with the purpose being to make a rough subchondral surface with the use of an awl to create several holes 3-4 mm apart and create conduits for marrow stromal cells (MSC) to migrate into the defect2. A list of potential problems with MFX include the possibility of an uneven repair fill, a mixed quality of resultant tissue2, and restriction in the clinical size of defects capable of being treated (95%) morphology, which together with the biocompatible composition fast resilient recovery and unique cross-linked controlled engineered degradation mechanism have the essential features for effective and efficient cell culture and organized tissue ingrowth making these unique biodegradable PSPU-U scaffold matrices the ideal platform for potential cartilage growth /repair.

Methods: In Vitro: A short term degradation scaffold and a long term degradation scaffold that will structurally collapse after 4 and 16 months, respectively, were used. The short and the long term scaffold were seeded with isolated articular chondrocytes. A concentration of 2x10^6 cells/ml were seeded onto scaffolds (dimensions 16mm diameter by 2mm thick) and allowed to thoroughly permeate scaffold to saturation. Samples were harvested at 3 and 8 days after culture, then were fixed and observed with scanning electric microscope (SEM). Cell proliferation was measured with the pico green assay. RNA was extracted for gene expression analysis. The differences were analyzed with two tail t test.

In Vivo surgical method: 30 male Sprague-Dawley rats were used. Each was anesthetized and placed in the supine position. Bilateral median parapatellar arthrotomies were made. A 1.5 mm osteochondral defect was created midline on the trochlea of the femur. Each knee was randomized to one of two treatment groups. The contralateral knee served as the empty control. The Treatment groups include: 1. Empty osteochondral defect (N = 30), 2. PSPU-U scaffold short term degradative profile (N = 15), and 3. PSPU-U scaffold long term degradative profile (N = 15). After seeding the scaffold, the rats were then allowed unrestricted activity as tolerated. They were sacrificed at 4, 8, and 16 weeks. The rat knees were then sectioned, decalcified, embedded in paraffin and cut into 7 µm sections for histologic examination with Safranin O/Fast green. The cartilage defect was measured and compared between implantation side and controlateral control side. The differences were analyzed with paired two tail t test.

Results: In Vitro: SEM observation showed nodules formation in the chondrocyte seeded scaffolds (Fig. 1). Pico green assay showed cell number slightly increased at day 8 compared to day 3 in both kinds of scaffolds. The short term degradation scaffolds had more cells than long term degradation scaffolds.
Long term degradation scaffold groups showed higher levels of aggrecan expression than short term degradation scaffolds. Long term degradation scaffolds had higher levels of type II collagen expression than compared with short term degradation scaffolds but the differences did not reach statistical significance. The expressions of both type II collagen and aggrecan maintained at a similar level between day 3 and day 8 in each group individually (Fig. 2).

In Vivo: At 4, 8, and 16 weeks the control knees exhibited fibrous tissue ingrowth at the cartilage defect. In the short term scaffold group, at 4 weeks the resilient conformal scaffold interfaces tightly with the surrounding tissue. At 8 weeks, newly migrated chondrocytes are seen at the defect-subchondral bone interface. At 16 weeks, the defects show new subchondral bone formation. The same is seen in the long term degradation group except at 16 weeks good bone repair is seen with less cartilage repair than compared with the short term. After measuring of the cartilage defects, the size of defects was seen smaller in the implantation groups compared to control groups (Fig. 3).

![Short term degradation scaffold](image1)

![Long term degradation scaffold](image2)

Fig 1. SEM observation of cells on scaffold for 8 days. Cells were seen growing on the surface and inside of both kinds of scaffolds.
Fig 2. Expressions of type II collagen and aggrecan of chondrocytes were maintained at a similar level between 3 days and 8 days in each group individually with in vitro culture. Both genes were seen had a higher level in long term degradation scaffolds. *: p<0.05; **: p<0.01
Discussion: In our study, we are able to demonstrate that chondrocytes will proliferate on both kinds of scaffolds. Short term scaffolds had more cells than long term degradation scaffolds after 3 days with lower level of expression of p16INK4a. This indicates cell proliferation on the short term scaffold is more active at the early stage of cell culture. Long term scaffold showed higher levels of type I collagen, type II collagen, and aggrecan expressions. Although increased levels of type II collagen were not significant. The expression of both of type II collagen and aggrecan maintained similar level between day 3 and day 8, which indicated no significant dedifferentiation of cells on the scaffolds.

Fig 3. Histologic slides of tissue ingrowth into the cartilage and subcondral bone defects at 16 weeks. The size of the cartilage defect was measured and the implantation side was compared to the contralateral control side. *: p<0.05; #: p<0.1.
A novel PSPU-U cartilage repair scaffold supports early migration of chondrocytes into articular cartilage defects. The short term degradative profile scaffold resulted in new subchondral bone formation and better cartilage repair than the long term degradative profile. Although the long term scaffolds had higher expression of type II collagen and aggrecan than the short term scaffolds in vitro, the in vivo study showed a better defect repair with short term scaffolds by 16 weeks. We consider that the degradation of the short term scaffold promoted the remodeling of the tissue with mature bone and cartilage at early time points post surgery. The growth of cartilage and subchondral bone was simultaneous. The porous structure of the scaffolds allowed stem cells from bone marrow to repair both cartilage and subchondral bone.

**Significance:** While microfracture is successful in the regeneration of small cartilage defects, it has a wide variance in the quality of the cartilage fill. Cartilage repair is thought to be directly proportional to the recruitment of MSC’s to the wound site. In future studies, we plan to evaluate the scaffolds in the context of MSC’s combined with growth factors. We plan to incorporate MSC’s onto the scaffolds, which may enhance a potential cartilaginous repair versus the scaffold alone. We hypothesize these future studies would demonstrate phenotypic conversion to cartilage of MSC’s compared to chondrocytes. The implications of the research are multifaceted, but the clinical significance will be achieved by ultimately incorporating the scaffold into the microfracture procedure, which effectively causes bleeding subchondral bone to attract MSC’s to the repair site.

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