Human Chondrocyte-Assisted Meniscal Healing Using A Platelet-rich Plasma Pretreated PLGA Scaffold

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Disclosures:

Introduction: The meniscal lesions occurring in the non-vascularized inner two-thirds heal poorly or not at all. A cell-based tissue-engineered construct can be employed for treating these intractable lesions [1-4]. Recently, we developed a dynamic cell-seeding technique and demonstrated the healing capacity of the swine chondrocyte-seeded PLGA mesh scaffolds in a novel in vivo swine meniscus repair mouse model [2, 3, 4]. Nevertheless, utilized as a scaffold, it should provide enhanced cell attachment and uniform cell distribution that will result in reducing donor cell number and optimal tissue repair and regeneration once implanted in vivo. Moreover, before clinical application of cell-based tissue engineered approach, it is essential to confirm that the similar healing capacity can be achieved using human cell and human meniscus. Platelet-rich plasma (PRP) is well-known as a storage vehicle for various growth factors and a good candidate substance for surface treatment of biomaterials [5]. In a previous unpublished study, we developed a novel surface modification technique for the PLGA mesh scaffold using centrifugal PRP treatment. The objective of this study was to test the hypothesis that platelet-rich plasma (PRP) pretreatment on a PLGA mesh scaffold enhances the healing capacity of the meniscus with human chondrocyte-seeded scaffolds in vivo, even when the seeded number of cells was reduced from 10 million to one million.

Methods: PRP and scaffold preparation: Allogeneic leukocyte-depleted PRP was obtained from our hospital blood bank. The mean platelet count in the PRP ranged from 1,000/ml to 1,300/ml. PRP of 5 donors was mixed and used for the experiments. The woven PLGA mesh scaffold (Vicryl™, Ethicon) measuring 20x8 mm (thickness, 0.2 mm) was prepared.

Centrifugal PRP treatment: The scaffolds were inserted into the bottom of 6 well plates and immersed into 1,000 μl of PRP. The plates were centrifuged at 150g for 10 min and incubated at 37 °C at an oscillation rate of 70 Hz for 60 min. Then, the scaffold was flipped 180° and the same procedure was done for the other side. After washing three times with DPBS (Dulbecco’s phosphate-buffered saline), the scaffolds were soaked into 1,000 μl of DMEM media at 37 °C for 10 min. The calcium in the media triggered the clotting cascade to form a fibrin network on the scaffolds.

Human chondrocyte isolation and seeding: Human articular chondrocytes were isolated from femoral head cartilage of 10 patients that underwent total hip arthroplasty after informed consent and approval of our IRB. At 80-90 % confluency after the first passage, the chondrocytes (P1) were harvested, mixed, labeled with Dil dye, and seeded to the scaffold. The scaffolds were placed in 15 mL polypropylene tubes containing one million chondrocytes in 5 mL of culture medium. The tubes were placed horizontally into a bidirectional rotator at 37 °C at an oscillation rate of 40 Hz for 7 days [4]. Scaffolds not pre-treated with PRP were also seeded with cells in the same manner, and serviced as a control.

Cell response evaluations: Cell distribution and viability on the scaffold was observed with a live/dead viability assay. Cell number for each cell-seeded scaffold was determined using a cell counting kit (CCK-8). The microstructural changes were observed with a field emission scanning electron microscopy (FE-SEM).

Animal experiment: After 7 days of culture, scaffolds were placed between human meniscal discs and were implanted subcutaneously in nude mice for 6 weeks (n=16 per group).

Statistics: The data was presented as average ± standard deviation and were compared via Mann Whitney test or Pearson chi-square test. Differences were considered significant for p values < 0.05.

Results: Fluorescence microscopy demonstrated that chondrocytes were uniformly attached throughout the scaffolds after 24 hours of dynamic seeding. The fluorescent images showed more cells on PRP-treated scaffolds at day 1 and day 7 of culture compared to non-treated scaffolds. Cell attachment analysis revealed a significantly increased number of chondrocytes on PRP-pretreated scaffolds than non-treated scaffolds (p<0.05). Mean fold increases of cell number on day 7 normalized with those on day 1 was not significantly different between the treatment and control groups. FE-SEM observation showed that chondrocytes attached to the PRP-pretreated scaffolds interconnecting their cellular process with the fibrin network at 24 hours and day 7 of culture. Of the 16 constructs containing PRP-pretreated scaffolds implanted in mice, six menisci healed completely, nine healed incompletely, and one did not heal. Histological evaluation demonstrated a continuous hypercellular new fibrous and cartilaginous tissue integrating into the native devitalized meniscus disc tissue in healed samples. Histologic results from the 16 control constructs revealed none had healed completely, four healed incompletely, and 12 did not heal. The histological outcome between the groups was significant (p<0.05) (Figure 1). Dil-labeled cells were present along the entire interface of the meniscal discs suggesting that healing by the cells seeded onto the scaffold (Figure 2).
Discussion: Human articular chondrocytes on PRP-pretreated PLGA mesh scaffolds demonstrated enhanced healing capacity of meniscus in a meniscal repair mouse model. PLGA scaffold modification using PRP pretreatment leads to similar healing capacity of the cell-seeded scaffolds in vivo even reduced number of cells was seeded compared to the previous study (1 million cells per scaffold vs. 10 million). These findings demonstrate that the PLGA scaffold modified by PRP pretreatment provides more biomimetic and biocompatible surface for chondrocytes and enables to use reduced number of donor cells.

Significance: Our results support a novel implant-assisted meniscal repair that can be used as a tissue engineered approach for meniscal tear lesions of the avascular zone.

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Figure 1. Photomicrographs of constructs after 6 weeks in vivo. (A) Construct containing PRP-pretreated scaffold demonstrating completely healed interface. (B) Construct containing non-treated scaffold demonstrating no evidence of healing between tissues. (Arrow heads indicate interface.) (Original magnification, 100×)

Figure 2. DiI-labeled cells (bright red) are present along the entire interface of the meniscal discs in both the upper and lower native meniscal disc tissues in a construct containing both PRP-pretreated scaffold (A) and non-treated scaffold (B). (Original magnification, 100×)