INTRODUCTION: The meniscus is vascularized only in the outer third and lesions occurring in the inner two-thirds heal poorly or not at all. Treatment for these types of lesions commonly involves partial meniscectomy, which can lead to long-term joint degeneration. A cell-based tissue-engineered construct can be employed for treating lesions occurring in the avascular inner zone of the knee meniscus [1-3]. In a pilot study, we have demonstrated healing of a meniscal lesion in a heterotopic in vivo mouse model using articular chondrocytes-seeded devitalized meniscus [1].

Woven Vicryl™ mesh (PLGA, Ethicon) is a biocompatible and biodegradable synthetic polymer used for abdominal wall defect closure or pelvic floor repair. This FDA approved material can be employed as a scaffold for meniscal repair because it is thin and flexible enough to interpose into the tear lesions. Recently, we developed a dynamic cell-seeding technique and a meniscus novel in vivo mouse model using a three-layered construct (chondrocyte-seeded PLGA mesh between discs) to assess the meniscal repair capacity of the scaffolds [2, 3].

The aims of this study were: 1) to assess the seeding and culture conditions (static and dynamic) for the scaffolds; 2) to assess the healing capacity of the cell-seeded scaffolds in a meniscal repair model; and 3) to assess in vivo cell labeling for tracking cells during the reparative process.

MATERIALS AND METHODS: Chondrocyte isolation and labeling: Under sterile conditions articular cartilage from 3-6 month-old swine was harvested, rinsed in PBS with antibiotics, and minced into 1-mm² pieces. The cartilage was digested in a 37 °C shaker for 12-16 hours in Ham's F-12 media containing 0.1% collagenase type 2 (Worthington Biochemical Co.). The exact cell count per milliliter was established using a hemocytometer and trypan blue to exclude dead cells. To be able to identify the cells implanted, the chondrocytes were labeled with PKH 26 dye (Sigma) according to the manufacturer’s instructions.

Scaffold and cell seeding: The scaffold used in this study was woven PLGA mesh (Vicryl™, Ethicon) cut into pieces measuring 20×8 mm (thickness, 0.2 mm). The scaffolds were placed in 15 mL polypropylene tubes containing 10 million labeled chondrocytes in 5 mL of culture medium. The tubes were placed horizontally into a bioreactor at 37 °C at an oscillation rate of 40 Hz for 24 hours.

In vitro culture: After dynamic cell seeding for 24 hours, medium was changed to discard unattached cells. Then, the half of cell-seeded scaffolds were cultured in static condition and the remaining half were cultured in dynamic condition at an oscillation rate of 40 Hz for 7 days. Cell distribution and viability on the scaffold was observed with Live/dead viability assay. Cellular DNA for each cell-seeded scaffold was determined using a Pico Green assay.

Meniscus preparation: Menisci were harvested from the knees of pigs under sterile conditions and cut into discs (6 mm diameter, 2 mm height). The meniscal discs were frozen at -20 °C then subjected to five cycles of freezing and thawing to kill all the native chondrocytes. Animal experiments: The IACUC of our hospital approved all animal procedures. Animal experiments were divided into four groups (A, B, C, D, n=8 for each group). Chondrocyte-seeded scaffolds were placed between devitalized meniscal discs and sutured in place with 7-0 prolene suture (A, dynamically cultured scaffolds and B, statically cultured scaffolds). Three-layered constructs in control group C were assembled with acellular scaffolds. Constructs from the negative control group D were sutured without placing an interpositional scaffold. All constructs were implanted into subcutaneous pouches in nude mice and harvested after a 6 weeks period.

Histologic analysis: From each explants, 5-µm-thick sections were obtained and stained with H&E. Frozen sections from explants containing PKH 26-labeled chondrocytes were examined with fluorescent microscopy.

RESULTS: The results from the Live/dead assays demonstrated that labeled chondrocytes were uniformly attached throughout the scaffolds after 24 hours of dynamic seeding. Although the cells covered all scaffolds completely, they were found to concentrate at the margins of the scaffolds at the end of the 8 days of culture in both dynamic and static cultured conditions. Live cells were more than 95% of total cells at both day 1 and day 8 of culture (figure 1-A). DNA measurement revealed a statistically significant difference in number of chondrocytes on the scaffolds for the dynamic versus the static conditions (figure 1-B).

DISCUSSION: Previous work has shown that static seeding of PLGA scaffolds resulted in poor cell attachment and non-uniform cell distribution [3]. These results demonstrate that the cells were attached uniformly throughout the scaffold using dynamic seeding conditions for as little as 24 hours. Culture conditions after seeding may not affect healing outcome since the articular chondrocytes-seeded woven PLGA scaffolds generate fibrocartilagenous healing tissue between meniscal discs placed in vivo in nude mice. The cells labeled with PKH 26 were visible in the repair tissue demonstrating that these cells are responsible for the healing process in the interface. These results suggest that lesions in the avascular zone of the meniscus can be repaired using isolated articular chondrocytes seeded onto a degradable PLGA scaffold providing a tissue engineering approach for the treatment of this frequently encountered clinical problem.

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