Gradient Microsphere Based Implants for Osteochondral Regeneration: A Long Term Study in Sheep

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Introduction: The study compared the performance of microsphere-based gradient implants to the conventional microfracture procedure for cartilage regeneration in sheep knee joints. The microsphere-based implants had gradients of material composition specific for osteochondral regeneration.[1] A single integrated gradient plug was designed to promote simultaneous regeneration of both bone and cartilage with proper integration at the interface toward treatment for human patients with focal joint cartilage lesions. Our hypothesis was that such a gradient plug would promote faster and complete regeneration compared to the conventional microfracture technique.

Methods: 18 female Rambouillet Columbia sheep approximately 3-4 years of age were used for the study. All procedures involving the use of live animals were approved by the CSU IACUC protocol #11-3150 and KU IACUC protocol #175-14. The gradient osteochondral scaffolds were made of continuous opposing gradients of poly(D,L-lactic-co-glycolic acid) - chondroitin sulfate (PLGA-CS) to poly(D,L-lactic-co-glycolic acid) - β-tricalcium phosphate (PLGA-TCP) microspheres from chondrogenic to osteogenic region. Group I consisted of 12 animals that received gradient implants and group II consisted of animals that underwent microfracture procedure. In Group I, osteochondral defects (6 mm diameter x 6 mm depth) were created in both the medial and lateral femoral condyles of right knee joints of the animals. In this group, animals received material gradient implants (Group I-A, n=6 knees), implants soaked in TGF β-3 (1 μg/implant) (Group I-B, n=3 knees) and implants soaked in IGF-1 (0.5 μg/implant) (Group I-C, n=3 knees). For Group II, the animals were subjected to a microfracture procedure (n=6 knees), where a standardized circular chondral lesion (6.0 mm diameter) was created in the medial and lateral femoral condyles. The cartilage within these limits was abraded down through the calcified cartilage layer, taking care not to penetrate the subchondral bone plate. Once the full-thickness cartilage lesion was created, 4 holes (~2 mm from defect edge and apart from one another) were created into the subchondral bone to a depth of 5-8 mm. The progress of regeneration was evaluated using 3T MRI at 4 months (n=3 knees). All groups had a single end point of 12 months, and the sheep were humanely euthanized according to the guidelines set forth by the American Veterinary Medical Association. The joints were retrieved, and the medial femoral condyles (MFC) and the lateral femoral condyles (LFC) were morphologically scored (max score = 10). Mechanical properties of the regenerated cartilage were evaluated with indentation stress relaxation (Instron 5848), and finite element analysis with the biphasic theory is in progress. Histology and immunohistochemistry will follow the mechanical evaluation.

Results: MRI Analysis: The 3D signal intensity of regenerated cartilage from the SPGR weighted sequences were compared to evaluate the quality of the cartilage. Figure 1 shows representative Sagittal Proton Density Fat Saturated images on the medial femoral condyles at 4 months. All the animals showed a similar response in the signal intensity of the regenerated cartilage. In all groups, two animals showed isointense signals while one animal showed mildly hypointense to normal signals in MFCs. In the LFC, the cartilage had mildly hypointense to normal signals in all the groups except the animals that received material gradients alone where the response varied from isointense to moderately hypointense.

Morphological scoring: Figure 2 shows a representative image of both condyles from each group at 12 months. The defect site is enclosed within the circle. In Group I, the animals that received material gradient scaffolds incubated in TGF-β3 performed better with an average of 82% and 89% tissue fill at the defect site on the MFC and LFC, respectively. The animals that received material gradients alone had a defect fill of 80% and 87% on MFC and LFC and the group incubated with IGF had a fill of 61% and 73%, respectively. The tissue fill was higher on the lateral side in all of the material gradient groups, while in contrast the microfracture group had higher tissue fill on the higher load bearing medial side.

The animals that received the material gradients alone had a higher morphological score of 7 in the LFC with the regenerated tissue appearing smooth and opaque, similar to native tissue and with 70% edge integration with the surrounding tissue, while the MFC had a total score of 6. Animals whose implants were incubated with TGF-β3 received a total morphological score of 6.7 on MFC and 6.2 on LFC, while those incubated with IGF had 6.3 on MFC and 5.7 on LFC. The tissue was opaque in all of the material gradient groups in both the MFC and LFC, while in the microfracture groups, the LFC condyles had repair tissue that was translucent.

In Group II, the average amount of repair tissue was 81% and 73% of the total defect area, and the total morphological score was 7 and 5.5 on the MFC and LFC, respectively indicating that they performed comparatively better on the higher load bearing MFC, but worse on the LFC. In a few of the lateral condyles, the repair tissue was translucent rather than opaque as observed in the medial condyle. In both the medial and lateral condyles, the repair tissue was slightly depressed and furthermore integrated.
Discussion: For the first time, the efficacy of microsphere-based gradient scaffolds with encapsulated extracellular matrix raw materials was presented in a load-bearing, large animal model. The implants performed better on the lateral side than on the medial side, and appeared to outperform microfracture on the lateral side. While soaking the implants in growth factor solution prior to implantation did not provide a clear benefit based on analyses so far, the mechanical testing data and immunohistochemistry will better evaluate relative differences.

Significance: The study compared the performance of microsphere-based gradient implants to the conventional microfracture procedure for cartilage regeneration in sheep knee joints. The microsphere-based implants had gradients of material composition specific for osteochondral regeneration. A single integrated gradient plug was designed to promote simultaneous regeneration of both bone and cartilage with proper integration at the interface toward treatment for human patients with focal joint cartilage lesions. Our hypothesis was that such a gradient plug would promote faster and complete regeneration compared to the conventional microfracture technique.

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