Histological and μCT Analysis of Biomaterial Scaffolds Implanted into Osteochondral Defects in Rabbit Knees with Rheumatoid Arthritis

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Statement of Purpose: Rheumatoid arthritis (RA) is generally believed to be caused by a combination of genetic, environmental, and hormonal factors but the exact mechanism of the autoimmunity initiation is not yet clearly understood due to its complicated etiology. To treat RA, various anti-inflammatory drugs have been employed. However, these drugs must be frequently injected into the patients for long time durations and is limited to treatment of those with minor RA symptoms, without restoration of tissue structure/function.

Tissue engineering has been recognized as a promising strategy for cartilage reconstruction and regeneration. In conjunction with great possibilities of minimally invasive and less complicated procedures for patients, cartilage tissue engineering has been motivated by the need of replacing lost or damaged tissue with an already structurally and mechanically functional implant that can be created *in vitro* using chondracytes in combination with biomaterials.

The objective of this research was to assess the influence of the RA situation on the host response to orthopedic biomaterials and to identify biomaterials which would be useful for tissue engineering in the RA situation as a basis on which to develop a TE strategy. Poly(lactic-co-glycolic acid) or agarose, with distinct and opposite effects on the phenotype of key antigen presenting cells, dendritic cells (DCs), which are involved in the initiation and propagation of RA, were used to form scaffolds (SCs) for implantation. The animal model presented here involves the combination of a biomaterial scaffold (SC) implantation into an osteochondral defect of the femoral condyle of a rabbit with existing induced RA. A rabbit antigen-induced arthritis (AIA) model was used to induce RA. In this study, the integration of the biomaterial SC and structure of joint tissue was assessed using histological assessment and micro-CT.

Methods: AIA RA was induced in male New Zealand white rabbits (3 kg) by immunization on day 0 with subcutaneous (SubQ) injection of ovalbumin (OVA) emulsified in Freund's Complete Adjuvant (CFA)[1]. On day 14, rabbits were boosted by SubQ injection of OVA and Freund's incomplete adjuvant (IFA), followed by arthritis induction by intra-articular injection of OVA/PBS into the right knee joint per rabbit on day 21[1]. One day later (day 22), porous PLGA or agarose SCs were implanted into the osteochondral defect formed in the right knee joint[2] (the left knee remained untreated as a within-animal control). On days 21, 22, 25, 29, and 36 (endpoint), joint swelling was measure to assess RA state, joint lavages and peripheral blood samples were collected to assess total leukocyte concentrations or differential leukocyte profiles in the joint or peripheral blood (these data are presented in a companion abstract). Histology and micro CT assessments of joints at the endpoint were performed and presented in a companion abstract. At the day 36 endpoint, whole intact knees were fixed in 10% formalin, decalcified in 10% formic acid, embedded in paraffin, sectioned at 5 μm thickness and stained for cellularity with H&E and for proteoglycan content with safranin-O. For micro-CT analysis, the distal femur of each knee joint was exposed and fixed. The samples were then incubated in 40% Hexabrix (contrast agent)/60% PBS. All scanning was performed in air using a μCT 40 (Scanco Medical) at a voxel size of 30 μm, E = 45 kVp, I = 177 μA, 200 ms integration time, and approximately 45 min acquisition time. Approximately 360 slices (4.3 mm) of each distal femur were scanned.

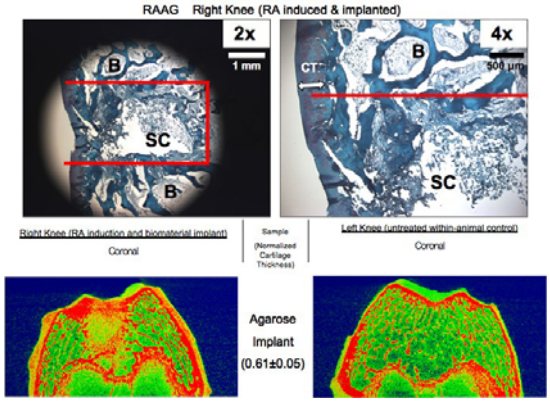

Figure 1. : Representative images of histological and micro-CT analysis on rabbit knee joint implanted with agarose with RA induction.

Results/Discussion: When comparing the right knee and the left knee, the control group of RA-induced rabbits exhibited higher value of x-ray attenuation in the right knee indicating a lower proteoglycan content. Furthermore, the right knee of rabbits with RA showed weak safranin-O staining for proteoglycan as compared to the contralateral knee. Interestingly, histological assessment showed that rabbits with induced RA with implanted agarose SCs exhibited a healed cartilage layer over the defect (Fig. 1) while defects implanted with PLGA SCs or left empty (sham) did not. Furthermore, rabbits with induced RA with implanted agarose SCs or without an implanted SC (empty defect sham) exhibited more pronounced bone healing into the defect site than for rabbits with RA and implanted PLGA SC. The micro-CT data showed a differential healing level and host response that was consistent with the histology data mentioned above.

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References:

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