Preparation of Artificial Biological Ligament and Experiment in vivo and Cell Culture.

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Statement of Purpose: Cruciate ligaments are an important component for intra-articular knee joints. Torn ligaments cannot repair themselves and will probably cause knee joint instability, resulting in articular degeneration, arthralgia and articular dyskinesia. Auto-graft reconstruction may have complications at the graft donor site, and allogenic grafting may result in disease and immunological rejection. There is also always the problem of not having enough auto- and allografts in supply. The goal of this project is to develop artificial ligaments to take the place of auto and allografts. We hope to develop an artificial biological ligament that can act as a scaffold for the anterior cruciate ligament(ACL) and will passively degrade, with the scaffold dissolving at the same rate as new ligament grows.

Methods: The raw material used came from Hyclone, USA., DMEM-LG nutrient fluid by Gibco, USA. Fresh pig tendons were tested for bioburden. Impurities were eliminated and pretreatments made. Cross-linking fixation was performed with epoxy, protein molecules were modified to raise the mechanical strength of the material, and diversified eliminations of antigen on the surface of the material were performed to make the material capable for adhesion to enriched growing fibers. The material was packaged, and sterilized using gamma rays, and thus yielded a finished product for ABL. Epoxy cross-linking fixation makes it a passive-degrading material.

Figure 1: Finished packaged product of ABL, scaffold for regeneration of host ligament.

In vivo: After they were anesthetized, the 28 goats operated on had their knees exposed via lateral incision and the ACL cut off. Drilling was performed with a 5 mm drill between the tibia and femur. Goats were sacrificed at 12, 26, 40, and 52 weeks after operation for sampling. Longitudinal and cross sections were also taken of the ABL and observed under SEM.

Cell Culture: The fibroblasts were cultured onto the material in polystyrene Petri dishes with media and allowed to grow at 37 □ C and 5% CO_2. The material was processed and stained with HE to observe cell growth after ~24, 48, and 72 hr. To test the effect of dynamic loading on the fibroblasts growing on the material, we applied dynamic stretching along the length of the material and observed the spread of the fibroblasts along the surface of the material. Force was applied at 1 s^{-1} and with 10-20% strain. The effects of dynamic loading were observed with histological analysis in HE staining and in control groups with no dynamic loading applied during the ~24-72 hr of growth.

Results: In vivo: One goat died the day after operation, while the rest stood up within 2-4 hr. Six of the goats showed post operative knee swelling which disappeared in 2-3 weeks. There was the greatest amount of synovial fluid at 12 weeks. At 26 weeks the synovial fluid was normal. At 40 weeks the toughness was close to that of a normal goat ligament, and at 52 weeks, the traces of scaffold had disappeared and the regenerated host ligament showed bright white. Under SEM observation, the goats sacrificed later had more collagen fibers parallel with the ABL, and at 40 weeks the collagen showed cross-linking.

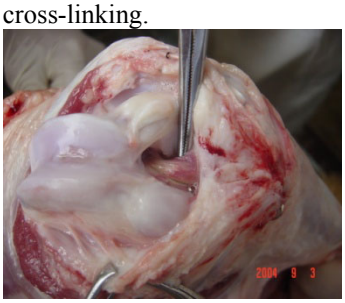

Figure 2: The observation of ABL at 3 months after implantation in a goat model.

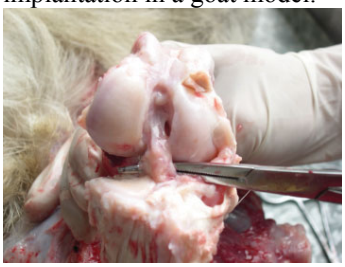

Figure 3: Observation of ABL at 12 months after implantation in a goat model.

Cell Culture: Primary cultured fibroblasts were able to grow within 24 hours of culturing on scaffold of the ABL. The cells adhered to the surface of the product. We observed that the fibroblasts were spread flatter on the surface of the material along the longitudinal force as dynamic loading was applied, compared to the control group.

Conclusions: In vivo: The porcine tendon-based natural tissue with multitreatment is a good substrate for the development of ABL. This material showed passive degradation behavior from epoxy crosslinking, making it suitable for an augmentation reconstruction of the ACL.

Cell culture: Dynamic loading on the substrate when cultured with fibroblasts will cause the fibroblasts to align in the direction of the dynamic load.

References:

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2. Wang K, Zhu L, Cai D, Zeng C, Lu H, Xu G, Guo X, Lin X, Chen S. Microsurgery. 2008;28:44-53.