

Tissue Engineered Human Anterior Cruciate Ligament Derived Cellular Patch for Partial ACL Repair

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Statement of Purpose: Partial tears of the anterior cruciate ligament (ACL) represent 10-28% of all ACL tears. Current treatment options are limited and include conservative modalities, thermal shrinkage of the remaining ACL, or ACL reconstruction. Studies have shown that up to 50% of patients with partial tears will progress to complete ACL insufficiency after non-operative management and fewer than 30% are able to return to their pre-injury level of activity^{1,2}. The viability of using biologics to enhance primary suture repair of partially torn ACLs has been an area of active investigation recently. Our lab has developed a protocol for the surgical retrieval, isolation and in-vitro expansion of human ACL derived cells (hACL) obtained from discarded ACL tissue. These cells were infused into an engineered 2D matrix to produce a patch intended to augment partial ACL repair and strengthen the torn ligament.

Methods: ACL stump specimens were obtained during routine knee arthroscopy. Tissue samples were minced into 1-2mm² pieces and digested with 0.4% Collagenase in DMEM/F-1 (50/50, IX) medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin (P/S) for 4-6 hours at 37° C. Cells were spun down in a centrifuge at 2000rpm for 5min and resuspended in the medium. Cells were washed and seeded in T-25 flasks. The media was changed when the cells attained desired morphology and became adherent. Once the cells were confluent, they were washed with PBS, trypsin was added and the cells were distributed amongst three T-25 flasks. The cells were then frozen using DMSO, FBS and the complete medium (DMEM/F-1 medium + 10% FBS + 1% P/S) in the ratio 1:2:7. Cells were stored in this medium at -80° C for the short term and in liquid nitrogen for longer durations. When needed for further use, the cells were thawed in a water bath at 37° C. The 2D PLAGA scaffolds were fabricated using a solvent evaporation method as described in the paper by Gupta et al¹. To evaluate biocompatibility, hACL derived cells were seeded on the PLAGA scaffolds and control TCPS (50,000cells/disk) and cultured over a 7day period. Cell adhesion, growth and morphology studies using Immunofluorescence staining and Scanning Electron Microscopy (SEM) were performed.

Results: The working model for surgical retrieval, tissue digestion and isolation of the hACL derived cells is shown in figure 1. The cells migrated from the explants and adhered to the T-25 flasks. These cells were cultured for 3 days and then were visualized under a light microscope. A confluent monolayer was obtained by day 7. The presence of healthy, viable cells indicated the successful retrieval and culture of hACL derived cells. SEM micrographs demonstrated that hACL derived cells adhered to and grew on PLAGA and control TCPS disks. Cells were almost confluent over the entire surface of the

scaffold and the TCPS. The morphology of the hACL derived cells was important to note in order to determine the cellular behavior on different surfaces. Immunofluorescence staining showed that hACL derived cells adhered to the polymer surface; grew and exhibited a normal, non-stressed pattern (spindle or elongated morphology) observed on both the experimental (PLAGA) and control (TCPS) surfaces.

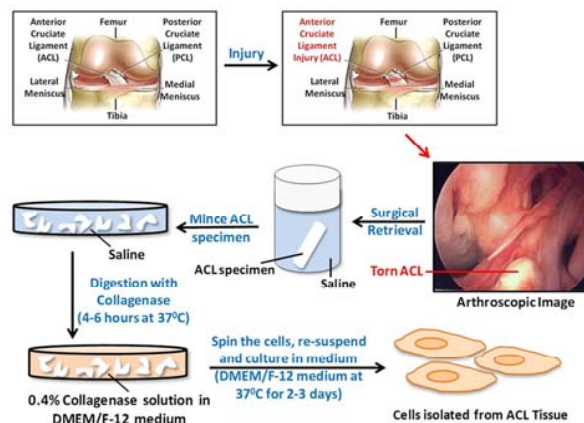


Figure1. Schematic representation of steps involved in the surgical retrieval, tissue digestion and isolation of cells from injured Anterior Cruciate Ligament (ACL).

Conclusions: Our results demonstrate that hACL derived cells can be isolated and expanded, and the PLAGA patch promoted growth. This technique is a reproducible and a reliable way to provide a potential cell source for a wide array of future investigations for ACL reconstruction. Our primary direction with the hACL/2D scaffold is for the future use as a patch to augment primary repair of partial ACL tears. Our protocol would allow a combination of suture repair with a 2D scaffold that offers a delivery system for hACL derived cells into the wound site while simultaneously protecting the cells from the synovial fluid environment. This could allow functional repair and healing of a partial tear, avoiding the comorbidities associated with ACL reconstruction. This technique demonstrates promising results and future investigation will display the potential of this technique for ACL repair and reconstruction.

References:

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3. Gupta A. J Orthop Res.2013;9:1374-1381.