0228 Strain-controlled organ culture of bone-ligament-bone human-derived anterior cruciate ligaments – an *ex-vivo* model to investigate degenerative and regenerative therapy

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Introduction:

Injuries of the anterior cruciate ligament (ACL) are among the most common ligament injuries.[1] Unresolved challenges in primary repair of ACL ruptures demanded the establishment of a culture system to study the regenerative capacities. The present study introduces an *in-vitro* culture model for human ACLs. Moreover, this study evaluates whether culturing ACLs in a strain-controlled dynamic loading bioreactor system is superior to a free-swelling static loading culture model.

Material and Methods:

A bioreactor to harbor and mechanically stimulate full human cruciate ligaments was designed and manufactured. Fresh and full ACLs with attached femoral and tibial bone were obtained from full-knee prosthesis surgery with written consent of the patients (ethically approved). ACLs were maintained for 7 days free-swelling in high glucose DMEM supplemented with 5% fetal calf serum (FCS) under either static or dynamic loading applying 7% dynamic strain with 0.2Hz. ACL cell activity and viability was determined close to the tibial region (T), the mid region (M) and close to the femoral region (F) by performing a resazurin salt cell activity assay and applying 3D stacks of confocal laser scanning microscopy (cLSM) on cells treated with a LIVE/DEAD staining kit. Additionally DNA content (Hoechst), collagen content (hydroxy-proline = HYP assay) and RT-qPCR, to check for relative gene expression of ligament specific markers, were screened.

Results:

Large differences in relative gene expression between each ACL sample were observed, indicating an inter-individual variability. Among the 3 different ACL zones of dynamically loaded ACLs, DNA content was higher in tissue close to the F zone than in the M and T zone. This trend was also noticed for cellular mitochondrial activity on day 1 (1480.9±415.4 vs. 844.7±155.8 RFU, respectively) as well as on day 7 (973.8±229.6 vs. 790.8±39.6 RFU, respectively). There was a trend that cell viability was higher in ACL cells cultured under dynamic loading conditions compared to cells cultured under static loading conditions (78.80±19.52% vs. 76.61±3.4% respectively, measured after 7 days). After 7 days of dynamic culturing, ACL-fibroblasts still expressed their ligament-specific genes (data not shown).

Discussion:

ACL cells are able to survive for 21 days and to maintain their phenotype in organ culture as shown by qPCR. DNA and cellular activity revealed that the ACL is an inhomogenous tissue in terms of cell density and cell phenotypes. The interest in tissue-engineered solutions grows leading to improved mechanobiological models of ACL culture, that allow testing of different regenerative approaches.

References:

1. Kiapour et al. (2014) Bone Joint Res 3:20-31.