

an influence on the differentiation of the cells. To simulate the forces exposed to native tissue dynamic culture experiment have to be done.

### 06.P07 Extracellular matrix based device for reconstruction of the temporomandibular joint (TMJ) meniscus

BN Brown, WL Chung, AJ Almarza, MD Pavlick, SN Reppas, MW Ochs, AJ Russell, SF Badylak  
University of Pittsburgh, USA

For a percentage of TMJ patients the only available treatment is meniscectomy, which results in wear of the articulating surfaces and leads to corrective surgeries. No alternatives exist for reconstruction, and removal without replacement is the 'gold-standard' for patient care. Urinary bladder extracellular matrix (UBM) was investigated as a scaffold for meniscus reconstruction. Five dogs were subjected to unilateral resection and replacement with UBM. Ten additional dogs were subjected to bilateral resection and replacement on only one side with UBM. The device remodeled rapidly and was indistinguishable from new host tissue. Remodeling was characterized by mononuclear and smooth muscle actin (SMA)+ cells at early time points changing with time to SMA- cells resembling those of the native meniscus. The remodeling process showed deposition of predominantly type I collagen, the density and organization of which resembled that of the native meniscus. Ingrowth of skeletal muscle tissue was observed at the periphery of the implant and was similar to that at the periphery of the native meniscus. Biomechanical testing showed that the mechanical properties were similar to those of native meniscus. No adverse changes in the articulating surfaces were observed in implanted joints. Results of this study suggest that the UBM device may represent an effective, off-the-shelf interpositional material while also serving as an inductive template for reconstruction of the TMJ meniscus.

### 06.P08 A novel triphasic composite silk fibroin scaffold with zonal release of multiple growth factors via microspheres delivery for integrated ACL-bone regeneration

YW Jiang, SL Toh and CH Goh  
Department of Bioengineering, National University of Singapore, Singapore

Development of an integrated ACL-bone tissue is always a challenge in orthopedics tissue engineering due to the composition of three different regions (ligament, fibrocartilage, and bone) at the interface. A promising strategy is to build a biomimic stratified scaffold with appropriate cell source for the matrix heterogeneity development which mimics the multi-tissue organization of the native ACL-bone interface. It is well known that the mesenchymal stem cell can undergo multiple lineages of differentiation under induction of different growth factors. Based on this, this study focuses on design of a novel triphasic composite silk fibroin scaffold with three distinct yet continuous layers, each layer being embedded with different growth factor loaded gelatin microspheres and the multi-lineage differentiation of the MSC on the triphasic scaffold. The zonal protein distribution, protein release kinetics and bioactivity were studied before cell culture on the scaffold and then cell proliferation, migration and differentiation were investigated and both genotype and phenotype results demonstrate the heterogeneity of matrix deposition as well as the formation of the fibrocartilage interface, which indicates the great potential of this biomimic triphasic composite silk fibroin scaffold for integrated ACL-bone tissue formation.

### 06.P09 Induction of tenogenic differentiation in human adipose stem cells by manipulating culture medium supplements

AI Gonçalves, MT Rodrigues, RL Reis and ME Gomes  
3B's Research Group, Univ. of Minho, AvePark, Portugal / ICVS / 3B's - PT Government Associate Laboratory, Portugal

*Introduction:* The limited ability of tendon to self-repair and the limitation of treatment regimes have hastened the motivation to develop cell-based strategies for tendon repair. Growth factors (GFs) such as EGF, FGF, PDGF and TGF- $\beta$  participate in tendon formation, ECM synthesis or healing, and may assist tenogenic differentiation. Thus, this work aims to establish culturing conditions that induce tenogenic differentiation of human adipose stem cells (hASCs) using these GFs.

*Materials&Methods:* hASCs were isolated and expanded in  $\alpha$ -MEM basic medium. Both freshly isolated and cryopreserved hASCs (P3) were further cultured with different supplements namely basic medium with glutamine (2 mM) and ascorbic acid (0.2 mM) plus i) EGF (10 ng/ml), ii) FGF (10 ng/ml), iii) PDGF (10 ng/ml) or iv) TGF- $\beta$  (10 ng/ml) for 7, 14, 21 or 28 days. hASCs differentiation into tenocytes was assessed by real time PCR analysis of the expression of collagen type I and type III, decorin, and scleraxis.

*Results:* In i) and ii) media, hASCs showed a tenocyte-like aligned distribution after 14 days, while in iii) and iv) it was only observed by 21 days. Overall, hASCs showed a higher gene expression in media i) and ii).

*Conclusions:* EGF and FGF evidence a higher potential to induce the expression of tendon-related markers in hASCs and the cryopreservation may influence the expression of these genes. This might be a useful approach to have high number of cells to use in cell-based tendon regeneration therapies.

### 06.P10 Effects of vibration on the proteome expression of anterior cruciate ligament cells

HJ Kim and CW Kim  
Korea University, Korea

Recent reports have suggested that vibration has beneficial effects on knee healing response; however, the biomechanism of these beneficial effects still need to be determined on the anterior cruciate ligament (ACL) cell level. In this study, we applied a 20 Hz vibration to ACL cells, which produced a 20% increase ( $p < 0.001$ ) in cell activity and 17% increase ( $p < 0.001$ ) in intracellular sulfated glycosaminoglycan (GAG) levels. In the 20 Hz vibration-stimulated ACL cells group, eight up-regulated (100–300%) protein spots were identified compared to the control group by proteomics analysis. Among these proteins, Annexin A2 and Prolyl 4 hydroxylase (PH4B) were shown to have a 71% and 16% higher expression, respectively, in the 20 Hz vibration-stimulated ACL cells group by western blotting ( $p < 0.001$ ). These results indicate that vibration produces a positive cellular environment, and Annexin A2 and P4HB are expected to help ligament repair and ACL cell proliferation by controlling cell membrane and extra cellular matrix formation.