P185
Preclinical studies of retrovirally transduced human chondrocytes expressing TGF-beta1 (TG-C)
I.S. Oh1, M.J. Noh1, Y. Yi1, R.O. Copeland2, K.B. Choi3, S. Hwang4, C. Lim5, V. Yip5, J. Hyun6, H. Lee7, K.H. Lee8;
1Orthopaedic Surgery, Inha Univ., Incheon, Korea, 2R&D, TissueGene, Inc., Gaithersburg, MD, United States of America, 3R&D, TissueGene, Inc, Gaithersburg, United States of America, 4R&D, TissueGene, Inc., Gaithersburg, United States of America, 5R&D, KOLON Life Science, Incheon, Korea

Purpose: TissueGene-C (TG-C) represents a cell-mediated gene therapy for localized delivery of allogeneic chondrocytes expressing TGF-β1 directly to the damaged knee joint. Untransduced human chondrocytes (hChon) cells have also been incorporated into the TG-C product in a 3:1 ratio with TGF-β1 expressing chondrocytes (hChon)6β7 in order to help fill in the defect and as target cells for the actions of the expressed TGF-β1.

Methods and Materials: We closely examined both the biodistribution and safety of these cells in appropriately sensitive animal models. The preclinical safety of TG-C was evaluated in a go-day biodistribution study in SCID mice, and intra-articular safety and efficacy studies in rabbits and goats. The safety and efficacy of TG-C was evaluated in rabbits for up to six-month post treatment following a single intra-articular injection. In order to examine safety in a weight-bearing animal model, a safety and efficacy study of TG-C was also performed in goats.

Results: In a series of studies with surgically induced cartilage defects, we demonstrated efficacy in inducing the formation of hyaline cartilage in vivo. The cartilage formed not only fills in the defect site, but also forms fully-differentiated hyaline cartilage without any adverse effects on the surrounding synovium, vessels, bone or vasculature.

Conclusions: TG-C has been extremely well tolerated in the knee joints of rabbits and goats following a direct intra-articular injection and dose administration directly into the knee joint. Based on the preclinical studies described above, we received authorization from the FDA to proceed with an initial Phase 1 clinical study of TG-C for degenerative arthritis.

P186
Cytokines stimulate growth of human articular chondrocytes in low glucose conditions
S.J. Duguay1, A. Parker2, M. Zhang3, S. Madden4, B.J. Seymour5, 1Genzyme Biosurgery, Cambridge, MA, United States of America, 2Biosurgery, Genzyme, Cambridge, United States of America, 3Gene Analysis, Genzyme, Framingham, United States of America, 4Technical Services, Genzyme Biosurgery, Cambridge, MA, United States of America

Purpose: Develop a serum-free medium for culture of autologous chondrocytes for use in repair of articular cartilage defects.

Methods and Materials: Microarray analysis was employed to compare gene expression of cells grown in SFM containing PDGF and bFGF versus cells grown in 10% FBS. More than 3,500 genes were found to be differentially expressed. Key word searching of Gene Ontology annotation identified 178 growth factors, hormones and other secreted factors as potential candidates for stimulating growth in serum-free medium.

Results: Testing identified the cytokines IL-6 and OSM as potent stimulators of chondrocyte growth in the absence of serum. A serum-free medium containing IL-6, OSM, PDGF and bFGF was compared directly to medium with FBS for the ability to stimulate growth of primary human chondrocytes through three passages, and the potential of the cells to redifferentiate was examined. Growth rate and total cell yield for cells grown in SFM was equal to or greater than that for cells cultured in medium with FBS. Cells grown in SFM formed proteoglycan-depleted colonies in agarose with the same frequency as cells grown with FBS. Expression of collagen 2 and aggrecan in alginate cultures was detected at lower levels in SFM-derived cells than cells cultured in FBS. In addition, cells grown in SFM senesced and displayed normal karyotypes.

Conclusions: These data indicate that SFM containing IL-6 and OSM is suitable for culture of human chondrocytes.

P187
Changes of biomechanical properties of chondrocytes isolated from normal and osteoarthritic rabbit cartilage
X. Wei1, X. Wang2, Q. Zhang3, W. Chen4; 1Orthopaedics, The Second Hospital of Shaxi Medical University, Taiyuan, Shanxi, China, 2Orthopedics, Second Hospital of Shaxi Medical University, Taiyuan, China, 3Applied Mechanics And Biomedical Engineering, Taiyuan University of Technology, Taiyuan, China

Purpose: The present study is to characterize the biomechanical properties of chondrocytes isolated from osteoarthritic and normal cartilage.

Methods and Materials: Sixteen NZW rabbits were randomly divided into two groups with each 8. The knee osteoarthritis was induced in 8 rabbits, and another 8 as normal control. After 20 weeks, rabbits were sacrificed. The cartilage of the left knees was used for histological study, and the right knees for isolation of chondrocytes. The diameter of chondrocytes in histological sections and after isolation was measured. The biomeanical properties of chondrocytes were determined using the micropipette aspiration technique.

Results: The cartilage showed obvious degeneration with higher Mankin’s score in experimental group compared with control (P<0.05). The diameter of chondrocytes in histological sections and after isolation was 14.4±2.9 µm and 14.9±2.2 µm (P<0.05). There were no differences between the normal and osteoarthritic chondrocytes in diameter (14.9±2.2µm and 14.4±1.9µm, P>0.05) and the Young’s modulus (0.569±0.429kPa and 0.538±0.398kPa, P>0.05). In response to a step pressure, chondrocytes exhibited viscoelastic solid creep behavior. The volume change after compression of chondrocyte, the diameter of chondrocytes in the pipette was smaller in the normal (57.7%) than in the osteoarthritic cells (23.5%) (P<0.0001). The viscoelastic properties were significantly different between the normal and osteoarthritic chondrocytes (P<0.0001).

Conclusions: Isolated chondrocytes show similar size with those in histological section. The diameter and Young’s moduli are similar in both normal and osteoarthritic chondrocytes. Different volume and viscoelastic changes of osteoarthritic chondrocytes compared with the normal suggest that the biomechanical changes play an important role in mechanism of osteoarthritis.

P188
Optimization of cell culture conditions to improve tissue engineered cartilage implantation properties
C.N Bengtsson1, S. Concaro2, C. Brantsing3, M. Brittberg4, A. Lindahl5; 1Department Of Clinical Chemistry And Transfusion Medicine, Institute of Biomedicine Sahlgrenska Academy, Gothenburg, Sweden, 2Department Of Orthopaedics, Gothenburg University, Gothenburg, Sweden, 3Department Of Clinical Chemistry And Transfusion Medicine, Gothenburg University, Gothenburg, Sweden, 4Orthopaedic Department, Cartilage Research Unit, G, Kungsbacka, Sweden, 5Clinical Chemistry And Transfusion Medicine, Biomedicine, Gothenburg, Sweden

Purpose: The aim of this study is to determine the appropriate differentiation grade that provides the best equilibrium between mechanical and biological properties at the time of implantation.

Methods and Materials: Human articular chondrocytes were isolated, culture expanded in monolayer and seeded in Hyaff 11 scaffolds. Three different media conditions were used. The different media were Media A: DMEM-F12 supplemented with 10% autologous serum; Media B: DMEM-F12 supplemented with ITS+TGFβ-1+4, dexamethasone and 10% autologous serum and Media C DMEM high glucose ITS, Linoleic acid, human serum albumin, TGFβ-1, dexamethasone and ascorbic acid. After 14 days the constructs were analyzed for the implantation properties, histoarchitecture, biochemical content and gene expression profile.

Results: Gene expression of collagen type I was non detectable in media A, highest in media C and intermediate in media B. Histology revealed that all media tested had a similar cell distribution pattern with adequate cell binding and matrix production. Glucosaminoglycan/DNA was highest in media C and intermediate in media B compared to media A. The surgeon evaluation showed that implants cultured with the media B presented the best implantation properties.

Conclusions: In our study we showed that we were able to produce implants with different properties by changing the media composition. The surgeon evaluation determined that the group that had an intermediate degree of differentiation had the best implantation properties. Further studies are needed to determine the in vivo integration properties of these constructs.