494 MONOLAYER EXPANSION MODALITIES INFLUENCES 3D CHONDROGENIC DIFFERENTIATION OF HUMAN MESENCHYMAL STEM CELLS SEEDED IN COLLAGEN SPONGES

C. Cournil-Henriinet 1, J. Goebel2, L. Galici3, C. Huselstein4, D. Mainard5, D. Bensoussan6, P. Netter5, J. Stoltz7, P. Gillet5, A. Pinzano-Watrin1, 1 UMR 7561 CNRS-Nancy Universite, Vandoeuvre les Nancy, FRANCE, 2 Chirurgie Orthopédique et Traumatologique, CHU Nancy, FRANCE, 3 UMR 7563 CNRS-Nancy Universite, Vandoeuvre les Nancy, FRANCE, 4 Unité de Therapie Cellulaire et Tisulaire, CHU Nancy Brabois, FRANCE

Purpose: Cartilage engineering requires a large population of cells that are seeded to scaffold. To this end, monolayer expansion is often used to increase cell number. The objective of this study was to test the primary influence of culture medium during monolayer expansion on the secondary chondrogenic differentiation and ancillary extracellular matrix (ECM) production of mesenchymal stem cells (MSCs) seeded in collagen sponges.

Methods: After isolation from human bone marrow, MSCs were cultivated in monolayer with or without bFGF from passages 0 to 2. At passage 3, just before MSC seeding into sponges, cells were divided into 5 groups: (1) differentiation medium (DM), (2) DM + chondrogenic supplement, (3) DM + bFGF, (4) DM + bFGF + chondrogenic supplements, (5) DM + bFGF + TGFβ1. After passage 3, MSCs were then seeded in collagen I sponge and cultivated for 28 days in vitro. Each group was cultured with 8 different mediums containing ITS (Insulin Transferrin Selenium) or FBS (fetal bovine serum) supplemented or not with TGFβ1 and bFGF. ECM production and chondrogenic differentiation of MSCs during 3D differentiation were evaluated at D28. Chondrogenic gene expression (COMP, Aggrecan, Versican, Coll 2, Coll 1, Coll 3, Sox9, Osteocalcin, Alkaline Phosphatase) was investigated by qPCR. Newly synthesized ECM was assessed histologically and immunohistochemically (Coll 1 and Coll 2).

Results: Medium composition during 2D expansion strongly influenced ECM production by MSCs during 3D culture in sponges: FGF supplementation alone or in combination (chondrogenic supplements or TGFβ1) increased ECM production inside the biomaterial. In 3D conditions, growth factor (TGFβ1 or TGFβ1+BMP) combined with ITS increased the quantity and quality of ECM. Conversely, BMP2 alone had no influence when added to ITS or FBS alone. In fact, TGFβ1 alone or the sequential exposure of TGFβ1 (D3-D14) followed by BMP2 (D15-D28) promoted both chondrogenic expression (COMP, Aggrecan, Coll 2+) and chondral phenotype as synthesized matrix contained proteoglycans as demonstrated histologically and by Coll1 + Coll2 immunostaining. In addition, polarized light microscopy depicted collagen network in the ECM. In contrast, when growth factors were associated to FBS during 3D cultures, there was a detrimental influence of this combination on chondrogenic differentiation of MSCs.

Conclusions: For cartilage engineering, chondrogenic differentiation of MSCs seeded in collagen sponges is promoted by a previous exposure to bFGF during expansion phase and by the contact to TGFβ1 alone (D3-D28) or switched by sequential BMP2 (D15-D28) during differentiation.

495 BONDING OF MENISCAL TISSUE WITH CELLULAR FIBRIN GLUE: A NUDE MOUSE STUDY

C. Scotti1, A. Pozzi1, L. Mangiavini1, C. Sosio1, F. Vitari2, C. Domenechhi3, G. Fraschini1, 1 San Raffaele Scientific Institute, Milano, ITALY, 2 Laboratory of Biological Structures Mechanics, Politecnico di Milano, Milano, ITALY, 3 Department of Veterinary Sciences and Technologies for Food Safety, Faculty of Veterinary Medicine, University of Milan, Milano, ITALY

Purpose: Meniscus repair is a current clinical challenge. Menisci play a fundamental role in knee biomechanics, but they lack intrinsic regenerative properties. Consequently, when a tear occurs and the meniscus is removed, even partially, cartilage changes in kneecap homoestasis take place, often leading to the development of early osteoarthritis. In the last decades tissue engineering approaches have been advocated to improve the reparative processes of joint tissues. The aim of this study is to test the capacity of isolated chondocytes embedded in fibrin glue to promote bonding of meniscal slices in a nude mouse model.

Methods: A swine chondrocytes-fibrin glue suspension was utilized as a biologic glue to improve bonding between two meniscal slices obtained from swine menisci. Tissue engineered tri-phasic sandwiches were obtained combining two meniscal slices with cellular fibrin glue and then wrapped with acellular fibrin glue. Radial slices were harvested from pig menisci and regularized with a scalpel. Chondrocytes were isolated from articular cartilage of pigs by collagenase digestion and then resuspended in fibrinogen solution. Cell solution was placed atop of a meniscal slice. The thrombin was added to form a fibrin glue gel embedding chondrocytes. During polymerization of fibrin glue, a second meniscal slice was put over the fibrin glue in order to form the tri-phasic sandwich. We prepared also control samples with acellular fibrin glue between the meniscal slices in order to evaluate the role of cells in the bonding process. All samples were wrapped in an acellular fibrin gel and then implanted in the subcutaneous tissue of nude mice for four weeks.

Results: At the end of the fourth week from implantation, samples were retrieved, macroscopically analyzed, tested for gross bonding and processed for histological evaluation. The fibrin gel embedding the samples was almost absorbed and the remnants appeared reorganized into a core-capusse wrapping each sample, rich of neovessels. Moreover, the meniscal slices did not show any shrinkage or signs of digestion demonstrating the effectiveness of the fibrin gel embedding as a “shield” against metalloproteinases digestion, while the fibrin gel itself appeared rich of cells likely deriving from the host animal. The gross bonding between the meniscal slices was tested with a pair of forceps and demonstrated a firm adhesion between the two slices in all the experimental samples. On the other hand, none of the control samples showed any sign of bonding. Histological evaluation (H&E) demonstrated the presence of an hypercellular fibrocartilaginous tissue at the interface between the slices. Interestingly, some penetration buds were present inside the meniscal slice coming from the cellular fibrin gel. No blood and no cellular tissue was found in the control samples. SEM confirmed the presence of a continuous tissue in the interface between the meniscal slices in the experimental samples.

Conclusions: These results demonstrated the potential of this model for improving meniscal bonding and confirmed the importance of cells in the bonding process of tissues. However, further orthopic studies in a large animal model are needed to evaluate its feasibility in clinical practice.

496 ENGINEERING AN OSTEochondRAL PLUG: ANALYSIS OF CHANGES FROM THE IN VITRO TO THE NUDE MICE CULTURE

G.M. Peretti1, L. Mangiavini1, C. Scotti1, D. Deponiti1, F. Boschetto2, A. Pozzi1, C. Sosio1, F. Vitari3, C. Domenechhi1, G. Fraschini1, 1 San Raffaele Scientific Institute, Milano, ITALY, 2 Laboratory of Biological Structures Mechanics, Politecnico di Milano, Milano, ITALY, 3 Department of Veterinary Sciences and Technologies for Food Safety, Faculty of Veterinary Medicine, University of Milan, Milano, ITALY

Purpose: Articular cartilage has a poor intrinsic regenerative potential. As a matter of fact, when a lesion occurs, the repair tissue is often fibrous, having insufficient biomechanical properties, which could frequently lead to the development of early osteoarthritis when physiologic mechanical forces act on this repair tissue. In the last decade, tissue engineering approaches addressed this topic with many cell-seeded biomaterials and some of them are currently used in clinical practice. The aim of this study was to assess the optimal time of in vitro development of osteochondral plugs before implantation in subcutaneous pouches of nude mice.

Methods: Osteochondral cylinders were developed combining a fresh chondrocytes-fibrin glue composite with a calcium-phosphate scaffold. Chondrocytes were isolated from articular cartilage of pigs by collagenase digestion and resuspended in fibrinogen solution. The thrombin was added to form a fibrin glue gel composite with cells. During polymerization of fibrin glue, the scaffold was put over the fibrin glue. Composites were placed in standard culture conditions and then retrieved after one, three and five weeks. At the end of all in vitro culture samples were analyzed and the other half implanted in subcutaneous pouches of nude mice for four weeks. Gross evaluation, histology, immunohistochemistry, biochemical analyses for DNA (Picogreen) and GAGs (DMB), biomechanical evaluation (compression and shear properties).

Results: The results demonstrated the presence of cartilage-like tissue maturing within the fibrin glue gel, and a macroscopic penetration of the cellular fibrin glue into the pores of the calcium phosphate scaffold. Moreover, GAGs seem to adhere to the scaffold and determining areas of integration between the calcium-phosphate and the cellular fibrin gel. Immunohistochemical assay demonstrated that collagen type 2 was present either at 1 week and 5 weeks with a more strong staining at 5 weeks and after in vivo culture. DNA quantisation demonstrated an increase in total DNA quantity.