compartment compared to control (femur and tibia; +225% $p<0.001$ and +42.9% $p<0.04$ respectively). No effect on the ungrooved lateral compartment was seen. The histological analysis of the cartilage corroborated these results. A significant increase in the OARSI score for the experimental medial compartment (+225% $p<0.001$ and +81,7% $p<0.006$, respectively for femur and tibia) was seen. Biochemical analysis showed a decreased PG content in the medial experimental compartment compared to control (femur -12.09% $p=0.038$ and tibia -7.32% $p=0.031$). There was a slight increase in synovial inflammation in the experimental joint ($p<0.003$). For all previous mentioned analyses, no changes in the experimental lateral compartment or the control joint were found.
Conclusions: The present study shows clearly that the development of joint damage is much more dependent on the biomechanical component, since all detectable damage is in the medial compartment. Joint homeostasis is disturbed, indicated by moderate synovial inflammation. However, this did not lead to generalised OA in this time period.

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REDIFFERENTIATION OF HUMAN DEDIFFERENTIATED CHONDROCYTES : AN INNOVATIVE COMBINATION FOR THE CELL THERAPY OF ARTICULAR CARTILAGE

F. Legendre [1], D. Ollitrault [1], M. Hervieu [1], C. Bauge [1], L. Maneix [1], H. Benateau [1], E. Renard [1], S. Leclercq [2], H. Chajra [3], C. Drougard [1], M. Briand [4], L. Poulain [4], F. Mallein-Gerin [5], K. Boumediene [1], P. Galera [1], M. Demoor [1]. [1] *MILPAT - Univ. of Caen/Basse-Normandie, CAEN, France;* [2] *Clinic St Martin, CAEN, France;* [3] *Symatèse Biomatériaux, Chaponost, France;* [4] *BIOTICLA - Univ. of Caen/Basse-Normandie, CAEN, France;* [5] *Lab. Biology and Engineering of Cartilage - CNRS FRE 3310, LYON, France*

Purpose: Since two decades, Autologous Chondrocytes Implantation (ACI) constitutes a therapeutic alternative in cartilage healing to return back to a functionally tissue after trauma or age related degeneration. Beyond the clinical aspect with its encouraging results, the major goal conditioning the success of ACI is to obtain a hyaline neo-cartilage with redifferentiated and phenotypically stabilized chondrocytes. Indeed, the inescapable amplification step dedifferentiates chondrocytes, which synthesize a type I non-functional collagen to the detriment of type II collagen and aggrecan phenotypic markers. To restart an active metabolism typical of a hyaline matrix, we developped a clinical suitable process which combine relevant physicochimic factors for chondrocytes culture such as collagen scaffold, BMP-2 and IGF-I as chondrogenic factors, physioxic conditions and transient RNA interference targeting COL1A1 and PSBA (a so-called protein implied in BMP-2 activity and matrix regulation).
Methods: Dedifferentiated Human Articular Chondrocytes (HAC) from macroscopically healthy zones but inflammatory environment of femoral heads from patients undergoing joint arthroplasty (age range 52-83) were cultured in type I collagen sponges, under low oxygen tension, and incubated with BMP-2/IGF-I. siRNAs were used to counteract type I collagen and to sustain chondrogenic factors activity. An extensive analysis at gene and protein levels relevant to differentiated, dedifferentiated and hypertrophic chondrocyte phenotypes was achieved and used to calculate the differentiation index corresponding to the ratio of COL2A1 mRNA/COL1A1 mRNA completed by the ratio of type IIB collagen/type IIA collagen mRNAs. Finally, we have evaluated the behavior of such differentiated chondrocytes in vivo, by subcutaneous implantation in the nude mouse model followed by immunohistochemistry analysis.
Results: Collagen sponges support metabolic activity of viable redifferentiated chondrocytes expressing type II B collagen and aggrecan, without sign of hypertrophy. The best differentiated phenotype profile was obtained after 7 days in culture with BMP-2 which enhanced transcription activity of COL2A1 gene by its specific enhancer region. The sustained BMP-2 action by siRNA treatment and COL1A1 down-regulation improved the differentiation index of chondrocytes, which synthesize a hyaline like-matrix 28 days after subcutaneous implantation.
Conclusions: HAC phenotype can be restarted and stabilized by this clinically suitable process with a biodegradable scaffold allowing the maintenance of chondrocyte characteristics and with a less invasive surgical method (patent request N°10 57683). This study will allow to develop new methods for autologous chondrocyte implantation in human.

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GOOD MANUFACTURING PRACTICES IN AUTOLOGOUS CHONDROCYTE IMPLANTATION: THE EXPERIENCE IN A PUBLIC HOSPITAL

L. Roseti, P.M. Fornasari, A. Maso, E. Storni, M. Serra, F. Canella, A. Tosi, C. Munno, B. Grigolo, A. Bassi. *Istituto Ortopedico Rizzoli, Bologna, Italy*

Purpose: Autologous chondrocyte implantation (ACI) is a widely diffused technique utilized for the treatment of articular cartilage damages. *In vitro* expanded chondrocytes for ACI procedure are considered advanced therapy medicinal products (ATMPs), as defined by the European Regulation. Consequently, it is mandatory that they have to be manufactured in accordance with the "specific medicinal rules" named Good Manufacturing Practices (GMPs) and in dedicated environments built as real "pharmaceutical factories". This study describes the GMPs development and characterization (validation) of chondrocyte-based medicinal products to be used in ACI.
Methods: Chondrocyte cultures were carried on in a production facility located in a Public Hospital and including clean rooms of different classification up to A in B work places, according to current GMPs (see figure). After informed consent, a cartilage biopsy was harvested from a non-bearing area of the knee of ten patients undergoing ACI. The tissues were enzymatically digested to isolate chondrocytes. Primary cultures were expanded in monolayer up to 3-4 passages and cells seeded at defined density onto matrices derived from collagen I/III (bi-dimensional). Engineered constructs were analyzed for the following parameters: sterility (microbiological control, Mycoplasma and endotoxins), cell number, viability and the expression of different phenotypic markers. Microbiological control was performed using an automated system allowing to reveal the presence of aerobic, anaerobic bacteria and fungi. Highly-sensitive Real Time Quantitative DNA PCR technology was the method used for detecting contaminating Mycoplasma. Bacterial endotoxin assays were performed by quantitative *Lymulus Amebocyte Lysate (LAL)*. Cell number and viability were evaluated with both an automated cell counter and the MTT test. Markers typical for hyaline cartilage (collagen II and aggrecan), fibro-cartilage (collagen I) and bone tissue (osteocalcin) were evaluated by immuno-histochemical analyses.
Results: Engineered chondrocytes were free of contamination from aerobic and anaerobic bacteria, fungi, Mycoplasma and endotoxins, viable (90-98%). The phenotipic analysis revealed that chondrocytes were able to express the typical hyaline cartilage molecules, collagen II and proteoglicans. Collagen I and osteocalcin, markers of fibroblasts a bone, respectively were not present.
Conclusions: Our results evidentiated the ability to manufacture chondrocyte-based medicinal products that are sterile, viable, phenotypically stable and therefore suitable for ACI application. After this initial validation, the cultures of 100 patients undergoing ACI were treated in our Cell Factory.