Purpose: Since two decades, Autologous Chondrocytes Implantation (ACI) constitutes the 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 developed a clinical suitable process which combine relevant physiocratic factors for chondrocytes culture such as collagen scaffold, BMP-2 and IGF-I as chondrogenic factors, physicoconditions 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 IIIB 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 immuno-histochemistry 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: Our results evidenced 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.