isolated from OA patients, were cultured in PLLA and PLLA-CHT1/2 scaffolds. Cell-constructs were cultured for 4 and 8 weeks, in a well-defined chondrogenic differentiation medium. Cellular metabolic activity was followed using the Alamar Blue™ assay. Histological examination was performed using hematoxylin-eosin, toluidine blue and safranin-O stainings, as well as immunohistochemistry analysis of types I, II and X collagen.

Results: Gravimetric analysis of the proposed scaffolds indicated porosities higher than 80%. PLLA presented spherical interconnected pores; incorporation of -CHT1, revealed a filling of the PLLA macro-porous structure, whereas in -CHT2, chitosan appears as a thin coating on the pore walls. The presence of a more hydrophilic component (CHT) renders a higher water absorption capability which could be related to lower mechanical properties, although in this case this is expected to confer PLLA, normally applied in bone, properties more appropriate to its application in soft tissues. Cells' metabolic activity was significantly increased, after 30 days, evidencing the biocompatible character of the scaffolds. The chondrogenic differentiation was followed, and after 4 weeks, cells secreted an extracellular matrix rich in proteoglycans (Figure 1), incorporating type II collagen and aggrecan. When comparing the different scaffolds, we could observe that PLLA and PLLA-CHT2 enabled the formation of a more stable tissue whilst PLLA-CHT1 induced a faster chondrogenic differentiation but at the same time a premature hypertrophy. Although the most noteworthy was the tissue organization found in the PLLA alone, that resembled the biomimetic zones of native articular cartilage.

Conclusions: PLLA alone or combined with CHT scaffolds properties and support for the chondrogenic differentiation and ECM deposition could be potential indicated for cartilage TE.

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BIOMOLECULAR CHARACTERIZATION OF CHONDROCYTES EMBEDDED IN A COLLAGEN I-MATRIX (CARES®) AT THE TIME OF TRANSPLANTATION

F. Halbwirth, E. Niculescu-Morzsa, H. Zwickl, C. Bauer, S. Nehrer. *Ctr. for Regenerative Med. and Orthopedics, Krems an der Donau, Austria*

Purpose: Early treatment of localized cartilage lesions is the prerequisite for avoiding the onset of progressive cartilage degeneration and osteoarthritis. One option is the transplantation of cell suspensions of autologous chondrocytes (ACT) at defect sites which has proved to efficiently support reconstitution of joint functionality. Nevertheless, this technique has some well-known drawbacks like the risks of leakage of cells from the implantation site or transplant hypertrophy as well as calcification and delamination due to the deployment of the periosteum. Many of those disadvantages can be circumvented by using autologous chondrocytes embedded and, hence, immobilized in matrices (matrix-coupled ACT). The goal of this work is to determine and establish biomolecular parameters accounting for the quality of collagen I-matrices seeded with autologous chondrocytes from a local biotech company. A special focus is set on cartilage-constituting, matrix-degrading, and cell surface proteins since they might influence the clinical outcome after implantation.

Methods: To characterize the chondrocytes in the transplants we detect chondrocytes- or differentiation specific markers (e.g., Aggrecan, Collagen 1A1, 2A1, 2AB, MMP 3, 13, Sox5, Sox6 and Sox9 by means of quantitative Real-Time-PCR (qPCR). To confirm the results of the (qPCR) we concentrate on surface markers to detect the chondrogenic capacity of the chondrocytes in the transplant by means of flow cytometry (FACS). There are well known surface markers like CD44 and CD90 which are expressed significantly higher in chondrocytes with a higher chondrogenic capacity. The amount of sulphated glycosaminoglycans (sGAG) correlates with the chondrocyte-specific expression of proteoglycans like aggrecan. In order to evaluate the chondrocytic phenotype and therefore the presence of the second dominant component of cartilage, we determined the amount of sGAG in transplants of different patients.

Results: Our results confirm the chondrocytic phenotype of matrix-embedded cells in collagen I transplants with respect to sGAG synthesis, gene products and CD markers. We, however, observed pronounced inter-individual differences in terms of amount of sGAG and gene products as well as the proliferation of the chondrocytes.

Conclusions: The biomolecular data permit the identification of quality determining parameters which can be used as a quality control of transplants and the chondrocytes in the collagen I matrix.

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CROSS-LINKED HYALURONIC ACID SCAFFOLDS: A POTENTIAL USAGE IN CARTILAGE REGENERATION?

C. Bauer [†], R. Baumgartner [‡], M. Hornof [‡], F. Halbwirth [†], E. Niculescu-Morzsa [†], H. Zwickl [†], D. Kern [†], S. Nehrer [†]. [†] *Danube Univ. Krems, Krems, Austria;* [‡] *Croma Pharma GmbH, Leobendorf, Austria*

Purpose: An important feature of biomaterials for cartilage regeneration is their influence on establishment and stabilization of a chondrocytic phenotype of embedded cells. For this biomaterials have to feature good biocompatibility, biodegradability and allow proper cell adhesion. In this study we examined the effects of a porous three-dimensional scaffold of cross-linked hyaluronic acid on the expression and synthesis performance of human osteoarthritic chondrocytes.

Methods: Human articular cartilage was obtained from osteoarthritic patients subjected to total knee arthroplasty. Cells were isolated from cartilage tissue, expanded until passage 2 and seeded onto the scaffold. This was followed by a three week cultivation period. Cell content within the scaffold was estimated by determination of the metabolic activity (XTT assay) and quantification of DNA (CyQuant cell proliferation assay). The expressions of chondrocyte-specific genes as well as the synthesis of sulphated glycosaminoglycans (sGAG) by embedded cells were analyzed in order to characterize the synthesis performance and differentiation status of the cells. Also electron and light microscope images on different time points were made.

Results: Within the scaffold, cells are arranged individually or in small cell clusters and the distribution was homogenous. Although DNA quantification indicated only partial loss of cells most probably due to matrix degradation, the metabolic activity within the scaffolds decreased dramatically. This might be attributed to a stop in cell proliferation combined with a switch of the cellular genetic program from cell division to differentiation towards a chondrogenic phenotype. Analysis of gene expression and sGAG synthesis substantiated this hypothesis as both chondrocyte specific gene expression and sGAG synthesis were increased and the differentiation index was clearly improved.

Conclusions: These results suggest that the investigated material has a chondroinductive effect on embedded cells.