Characterization of cross-linked hyaluronic acid scaffolds in vitro and in vivo

Christoph Bauer[1], Eugenia Niculescu-Morzsa[1], Sonja Höller[2], Margit Hornof[2], Daniela Kern[1], Vivek Jeyakumar[1], Stefan Nehrer[1]
[1]Danube University Krems, Krems, Austria
[2]Croma Pharma GmbH, Leobendorf, Austria

Disclosures: Christoph Bauer (N), Eugenia Niculescu-Morzsa (N), Sonja Höller (3C-Croma Pharma GmbH), Margit Hornof (3C-Croma Pharma GmbH), Daniela Kern (N), Vivek Jeyakumar (N), Stefan Nehrer (N)

ABSTRACT INTRODUCTION:

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 three-dimensional porous scaffold of cross-linked hyaluronic acid on the expression and synthesis performance of human osteoarthritic chondrocytes. After the positive in vitro results, the scaffold was used in an animal study. For this, a chondral damage was induced, and a hyaluronan thiomer gel alone or in combination with the scaffold was subsequently implanted in the trochlea of the femur of female NZW rabbits.

METHODS:

For the in vitro study 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 two 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 expression of chondrocyte-specific genes, as well as the synthesis of sulphated glycosaminoglycans (sGAG) by embedded cells, were analyzed in order to characterize the ECM production and differentiation status of the cells. In addition scanning electron microscope images were made. For the animal study, 18 animals were divided into 3 groups (untreated, hyaluronan gel alone and scaffold in combination with the gel). The observation period was 4 and 12 weeks. At the end of each period macroscopic findings using Brittberg score, histopathological findings using O'Driscoll score and the synovial smear were analyzed.

RESULTS SECTION:

Electron microscope images showed that, within the scaffold, cells are arranged individually or in small cell clusters and distributed homogeneously. Although DNA quantification indicated only the partial loss of cells, possibly due to matrix degradation. This can be further supported by the decrease in metabolic activity. This can be attributed to stop cell proliferation and switch the cellular genetic program from cell division to differentiation towards a chondrogenic phenotype. Analysis of gene expression and sGAG synthesis substantiate our hypothesis, which both the chondrocyte-specific gene expression and sGAG synthesis were increased, and the differentiation index was clearly improved.
In the animal study, macroscopic findings varied greatly during the first observation period (4 weeks), while 12 weeks after the implantation the results were more homogenous and nearby a normal regeneration was visible. The O'Driscoll score for histopathological findings showed a slightly better result at the end of 12 weeks for the scaffold in combination with gel.

DISCUSSION:

Our data rule out that, the investigated material, a novel cross-linked hyaluronic acid scaffold has the potential to modulate the chondrocyte phenotype in a way favoring a more differentiated status. *In vitro*, it has a positive impact on the synthetic performance of embedded chondrocytes with respect to the production of cartilage specific components like collagen type II, aggrecan, and sulphated glycosaminoglycans. However, *in vivo* no test item related macroscopic findings were noted in the animals, and the chondral damage was not negatively influenced by one of the treatment schemes. Histopathologic analysis was nearly the same in all the groups but showed a slightly better tissue repair for the scaffold in combination with gel. As the effect is not significant, it should be tested in large animal models like sheep or goat.

SIGNIFICANCE:

The study addresses the problem of finding an appropriate biomaterial for tissue repair in cartilage regeneration. The material should be biocompatible, degradable and should lead the cells to a good synthesis performance of cartilage specific components.

IMAGES AND TABLES: