Basic Research

# Scientific Evidence

## HYDROLYZED COLLAGEN: AN *IN VITRO STUDY*

The *in vitro* effects of CHondroGrid, a possible adjuvant in the treatment of osteoarthrosis symptoms.

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Joints affected by arthrosis show a range of structural, tissue, cellular and biochemical changes. The expression in the joints of certain inflammation mediators, such as interleukins 1 α and 6 (IL-1 α and IL6), or *tumor necrosis factor-beta* (TNF-β), causes the activation of enzymes that degrade the cartilage, including certain metalloproteinases and disintegrins. These enzymes degrade the extracellular matrix, including its collagen component. This fact led to speculating that endogenous, intra-articular administration of collagen might have beneficial effects. The first *in vitro* studies confirmed this hypothesis, showing that synovium or cartilage cells release fewer inflammation mediators, while increasing the production of hyaluronic acid, a key component of the cartilage matrix and of the synovial fluid[1]. Studies on animal models showed that intra-articular injection of simple tripeptides obtained from collagen made for a significant decrease in the degradation of the joint cartilage that had been induced to simulate arthrosis, while simultaneously significantly increasing the number of chondrocytes positive for the synthesis of type II collagen, a fundamental component of the cartilage matrix[2]. Bioteck recently placed on the market an injectable device made with hydrolyzed collagen, CHondroGrid, for the treatment of the pain and functional symptoms associated with arthrosis. The objective of this sheet is to summarize a pre-clinical *in vitro* study aimed at investigating the effects of CHondroGrid on human chondrocytes in culture.

1 Comblain F, Dubuc JE, Lambert C, Sanchez C, Lesponne I, Serisier S, Henrotin Y. Identification of Targets of a New Nutritional Mixture for Osteoarthritis Management Composed by Curcuminoids Extract, Hydrolyzed Collagen and Green Tea Extract. PLoS One. 2016 Jun 8;11(6):e0156902.

2 Naraoka T, Ishibashi Y, Tsuda E, Yamamoto Y, Kusumi T, Toh S. Periodic knee injections of collagen tripeptide delay cartilage degeneration in rabbit experimental osteoarthritis. Arthritis Res Ther. 2013 Feb 22;15(1):R32.

## Materials

The study at first entailed isolation and then expansion in culture of human chondrocytes obtained from donors suffering from arthrosis of the hip. The cells were then exposed to different concentrations of CHondroGrid.

Their viability was measured with the Alamar Blue assay, while their proliferation was measured via fluorescence quantification. To simulate an inflammatory environment, the chondrocytes were treated with interleukin 1-beta (IL-1β) for 48 hours. To ascertain the possible anti-inflammatory effect of CHondroGrid, the cells were then exposed to CHondroGrid, assessing the expression of metalloproteinases MMP1 and MMP3 and of their inhibitors, TIMP1 and TIMP3, using the real-time PCR technique and the technique of trophic/pro-angiogenic factors TGFβ1, IGF-1 and VEGF through ELISA assays.

Lastly, the cells underwent an immunohistochemical analysis via a semi-quantitative assessment of the expression of type I and II collagen.

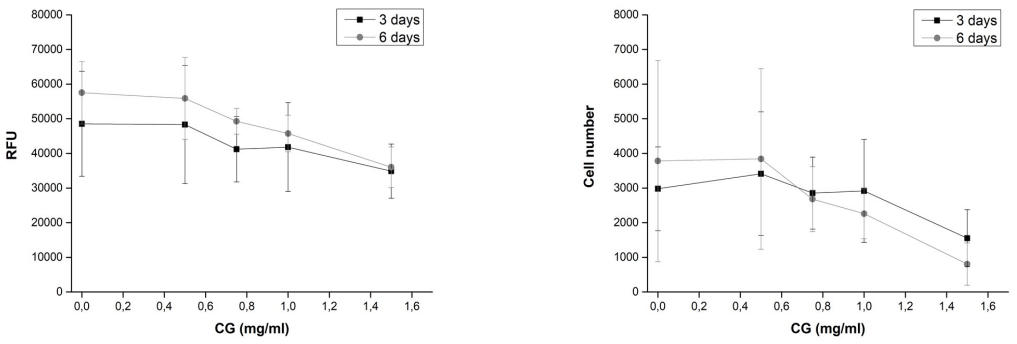


***Fig. 1*** *- Cell viability (left) and proliferation (right) of human chondrocytes exposed to various concentrations of CHondroGrid. The only concentration at which a significant reduction of both parameters is observed is 1.5 mg/ml, a concentration that can never be observed in clinical application.*

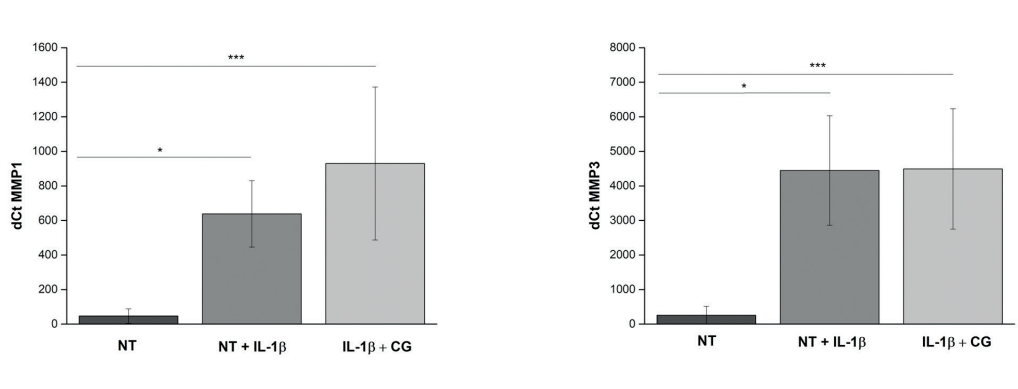


***Fig. 2*** *- Expression of metalloproteinases MMP1 (left) and MMP3 (right) of non-treated (NT) chondrocytes, following inflammation induced with IL-1β (NT + IL-1 β), and after treatment with CHondroGrid (IL-1β + CG). The exposure to CHondroGrid does not modify the expression of metalloproteinases; the same applies to metalloproteinase inhibitors TIMP1 and TIMP3 (not shown).*

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## Results

The cell viability test showed that CHondroGrid does not have a negative effect on cell viability except in high concentrations (1.5 mg/ml), thus confirming the non-cytotoxicity data that had already been collected during the pre-clinical evaluation of the device performed by the manufacturer. We should point out that this concentration can never be reached during clinical application, owing to the doses applied and the average volume of synovial fluid.

No other significant effects of CHondroGrid were observed on the expression of metalloproteinases and their inhibitors; it was also proved that CHondroGrid has no effect on the expression of the trophic and pro-angiogenic factors analyzed. CHondroGrid, therefore, has no direct anti-catabolic or anti-inflammatory effect; this result is consistent with the action mechanism that underlies the therapeutic effect of CHondroGrid, i.e. a mechanical action reinforcing the cartilage matrix rather than a pharmacological, immunological or metabolic action. Indeed, CHondroGrid peptides, whose low molecular weight promotes their dissemination through a liquid medium, such as the injection solution and the synovial fluid, can easily disperse on the cartilage surface, thus mechanically reinforcing the portions that have been damaged by the inflammatory process of arthrosis.

From a histological point of view, the chondrocytes cultured in a chondrogenic culture medium, in the presence of CHondroGrid, deposited significantly higher quantities of extracellular matrix compared to cells that were neither exposed to CHondroGrid nor were cultured in a chondrogenic medium. Cells cultured in the presence of a chondrogenic medium and CHondroGrid showed a more rounded morphology, indicating better trophism, and a higher expression of type II collagen attended by a lower production of type I collagen.

Seen as a total, these results show that CHondroGrid is intrinsically safe and suggest that its application may induce chondrocytes to produce hyaline articular cartilage, countering the normal reparative response that would lead to the formation of fibrous tissue.

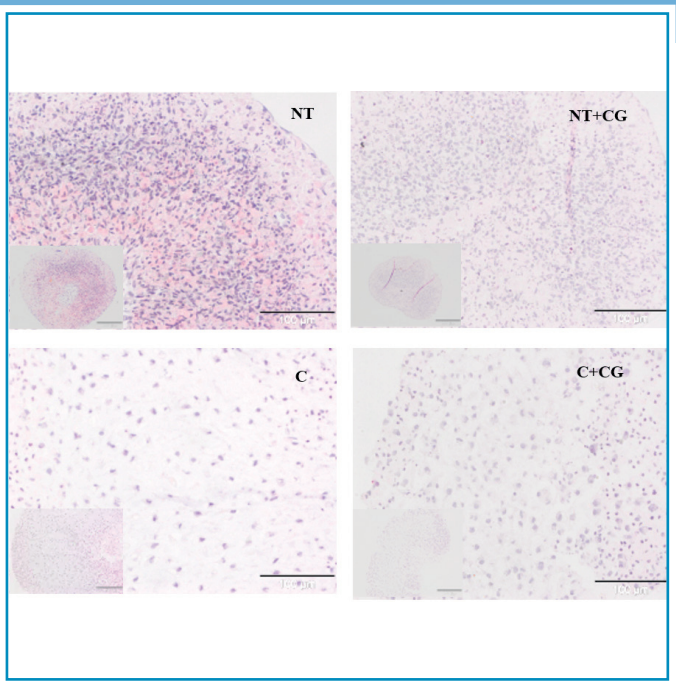


***Fig 3*** *- Histology of non-treated (NT) chondrocyte cultures, with CHondroGrid (NT + CG); with chondrogenic medium only (C), with chondrogenic medium and CHondroGrid (C + CG). In the presence of CHondroGrid the cells produce larger quantities of extracellular matrix and present a more rounded morphology, indicating better trophism.*

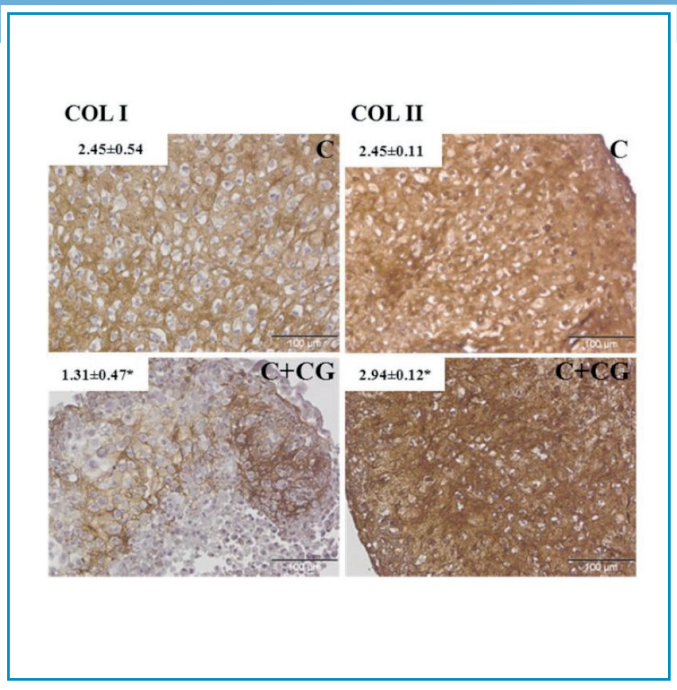


***Fig 4*** *- Immunohistochemical analysis of chondrocytes exposed to chondrogenic medium only (C), or to chondrogenic medium and CHondroGrid (C + CG); in the presence of CHondroGrid, the cells show lower expression of type I collagen (COL I), typical of fibrous tissues, and increased type II collagen expression (COL II), typical of the cartilage matrix.*

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