

## VARIABLE EFFECT OF 3 DIFFERENT CHONDROITIN SULFATE COMPOUNDS ON CHONDROCYTES SECRETED PROTEINS PROFILE REVEALED BY SILAC TECHNIQUE.

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Although chondrocytes are widely used as *in vitro* model of cartilage diseases such as osteoarthritis (OA), their secretome is still in large part unknown. Thus, chondrocyte secretome could represent an attractive sub-proteome for elucidating pathophysiological mechanisms underlying cartilage degeneration during OA process, for the discovery of novel candidate OA biomarkers and for the development of new OA drugs. In this work we applied SILAC technique to study secreted protein profile of human chondrocytes treated with 3 different types of chondroitin sulphate (CS), a therapeutic agent widely used in clinic. Chondrocytes released from 3 OA cartilages were recovered and plated at low density in SILAC medium. When complete labelling was achieved, chondrocytes were treated with 200µg/mL of CS1 (porcine, purity 90.4%), CS2 (bovine, purity 96.2%) and CS3 (bovine, purity 99.9%). 48 hours later, conditioned media were collected and their proteins were concentrated and quantified. Heavy and light samples were mixed 1:1 and a first-dimension separation by 1D gel electrophoresis was performed. 5 µg of each mixed sample were in-gel reduced, alkylated and digested with trypsin prior to nanoLC-MS/MS analysis. The identification and quantification of proteins was carried out with Protein Pilot software. Database search allowed us the identification of 96 different proteins in the CS1 secretome, 95 in CS2 and 104 in CS3. CS1 modulated the expression of 21 proteins: 15 resulted upregulated and 6 downregulated. CS2 modulated 13 proteins: 5 upregulated and 8 downregulated. Finally CS3 modulated 9 proteins: 8 upregulated and only 1 downregulated. Our results show that not all chondroitin sulfates act similarly: CS1 even upregulates catabolic factors and decreases anabolic factors. This clearly illustrates that not all brands of CS prove to be efficacious and confirms the disparity in effects revealed by literature, probably due to differences in purity and production process.