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IN VITRO PERFORMANCE OF K-CARRAGEENAN HYDROGELS COMBINED WITH DIFFERENT TYPES OF CELLS AIMED AT APPLICATIONS IN CARTILAGE REGENERATION

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Objectives: Injuries of the articular cartilage are one of the most challenging issues of musculoskeletal medicine due to the poor ability of this tissue for repair. Cartilage tissue engineering strategies require the presence of cells and a scaffold material, typically a hydrogel. In this work we have analyzed the *in vitro* performance of k-carrageenan, an ionic hydrogel recently proposed for TE approaches, with encapsulated cells of different types, namely a chondrocytic cell line, primary chondrocytes cells and human adipose stem cells, often proposed for cartilage regeneration strategies.

Methods: The k-Carrageenan hydrogels were produced using an ionotropic gelation method and cells, namely ATDC5 cells, human nasal primary chondrocytes (hNCs) and human adipose stem cells (hASCs), were encapsulated at a density of $5*10^6$cell/mL and cultured for 21 days. The cells viability and proliferation was determined by fluorescence staining, DNA quantification. Chondrogenic differentiation of the different cells encapsulated in the hydrogels was characterized by GAGs quantification, typical histological staining and real time qRT-PCR analysis (Sox9, aggrecan collagen type I, type II and type X).

Results: The biological evaluation of k-carrageenan hydrogel revealed that this polymer enables long-term viability and proliferation of different cells. During 3 weeks of culture, cells encapsulated within the hydrogel developed a cartilage-like extracellular matrix rich in proteoglycans and type II collagen. Cartilage-like ECM deposition and production was found throughout all culturing periods indicating a stable chondrocyte phenotype in encapsulated cells. Nevertheless, encapsulated hASCs exhibit the highest proliferation rates and highest levels of chondrogenic markers expression.

Conclusions: K-carrageenan hydrogels enable the viability and proliferation of different cell types during long-term cell culture. The results obtained indicated the feasibility of using these hydrogels in cartilage tissue engineering approaches due to its ability to support chondrogenic features of different cells types, particularly the hASCs.