Cryopreservation of cell laden natural origin hydrogels for cartilage regeneration strategies

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Statement of Purpose: An increase of scientific published literature and clinical experience supports the requirement of providing products like cultured cells and tissues to the market. One of the main prospects of cartilage tissue engineering is the possibility of developing custom-made regenerative medicine solutions on an individual patient basis. The efficient preservation and storage procedure will provide products available as needed which could be adapted to an autologous immediate solution. Thus, the aim of this study was to examine the effects of the cryopreservation on the chondrogenic differentiation characteristics of human mesenchymal stem cells isolated from adipose tissue (hASCs). Furthermore, we also propose to determine hASCs-hydrogel stability and confirm the potential of these bioengineered constructs to be applied in cartilage regeneration. The results obtained show that the hydrogels withstand the cryopreservation process maintaining their structural integrity, with good cell content after cryopreservation. Thus, cell encapsulation systems of natural based hydrogels may be an interesting approach for the long term preservation of cartilage tissue engineered products.

Methods: The κ-carrageenan (κCR) hydrogels were produced using an ionotropic gelation method. Then, stem cells, namely human adipose derived stem cells (hASCs), were encapsulated in κCR discs (5 mm dia. x 3 mm height) at a density of $5x10^6$ cell/cm^3 and cultured for 21 days in standard basal (BM**)** or chondrogenic media (ChM). The cell hydrogels were cryopreserved in liquid nitrogen for up to 30 days. The overall morphology of κCR discs with encapsulated hASCs was observed under light microscope. hASCs viability and proliferation rate was determined by double stand DNA quantification. Additionally, chondrogenic differentiation of hASCs encapsulated in the hydrogels is being characterized by histological staining for selective cartilage staining and real time PCR analysis (Sox9, aggrecan, and collagen: type I, type II and type X). DMA analysis allowed determining the mechanical properties of κ-carrageenan hydrogels, namely storage (elastic) and loss (viscous) while immersed in wet state at 24 °C and throughout a physiological relevant range of frequencies. The described characterization assays were performed both before (BC) and after cryopreservation/freezing (AC) time points.

Results: The cell morphology, distribution and appearance of the hydrogels are clearly observed from the microscopic light images (Figure 1A). It is possible to observe the smooth and homogeneous surface, the well defined and stable shape before and after the freezing process. Encapsulated hASCs were able to maintain cellular content, despite an expected decrease observed upon cryopreservation (Figure 1B), which is associated to a recovery time after thawing. The microscopic images and biological evaluation of κCR hydrogel revealed that the cryopreservation process did not change the cellular morphology; the surface and integrity of the hydrogel disc and enables maintenance of hASCs after exposure to low temperatures environments.

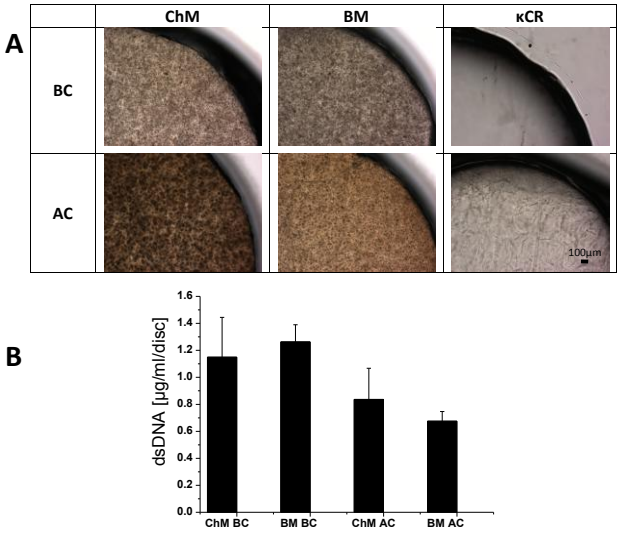

Figure 1. (A) Representative optical micrographs of hASCs encapsulated in κCR hydrogels and cultured in ChM and BM before and after cryopreservation and (B) cell proliferation results, based on the quantification of dsDNA content. Scale bar represent 100 μm.

Conclusions: The results obtained so far indicated the feasibility of hASCs-κCR system in cartilage tissue engineering regeneration strategies due to its ability to support hASCs viability before and after cryopreservation. Ongoing studies on the assessment of chondrogenic features of these cryopreserved systems will provide information on the effect of cryopreservation indicative of a stable chondrocyte phenotype. In summary, this study provided information on the potential of ASCs-hydrogel constructs for a long term storage and ready to use bioengineered tissue substitutes for cartilage regeneration strategies.

References:
(Popa EG. Biomacromolecules 2011;12:3952-3961)