BIOFUNCTIONAL HYALURONIC ACID BASED INJECTABLE HYDROGELS FOR THE REPAIR OF CARTILAGE LESIONS

E. Tsanaktsidou[1,2], O. Kammona[2], V. Zaspalis[1,2], C. Kiparissides[1,2,*]

[1]Department of Chemical Engineering, Aristotle University of Thessaloniki, Thessaloniki, Greece

[2]Chemical Process & Energy Resources Institute, Centre for Research and Technology Hellas, Thessaloniki, Greece

*(*costas.kiparissides@cperi.certh.gr)*

ABSTRACT

The present study focuses on the synthesis and characterization of hyaluronic acid based injectable hydrogels with desirable properties, in combination with cells and biomolecules, for the repair of cartilage lesions. Injectable methacrylated hyaluronic acid (MeHA) hydrogels were synthesized using two different crosslinking methods, namely, a redox initiation system (i.e., ammonium persulfate, APS and N,N,N,N'-tetramethylenediamine, TEMED) and a matrix mataloproteinase 7 (MMP7)-degradable peptide. The effects of molecular weight of HA, degree of methacrylation (DM) or/and biofunctionalization of MeHA (i.e., with a chondroitin sulfate (CS) binding peptide) on the end-use properties of synthesized hydrogels were experimentally investigated. It was found that the storage modulus (G`) of the hydrogels synthesized, using a redox initiation system (i.e., APS and TEMED), increased as the DM of MeHA or/and the molecular weight of HA increased, resulting in the formation of more rigid hydrogels exhibiting a lower degree of swelling and a slower hydrogel degradation rate. The functionalization of MeHA with a CS binding peptide resulted in an increase of the gelation onset time as well as in a decrease of the hydrogel crossilinking density (i.e., resulting in the formation of softer hydrogels). Moreover, it was found that as the concentration of the peptide crosslinker increased the hydrogel onset time decreased as well as the degradation rate of the synthesized hydrogels while their storage modulus (G') increased. Additionally, MeHA crosslinking reaction was also carried out in a cell growth medium, in order to create an appropriate environment for the growth of the embedded cells. The storage modulus of this hydrogel increased to a value that favored the differentiation of human mesenchymal stem cells (hMSCs) to chondrocytes. hMSCs were subsequently encapsulated in (CS)MeHA hydrogels formed with an MMP7-degradable peptide as crosslinker. The effect of MeHA on cell viability and proliferation was examined and an increase in their viability and proliferation was observed. Furthermore, real-time polymerase chain reaction (real-time PCR) was performed for chondrogenic and hypertrophy markers. The gene expression of the aforementioned markers indicated that the formed hydrogels favored the chondrogenic differentiation of hMSCs and inhibited or delayed their differentiation towards a hypertrophic phenotype. The cell-laden MeHA hydrogels were also characterized with respect to DNA, sulfated glycosaminoglycans (sGAG) and collagen content. The amount of DNA was found to be stable throughout the culture period, whereas the collagen and sGAG content increased. Finally, the ability of MeHA hydrogels incorporating chondrocytes to induce repair of cartilage lesions was tested in an *ex vivo* cartilage model (i.e., osteochondral explant isolated from porcine knee). The histological analysis (Safranin-O/Fast Green Staining) results indicated the formation of extracellular matrix (ECM).