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# FROM SEA TO JOINT: FORMULATING INJECTABLE MARINE ORIGIN CRYOGELS BASED ON COLLAGEN, CHONDROITIN SULFATE AND HYALURONIC ACID

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## Abstract

Marine biomaterials have emerged as promising alternatives to the use of mammalian-derived compounds in regenerative medicine due to their bioactivity, safety, and sustainable origin. In this work, we explored three marine-derived biopolymers -collagen, chondroitin sulfate (CS), and hyaluronic acid (HA)- aiming at the formulation of injectable cryogels for articular cartilage therapies.

Collagen and CS were obtained from *Prionace glauca* skin and cartilage, respectively, and HA via bacterial-fermentation using marine peptones. After a thorough physicochemical characterization, blends were formulated by combining biopolymer solutions and inducing gelation at low-temperature, exploring different polymer concentrations, ratios, and crosslinking strategies. Cohesiveness and injectability were evaluated concerning composition.

FTIR spectroscopy confirmed functional groups, including amide-bands in collagen, sulfate-peaks in CS, and a carboxylate-band in HA. Circular dichroism confirmed the triple-helix structure of shark collagen. Rheology showed shear-thinning behavior in both collagen and HA, with the latter increasing in viscoelasticity at higher concentrations. DMMB assay revealed higher sulfation in marine-CS ( $1.26 \pm 0.32 \mu\text{g/mL}$ ) compared to bovine-CS ( $1.00 \mu\text{g/mL}$ ). Zeta-potential analysis of HA showed a strong negative surface charge ( $-45.1 \text{ mV}$ ), indicating high-electrostatic stability.

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These findings supported the use of marine biopolymers in cryogel formulations. Playing with polymers concentration and ratios, also exploring further covalent crosslinking with EDC or genipin, it was possible to generate hydrogels with different cohesiveness and structural stability in PBS. Consequently, the injectability of the produced hydrogels was also influenced by the formulation details, enabling the selection of more promising matrices as injectable biomaterials potentially suitable for minimally invasive therapies in joint diseases.

**Keywords:** Cryogels, shark collagen, chondroitin sulfate, hyaluronic acid, cartilage therapies