

## Development of injectable thermosensitive hydrogels based on marine exopolysaccharide for cartilage tissue engineering

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Infernan, an anionic slightly sulfated exopolysaccharide (EPS) secreted by *Alteromonas infernus*, a bacterium from deep-sea hydrothermal vents, displays some biological properties similar to glycosaminoglycans of mammalian tissues<sup>1-2</sup>. For its use in cartilage tissue engineering as thermoresponsive and injectable hydrogel, uronic acids (R-COOH) of infernan were covalently grafted by the amine terminated poly(*N*-isopropylacrylamide) (pNIPAM-NH<sub>2</sub>) via carbodiimide chemistry<sup>3</sup>.

By varying COOH:NH<sub>2</sub> molar ratio, four grafted polysaccharides were obtained, having distinct EPS rate (9% to 33% w/w) and different grafting density (2% to 16%). Physico-chemical properties of grafted EPS were subsequently characterized at molecular scale using a multidisciplinary approach. At room temperature, grafted EPS chains were organized in the form of very high molar mass aggregates (> 10<sup>9</sup> g/mol), established by inter-chain interactions between grafted pNIPAM chains (Force Flux Fractionation, AF4). Above the sol-gel transition temperature of 32°C, grafted EPS formed stable hydrogels with elastic moduli from 2 to 5 kPa, measured in fully hydrated state by Atomic Force Microscopy (AFM). Finally, the results of the viability test of seeded control cells showed that EPS-pNIPAM hydrogels with a less dense and more EPS-rich network were cytocompatible.

All these outcomes allowed a better understanding of the relationship between the structure of infernan grafted by a thermosensitive polymer and the functional, mechanical and biological properties of the resulting EPS hydrogels. This knowledge remains essential for future use of these thermosensitive infernan-based hydrogels in cartilage tissue engineering.

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<sup>1</sup>Rederstorff, E. *et al.* Enriching a cellulose hydrogel with a biologically active marine exopolysaccharide for cell-based cartilage engineering *J. Tissue Eng. Regen Med* **2017**, *11*, 1152-1164. <sup>2</sup>Gélébart, P. *et al.* Microgels based on Infernan, a glycosaminoglycan-mimetic bacterial exopolysaccharide, as BMP-2 delivery systems *Carbohydr. Polym* **2022**, *284*, 119191. <sup>3</sup>Fillaudeau, A. *et al.* Glycosaminoglycan-mimetic infernan grafted with poly (*N*-isopropylacrylamide): Toward a thermosensitive polysaccharide *Carbohydr. Polym* **2024**, *326*, 121638.