BIO-ORTHOGONAL DOUBLE-CROSSLINKED ALGINATE-GELATIN/MXenes HYDROGELS AS BIOMIMETIC VISCOELASTIC AND ELECTROCONDUCTIVE SUBSTRATES SUPPORTING CARDIAC REGENERATION

Testore D.[1], Zoso A. [1], Paoletti C. [1], Groppo S. [1], Marcello E. [1], Rafieerad A.[2], Dhingra S. [2], Chiono V. [1]

[1]*Department of Mechanical and Aerospace Engineering, Politecnico di Torino, Turin, Italy.*

[2]*Institute of Cardiovascular Sciences, St. Boniface Hospital Albrechtsen Research Centre, Department of Physiology and Pathophysiology, Max Rady College of Medicine, Rady Faculty of Health Sciences, University of Manitoba, Winnipeg, Manitoba, R2H 2A6, Canada*

Myocardial infarction is one of the leading causes of death worldwide and represents a major clinical challenge [1]. In recent works, cell culture hydrogel substrates enhanced miRNA-mediated direct reprogramming efficiency of human cardiac fibroblasts (HCFs) into induced cardiomyocyte (iCMs) [2][3]. Notably, these hydrogels were purely elastic or highly soft, whereas several studies highlighted the importance of ECM-like stress-relaxing viscoelastic hydrogels in regulating cell behavior [4]. Furthermore, electroconductive hydrogel substrates enhanced the maturation of cardiac contractile cells [5]. Herein, alginate-gelatin/MXenes hydrogels based on bio-orthogonal click-chemistry, with tunable viscoelastic and electroconductive properties were developed with the aim to support cardiac regeneration by enhancing direct reprogramming of HCFs into iCMs.

Firstly, a solution of alginate-azide and gelatin-azide conjugates, was mixed with a 4-arm-PEG-DBCO solution to form spontaneous hydrogels via bio-orthogonal strain-promoted azide-alkyne click reaction (SPAAC). Double-crosslinked hydrogels were obtained, through additional ionic cross-linking by Ca^{2+} ions. Furthermore, MXene quantum dots (MQDs) were incorporated into hydrogels, to impart electrical conductivity. Physicochemical properties of hydrogels were deeply investigated. *In vitro* cell viability and adhesion were performed by embedding HCFs into hydrogels.

Bio-orthogonal alginate-gelatin hydrogels were successfully obtained through SPAAC click reaction. By varying azide:DBCO molar ratio, physicochemical properties of hydrogels were modulated. At increasing azide:DBCO ratio, hydrogels showed increased mechanical stiffness and more pronounced elastic response, mimicking healthy cardiac tissue. All hydrogels showed physiological rates of stress relaxation. Particularly, substrate viscoelasticity could be tuned by double-crosslinking strategy, which enabled viscous stress dissipation by unzipping of ionically packed molecules. MQDs could be finely dispersed within the hydrogel network enhancing substrate electroconductivity. Thanks to SPAAC bio-orthogonality, all hydrogels supported cell viability, adhesion and spreading. The study of *in vitro* direct reprogramming of HCFs into iCMs in contact with the developed hydrogels is ongoing.

As a conclusion, bio-orthogonal double-crosslinked alginate-gelatin/MXenes hydrogels developed in this study are promising candidates as biomimetic substrates for cardiac direct reprogramming applications, deserving future investigations.

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BIORECAR project has received funding from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme grant agreement No 77216