Alginate and Gelatin-based hydrogels for 3D bioprinting of skeletal muscle tissue

Giovanni Paolo Stola [1] , Elena Marcello [1] , Alice Zoso [1] , Gerardina Ruocco [1] , Valeria Chiono [1]

[1]Department of Mechanical and Aerospace Engineering, POLITO Biomedlab, Politecnico di Torino, Torino, Italy

giovanni.stola@polito.it

Introduction

Skeletal muscle is crucial for locomotion and metabolic regulation. Conditions like Duchenne muscular dystrophy and volumetric muscle loss lead to severe damage, impairing function and quality of life. 3D bioprinting has emerged as a promising strategy to address the limitations of conventional tissue engineering techniques enabling precise control over the spatial arrangement of cells and biomaterials [1]. Among biomaterials, alginate (Alg) has been widely studied due to its ease of printability and cost-effectiveness. However, since Alg lacks inherent cell adhesion properties and exhibits poor *in vivo* degradability, this study combines alginate dialdehyde (ADA) and gelatin (Gel) to develop biodegradable and cell-adhesive bioinks for 3D *in vitro* skeletal muscle models.

Experimental methods

ADA was produced through partial oxidation of alginate using sodium metaperiodate ($NaIO_4$). ADA oxidation degree (OD), total polymer concentration (2 - 8% w/v) and the ADA:Gel weight ratio (30:70 and 50:50) were optimized to achieve desired viscoelastic properties and printability. Additionally, two external cross-linking methods were explored: calcium chloride (0.1 M $CaCl_2$) alone and in combination with microbial transglutaminase (2.5% w/v mTG). *In vitro* cell studies using C2C12 mouse myoblasts were carried out to assess ADA/Gel cytocompatibility and cell adhesion properties.

Results and discussion

ADA with four OD (25, 10, 5 and 2.5%) was successfully produced. Bioink formulation with ADA 5% OD, a total polymer concentration of 6% w/v and ADA:Gel 50:50 was selected as the optimal composition. The combination of $CaCl_2$ and mTG for crosslinking significantly enhanced ADA-Gel mechanical properties, obtaining an increment in both storage and elastic modulus (G': from 0.7 kPa to 3.7 kPa; E': from 2 kPa to 11 kPa), compared to $CaCl_2$ alone, and achieving properties within the optimal range of skeletal muscle (10-17 kPa) [2]. Additionally, increased hydrogel *in vitro* stability (from 14 days up to more than 60 days) and improved Gel retention within the hydrogel matrix was observed. Finally, *in vitro* cell studies using C2C12 showed that ADA/Gel hydrogels crosslinked with $CaCl_2$+mTG promoted

favorable adhesion and spreading of cells over time. Future research endeavors will evaluate the long-term behavior of cultured cells within the bioink constructs, further investigating cell proliferation and myogenic differentiation.

Conclusion

In this work, we successfully developed a novel biodegradable and cell-adhesive bioink for 3D *in vitro* skeletal muscle models by combining ADA and Gel. Future works will explore the integration of bioactive cues within the bioink matrix to mimic the anisotropic architecture of skeletal muscle and provide multidimensional stimulation to embedded cells.

References

[1] K. Y. Lee et al., “Alginate: Properties and biomedical applications”, 2012.
[2] M. Volpi et al., “Hydrogel-Based Fiber Biofabrication Techniques for SMTE”, 2022.

AcKnowledgments This project is supported by ERC BIORECAR-EU H2020 GA 772168