Tuning of mechanical properties in photopolymerizable gelatin-based hydrogels for *in vitro* intestinal epithelial models

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INTRODUCTION

The mechanical microenvironment is a critical factor in the evolution of colorectal cancer (CRC) [1]. In order to set and study different scenarios, hydrogels have emerged as versatile biomaterials with a huge potential to behave as two- and three dimensional *in vitro* models owing to their unlimited functionalization possibilities. Gelatin methacryloyl (GelMA)- based hydrogels are of particular interest due to the peptidic backbone which provides cell adhesion sites [2] and the on-demand photopolymerization alternatives to create covalently crosslinked networks. In addition, photo-induced thiol-based reactions own many of the advantages of click chemistry, including robustness, simplicity and the formation of homogeneous networks [3]. Thus, it is highly relevant to develop a platform of bioscaffolds with different chemical composition and tunable stiffness in order to generate *in vitro* intestinal epithelial models for the study of CRC.

EXPERIMENTAL METHODS

In the present work, two types of biomaterials have been prepared and characterized: GelMA hydrogels were generated through UV-photopolymerization and GelMA-SH hydrogels followed a "mixed-mode" mechanism combining radical photopolymerization and thiol-ene step-growth reaction. Swelling properties, pore size and stiffness are reported for hydrogels prepared in PBS. In addition, hydrogels have also been prepared in DMEM and characterized in terms of swelling behaviour and stiffness. In order to check the biological relevance of these biomimetic materials, *in vitro* cell culture experiments were performed by seeding Caco-2 cells on the surface of four different substrates. Cell viability, adhesion and proliferation were reported. Lastly, immunofluorescence staining and SEM imaging were performed. Between three and five replicas were fabricated for mechanical characterization and cell culture experiments.
Student's t-tests were used to determine significant differences. Non-normally distributed populations were analyzed with the Mann-Whitney method.

RESULTS AND DISCUSSION

First of all, gelatin was successfully functionalized achieving almost 85% of amines derivatized into methacrylamide groups. UV irradiation of GelMA and GelMA-SH mixtures in PBS led to hydrogels exhibiting similar stiffness but with different curing times. Besides, the thiol click chemistry was found to be an effective tool to easily tune mechanical properties. GelMA-30, GelMA-150, SH-10 and SH-30 were the selected scaffolds to perform a deeper characterization study. While the crosslinking strategy and the irradiation time had no significant effect on hydrogels morphology, it was observed that thiol-ene systems showed higher swelling properties. Hydrogels prepared in DMEM exhibited markedly different values of Young's Modulus compared to PBS although the swelling trend was similar.
Regarding cell experiments, Caco-2 cells showed the best substrate adhesion after 24h on GelMA-150 whereas they hardly adhered to SH-10 scaffolds. A good cell proliferation was achieved in GelMA-30, GelMA-150 and SH-30 since the three conditions supported a confluent monolayer at day-14. Finally, SEM imaging of cell-seeded hydrogels showed a proper apical-basal polarization of cells according to a villus-forming monolayer, exposing structural differences among the different substrates.

CONCLUSION

This research describes a precise stiffness modulation of covalently crosslinked GelMA-based hydrogels by varying the photoirradiation time. A platform of bioscaffolds with different chemical composition and stiffness values covering a wide range from healthy tissue to cancerous stages, have been prepared. 2D *in vitro* studies showed successful cell viability, proliferation and polarization of Caco-2 cells-seeded hydrogels. This work demonstrates the formation of adequate epithelial models and stablishes the basis for future studies focused on more complex stroma models and deeper epithelium characterization.

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ACKNOWLEDGMENTS

Funding for this work was provided by the Spanish "Ministerio de Ciencia, Innovación y Universidades (MCIU)" through AEI/FEDER(UE) project PID2020-118485RB-I00; Gobierno de Aragoń project LMP221_21, FEDER (EU); through the "Fondo Social Europeo" (DGA E15_20R and E47_20R). RPC acknowledges Gobierno de Aragón for a predoctoral fellowship (2017-2021).