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3D Bioprinted patches embedding probiotics for wound healing

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Introduction

Due to the serious spread of antimicrobial resistance, the single use of antibiotics to fight chronic diabetic wounds, such as diabetic foot ulcers (DFUs), is not resolutive, and the identification of alternative therapeutic strategies able to replace or support the use of antibiotics, is a largely recognized priority. One of the most attractive, yet not fully explored, options is represented by bacteriotherapy, which relies on the use of harmless bacteria (i.e., probiotics) to compete with pathogens, with the aim of displacing them, suppress the expression of their virulence factors and contrast the microbial ability of inducing tissue injury and chronic inflammation [1]. Despite bacteriotherapy being a valuable strategy to prevent or treat various diseases, vehicles for an efficient bacteria delivery to the target site are not yet established. Recently, 3D bioprinting has emerged as a fascinating biofabrication strategy to manufacture multi-dimensional constructs encapsulating living organisms for therapeutic applications [2]. Nevertheless, there are few literature sources describing the encapsulation of bacteria using this method.
The present work aims at fabricating a novel hydrogel material with incorporated probiotics to heal or prevent the occurrence of DFUs. For this purpose, an alginate/glycerol-based bioink loaded with commercial probiotics was formulated and employed for the 3D bio-printing of patches with different geometries to be used topically on the inflamed skin.

Experimental

Two different bioinks were used for extrusion-based 3D printing using Bio X Bioprinter (Cellink): a commercial alginate-based bioink (Cellink) and a custom-made bioink. The latter was prepared dissolving 4 w/v% alginate (Mw = 427 kDa, M/G ratio = 0.7) in a water-glycerol mixture (25 v/v% glycerol) solution. 3D constructs were printed with both bioinks and cross-linked using 1 w/v% calcium chloride solutions. Several tests were conducted to identify the optimal printing conditions and the printability of both the bioinks was evaluated and compared. A degradation test in phosphate buffered saline (PBS) at T = 37°C was performed for probiotic-free constructs to assess their stability in simulated biological conditions. Commercial probiotics were prepared by dissolving the probiotics in the custom-made bioink.

Results and Discussion

3D constructs with different shapes and dimensions were obtained with both bioinks, however, the custom-made bioink showed best printability, and good structural stability for up to two weeks.

Conclusions

The custom-made bioink can be used to print 3D structures with customizable features, with good fidelity and structural stability. Therefore, this method is promising to produce 3D patches to treat complicated wounds. However, further studies aimed to assess the survival of probiotics to the process conditions, as well as the *in vitro* and *in vivo* efficacy of bacteriotherapy, are necessary.

References

[1] Pereira, S. G., Moura, J., Carvalho, E., & Empadinhas, N. (2017). Microbiota of chronic diabetic wounds: ecology, impact, and potential for innovative treatment strategies. Frontiers in microbiology, 1791.

[2] Mallick, A., Quilès, F., Francius, G., Burgain, J., Gaiani, C., Scher, J., ... & Alem, H. (2022). An easy and robust method of preparation of capsules for delivering probiotic bacteria by a 3D bioprinting. Food Hydrocolloids for Health, 2, 100088.

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Biography

Roberta Rovelli is a master's degree student in Materials and Nanotechnology at University of Pisa – Scuola Normale Superiore and currently engaged in her thesis project.

She received a bachelor's degree in Chemical Engineering from University of Pisa. She is interested in polymeric hydrogels and their use for drug delivery and tissue engineering applications.