3D bioprinting integrated touch-spinning of composite bioink – nanofiber constructs

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INTRODUCTION

Biofabrication aims at the engineering of biologically relevant constructs that mimic the complex building blocks of tissues and organs. A promising technique is bioprinting, which is a biofabrication technology that uses bioinks to fabricate constructs. Hydrogels have been widely used as bioink materials in the field of tissue engineering for decades. Hence, hydrogels are well-known for their high-water retention capability, which provides the cells with a hydrated environment that supports cell proliferation and subsequent tissue growth.[1], [2] However, hydrogels do not mimic the complexity of tissue extracellular matrix, which is composed of both gel-like and fibrous components. In addition to that hydrogels are isotropic in nature, soft, and have poor mechanical properties for high-load tissue engineering applications such as muscles.[2], [3] Additionally, the isotropic nature of hydrogels does not resemble the anisotropic nature of tissues, which in most cases results in non-controlled or random cell orientation. The orientation and arrangement of cells are crucial for the proper functioning of many tissues such as muscles, in which the muscle fibers are natively highly oriented. A wide range of approaches has been employed for the fabrication of these hybrid structures along with bioprinting such as electrospinning and melt electrowriting. [2], [4], [5] These techniques have shown limitations such as the use of high voltages and temperatures for the fabrication of fibers. Thereby, touch-spinning was used in this study, which is based on mechanically pulling nanofibers from polymer solutions to deposit highly aligned fibers on a stationary substrate without the need for high voltages and/or temperatures and with a relatively faster fiber production rate.[5], [6]

MATERIALS AND METHODS

Comprehensive rheological and flow properties of alginate-based (alginate, carboxymethyl cellulose, laponite, and their blends) inks in two different solutions were investigated. The bioinks were prepared by mixing fibroblasts with the already prepared hydrogel solutions to reach a final cell density of 60 million cells/mL. The composite multilayered bioink-nanofiber construct was prepared by using the hands-free sequential bioprinting integrated touch-spinning approach. In this approach, nanofibers are first spun on a substrate followed by bioprinting of the bioink, forming a composite layer that is sequentially repeated. Finally, cell orientation, viability, and motility were studied.

RESULTS AND DISCUSSION

The biofabricated constructs have shown a preferred orientation of fibroblasts body along the fiber's main direction, in which an alignment degree of higher than 60 % and reaching a maximum of 93 %. Additionally, all bioink formulations have shown a high cell viability of higher than 80 %, confirming the non-toxicity of the system. Finally, time-lapse videos of fibroblast have shown an average displacement of 3.5 μm s^{-1} with a directed movement along the fibers.

CONCLUSIONS

It was concluded that biomaterial inks play a crucial role in fibroblast behavior. In which, cells show a preferred orientation along the nanofiber's main direction, while cells tend to form aggregates in bioinks with higher viscosity. Finally, fibroblast motility using time-lapse microscopy shows a directed movement of the cells along the fibers.

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