3D bioprinted polysaccharide-based constructs for endothelial tissue engineering

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Introduction

Kappa-carrageenan is a natural linear polysaccharide derived from red seaweed with remarkable biocompatible properties [1]. Gellan gum is a biocompatible material produced by the bacterium Sphingomonas paucimobilis, which promotes the cell adhesion and proliferation capacity of various cells [2]. In recent years, a variety of different research studies in tissue engineering and regenerative medicine focuses on the construction of bioinks, which are structures comprising cells and other cellular constituents combined with biocompatible materials [3]. Our current work is based on the fabrication of a bioink containing kappa-carrageenan and gellan gum, which is tailored by employing extrusion 3D bioprinting to fabricate scaffolds with specific geometry facilitating a favorable 3D micro-environment for endothelial tissue growth.

Experimental methods

Two different blend compositions were prepared by mixing 4% w/v gellan gum (GG) with either 1.5% or 2% w/v kappa-carrageenan (K) in H_2O at 90°C for 4 h. The produced blend compositions are designated as GG-K1.5 and GG-K2 and used as inks. Subsequently, after cooling them at room temperature, the blends were mildly mixed with a volume of cell suspension ($30x10^6$ cells/ml) in a ratio of 10:1 and the bioinks were loaded into extrusion cartridges for the 3D bioprinting process by means of a bioprinter (Inkredible+, Cellink). The produced bioinks have been placed in αMEM culture medium and stored in a humidified incubator at 37°C. Live/dead assay has been conducted with L929 fibroblasts to determine the cell viability and proliferation inside the bioink on days 1 and 7. Biodegradation studies have been performed on days 0, 7, 14 and 21 to assess the bionks degradation rate in the presence of cells. Rheological analysis protocols have been executed to deduce the printability efficiency and the mechanical properties of the scaffolds including dynamic strain sweep (DSS), dynamic frequency sweep (DFS), recovery capability and viscosity of the developed bioinks. Ongoing experiments, evaluating functional endothelial markers including PECAM1 in bioprinted constructs are in progress.

Results and discussion

High cell viability of 90% has been validated for both scaffold compositions on day 1, while between days 1 and 7, a three-fold increase in cell number has been observed. No significant differences were detected between the two compositions regarding their biocompatibility. Additionally, the GG-K1.5 bioink depicted a biodegradation rate of 19% and 37% on days 7 and 21, respectively. The GG-K2 bioink exhibited lower biodegradation values of 15% on day 7, and 26% on day 21. Both blends displayed shear-thinning behaviour, a necessary characteristic for extrusion bioprinting. From the DSS tests, the yield points of the blends were calculated. The GG-K1.5 blend showed a yield point at 18 kPa, while the GG-K2 blend at 28 kPa. Both blends shared similar ranges for the loss tangent, 0.07-0.11 for the GG-K1.5 and 0.08-0.12 for the GG-K2 composition, indicating the predominance of elastic nature of both blends. A crucial condition for extrusion bioprinting is the material's ability to rapidly recover from the applied shear stress. The recovery of viscosity after 10 sec was measured at 97% for the GG-K1.5 and at 94% for the GG-K2 blend.

Conclusion

Two different bioink compositions containing kappa-carrageenan and gellan gum were fabricated through 3D extrusion bioprinting and promoted the growth of fibroblasts. Physicochemical and preliminary biological investigations confirm the constructs suitability for 3D bioprinting for soft tissue engineering.

References

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