Developing clinically relevant chondrogenic biomaterial ink for extrusion bioprinting

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INTRODUCTION

The articular cartilage in adults cannot regenerate and even minor damage can result in progressive joint degeneration and osteoarthritis, a leading cause of inability in Europe. Effective regenerative procedures for treating cartilage lesions larger than 3.5 cm^2 are needed. The bioprinting of a hyaline cartilage tissue is a promising approach. In particular, the suspended 3D printing enables the engineering of tissues with a 20 µm resolution [1] and it is compatible with bioprinting living cells [2]. Also, collagen type I is suitable for bioprinting primary chondrocytes which then form *de novo* cartilage tissue *in vivo* [3]. Thus, we hypothesize that the suspended bioprinting of a collagen ink will enable the bioprinting of a biomimetic hyaline cartilage implant.

MATERIALS AND METHODS

Collagen biomaterial ink formulation and functionalization. Acid-soluble collagen was extracted from bovine tendons by a procedure, compliant with Regulation (EC) No 142/2011, at a facility, registered with the competent authority. The resulting biopolymer's quality was ascertained by rheology, SDS-PAGE, biuret analysis, turbidimetric analysis for solubility, and others. The extrusion printability of 1.0 to 7.0% neutral collagen inks was evaluated by direct and/or suspended 3D printing. The ink was prepared by first dissolving the collagen in dilute acetic acid, adding a saline buffer, and then neutralizing. The collagen ink was also functionalized by physisorption and/or *in situ* conjugation, using the 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride and N-hydroxysuccinimide, of TGFβ-1 to collagen, verified by ^{1}H NMR, Fluorescamine assay, and ELISA.

Development and validation of a suspension medium. A gelatin-based suspension medium [1] was optimized for 3D printing of neutral collagen ink as well as sterility and lack of cytotoxicity, per MTT assay. The medium was investigated by rheology and suspension 3D printing. Suspended 3D printing was performed on a mechanical extrusion system with a printing speed of 10 mm/s, a flow rate of 0.26 ml/h, a 22 G nozzle, and a layer height of 0.1 mm. Following printing, the medium was melted at 37°C.

RESULTS AND DISCUSSION

Functional collagen biomaterial inks for a direct and suspended extrusion bioprinting using high purity collagen extract were developed. Over 90% of the extract's mass is protein while its SDS-PAGE pattern corresponds to that of collagen type I. Its good solubility results in a neutral 0.3% collagen solution with a low turbidity of 32 NTU. A characteristic kinetic curve during the thermal crosslinking also shows a high-quality extract. The inks' dynamic viscosity increases with the collagen concentration from 1 to 7% ranging from 23 up to 997 Pa.s at a shear rate of 1 s^{-1}. Good quality direct 3D printing is achieved with an ink which is 2% or more while the 1% ink is suitable for suspended 3D printing. Suspended printing with a 22G nozzle enables a resolution of 100 µm. The physisorbed TGFβ-1 is released from a thermally crosslinked collagen only after 72 hours and most of the growth factor is not released within the first week. Reduction in free -NH_2 groups, per ^{1}H NMR and Fluorescamine assay, reflect successful conjugation. Conclusive determination is done by ELISA. Precise determination of degree of conjugation is ongoing. Similarly, data from a long-term study of the release rate of conjugated TGFβ-1 are still being collected.

A suspension medium suitable for high fidelity extrusion bioprinting was developed. The compactification of the suspension medium up to RCF of 1800 g increases its true yield stress up to 846 Pa and decreases its thixotropy resulting in higher fidelity printing. UV irradiation of the starting dry substances allows the preparation of a sterile suspension medium without significantly affecting its rheological characteristics. Finally, the medium is not cytotoxic making it suitable for bioprinting with live cells.

CONCLUSIONS

We have developed and extensively characterized collagen biomaterial inks, functionalized with a chondrogenic growth factor, suitable for direct and suspended extrusion bioprinting, as well as an optimized gelatin-based suspension medium. As a result, we have demonstrated the required resolution and fidelity for the bioprinting of a biomimetic hyaline cartilage, our next step.

REFERENCES

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