Bioprinting of nerve guide tubes using a DLP technology

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INTRODUCTION

In clinical practice, autografting remains the gold standard for the treatment of moderate/large gaps in peripheral nerves (PNs). However, this practice is associated with complex and lengthy surgeries to harvest the donor material (healthy nerve), donor site morbidity, and neuroma formation. A viable substitute for nerve grafts is the insertion of tubular structures (called nerve conduits, NGCs) made of biological/synthetic polymers that fill the nerve gap, provide nutrients to the axons, and form a barrier to scar tissue growth. Although NGCs are already used in clinical practice, they are still limited to PN injuries with subtle abnormalities and short gaps. To finally address the problems in the treatment of PN injuries, our research group has developed a revolutionary polymer formulation for the fabrication of guide tubes that could accelerate nerve regeneration in a rat model with sciatic nerve injury. [1] In this context, this work aims to produce bioprinted cellularized tubes suitable for a personalized clinical strategy to further stimulate the biological processes of PN regeneration. To this end, 3D printing technology with digital light processing (DLP) will be used to enable precise positioning of functionalized dextran-polycaprolactone (PCL) copolymer and biological entities (neurons and supporting cells) for the fabrication of NGCs with anisotropic properties and fine-tuned dimensions. Therefore, our strategy will enable on-site manufacturing of devices specifically tailored to the needs of individual patients and enable faster recovery after short/long injury breaks.

MATERIALS AND METHODS

Synthesis of the polymeric precursors: The polymers with photopolymerizable moieties, Dextran (M_w = 70'000 g mol^{-1}) functionalized with glycidyl methacrylate (GMA) and PCL-diol (Mw = 550 g mol^{-1}) modified with 2-isocyanoethyl methacrylate (IEMA) were prepared as reported elsewhere.[2]

Ink preparation: A mixture of Dextran-GMA and PCL-IEMA in different ratios were dissolved in DMSO to obtain a solution with a polymer concentration above 30%. To this mixture, previously dissolved LAP and tartrazine were added and let to solubilize at room temperature for 6 hours. The inks were kept away from light to avoid premature gelation and used as prepared.

Printing procedures: Predefined structures were fabricated using the previously described photocurable ink with a DLP 3D bioprinter (Lumen X, DLP bioprinter from CELLINK). After optimizing the printing conditions, the following DLP printing parameters were used: The exposure time for each 100-µm layer was 8.25 s, and the time scaling factor for the first layer was set to four times. The power of the projector was set to 55% (21 mW/cm2). After printing, all 3D-printed structures were immersed in distilled water for 3 days to remove unreacted products and allow complete solvent exchange. Finally, the 3D-printed structures were placed in a drying oven at 40 ºC until a constant weight was achieved.

RESULTS AND DISCUSSION

Biocompatible photopolymerizable precursors were used to fabricate 3D-printed constructs with tailored physicochemical properties. The presented ink formulation enabled printing of various structures with high resolution by DLP. More importantly, the ink formulation enabled 3D printing of cylindrical constructs with a clear lumen that can be used as nerve conduction tubes for PN regeneration. The 3D printed tubes exhibited good mechanical durability as determined by uniaxial compression tests. In addition, the printed tubes were found to be transparent and exhibited varying degrees of flexibility, which is essential for improved clinical handling and implantation. Preliminary in vitro cytotoxicity testing showed that the printed constructs were cytocompatible towards a fibroblast cell line.

CONCLUSIONS

A new bioink formulation using photopolymerizable biodegradable polymers has been successfully produced. This innovative combination of polymers has allowed us to obtain transparent and customizable NGC. The reported promising results thus represent a step towards the development of NGC specifically tailored to the needs of individual patients.

REFERENCES

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