Laser patterning bioprinting using a light sheet-based system equipped with light sheet imaging produces long-term viable skin constructs

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INTRODUCTION

Light-based 3D bioprinting has emerged as a pioneering technique in tissue engineering and regenerative medicine, offering the promise of fabricating complex, functional three-dimensional (3D) tissue constructs with precise control over cellular organization and spatial distribution. This research introduces a new 3D bioprinter that incorporates live imaging of the bioprinted tissue with high resolution and high-speed capabilities. The printer employs a light sheet-based system to photocrosslink polymers into hydrogels at a printing speed of up to 0.66 mm^3/s with a resolution of 15.7 µm. A significant advancement of this bioprinter is its ability to track cells and bioink during crosslinking, which enables real-time evaluation of the 3D-bioprinted structure's quality.

MATERIALS AND METHODS

The light sheet bioprinter was built by means of optical and electrical engineering on top of an optical breadboard. Custom designed cuvettes were thermoformed and filled with photocrosslinkable hydrogels and human Hs27 and HaCaT cells. Bioprinting took place at 37° C and constructs were extracted afterwards into a well plate for further investigation. Cell viability was assessed at several timepoints and immunofluorescence staining of markers were conducted. Fluorescence recovery after photobleaching was carried out to determine the crosslinked hydrogel's properties. Image processing was done in Fiji by ImageJ. The statistical analysis and plotting were conducted on Python 3.9. Normality was tested with a Shapiro-Wilk test ($p>0.01$). Statistical comparison between two groups was tested with Welch t-test ($p<0.01$).

RESULTS AND DISCUSSION

The custom-made light sheet bioprinter reads common G-code files used in 3D printing, hence any 3D structure can be bioprinted. For proof of concept, a wheel of resolution, with spokes ranging from 1 to 120 µm, a liver lobule and a torus were 3D printed. Then, fibroblast cells (Hs27) were encapsulated using this method, and the viability was evaluated directly after bioprinting and seven days after encapsulation, which was found to be high (83% ± 4.34%). Furthermore, a full-thickness skin construct (with Hs 27 and HaCaT cells) was bioprinted and maintained in culture for 6 weeks, demonstrating the long-term viability and physiological relevance of the bioprinted tissue in terms of stratification and gene expression.

CONCLUSIONS

This novel, light based bioprinting technique enables fast and high resolution biofabrication, coupled with the possibility to capture cells and hydrogel before, during and after the bioprinting procedure via light sheet fluorescence imaging. High viability and physiological human full thickness skin constructs were bioprinted and cultivated over the course of 42 days.

REFERENCES

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