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Abstract

Jet-based bioprinting of viable human cells

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This work demonstrates that viable human cells may be deposited in predefined geometries by an HP (HP inc.) TIPS (Thermal Inkjet Pipette System) jet-based bioprinter. Though pioneering work in the realm of jet-based bioprinting has been done with modified conventional jet printers, as described elsewhere [1], this prototype seeks to make the step towards creating a dedicated bioprinter for commercial and research use. The aim of these experiments is the incipient use of this bioprinter for printing human cells and verification of the cell viability following printing. The controller, or printhead, was provided by HP inc., and was mounted on a Creality™ (Shenzhen Creality 3D Technology Co, Ltd., Shenzen, China) printing stage, allowing for three-dimensional motion capabilities that are typical of a conventional 3D printer. Human embryonic kidney (HEK293) cells were suspended in a low-calcium cell media suspension to form a bioink—a suspension of cells in a carrier solution for use in jet-based bioprinting. The bioink solution is a 0.01 mM Ca^{2+} modified DMEM solution (Gibco, Thermo Fisher, Waltham, MA, USA). The bioink was then filled into the 150 µl jetting cartridges that are subsequently inserted into the printhead to allow for controlled extrusion via the thermal jet-based nozzles that are attached to the cartridges, allowing printing of droplets of bioink through a 2x5 array of 80 µm nozzles. A concentration of 3.5 x 10^6 cells/ml has been shown to be an optimal cell concentration from the bioink, allowing for normal cell proliferation post-print while avoiding clogging the printer tip. Cell viability post-print was determined using Calcein AM dye (Invitrogen, Thermo Fisher, Waltham, MA, USA) – cells were dyed while still confluent and adherent. The fluorescently loaded cells were then trypsinized and prepared in suspension in the carrier bioink solution as mentioned above for printing. After printing 1 cm^2 squares, containing a cell bioink volume of approximately 40 µl per square, into a 6-well plate (Corning Falcon, Corning, NY, USA), 2 ml of cell media were added to each well. The printed cells were then excited with a 480 nm light source and imaged at 535 nm. These fluorescence images show cell viability of over 90% immediately post-print, aligning with the results of a similar study by Boland et al. [2]. Printed cells reached approximately 70-100% confluence per well five days post printing. The bioink described thus far has a very low viscosity, causing the printed squares have little structural fidelity. Cells migrate upon adding cell media immediately post print – a necessary step to prevent evaporation and support cell proliferation. To combat this, HyStem®-C (Advanced BioMatrix, Carlsbad, CA, USA), a hydrogel containing hyaluronic acid, gelatin, and polyethylene glycol diacrylate (PEGDA) was used to add a supportive structure to the printed cells. The hydrogel solution was diluted with Milli-Q® water until it could be printed with the jet bioprinter but still allowing for crosslinking following jet-bioprinting. The diluted hydrogel was then deposited with the bioprinter in alternating layers with the bioink, creating a cell-laden three-dimensional biological structure that can be maintained in cell media after crosslinking and retain its shape.

Author's statement

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References

[1] Wilson Jr, W.C. and T. Boland, *Cell and organ printing 1: Protein and cell printers.* The Anatomical Record Part A: Discoveries in Molecular, Cellular, and Evolutionary Biology, 2003. 272A(2): p. 491-496
[2] Xu, T., et al., *Inkjet printing of viable mammalian cells.* Biomaterials, 2005. 26(1): p. 93-99.

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