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Utilization of cryogenic temperatures to reduce line width variability in 3D bioprinted hydrogel lattices

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Abstract: The 3D bioprinting of acellular hydrogel scaffolds holds enormous potential for wound healing and the regeneration of tissue. The precision of hydrogel scaffolds is limited by the diffusion and fusion of soft hydrogels during the printing process. The use of cryogenic temperatures may reduce the unequal diffusion of 3D printed hydrogel and thus protect pore morphology. Our results suggest that hydrogel lattices printed at cryogenic temperatures display less variation in line width along the length of the printed segments as well as sharper outer corners.

I. Introduction

The 3D bioprinting of acellular hydrogel scaffolds holds enormous potential for wound healing and the regeneration of tissue. Like all 3D printed objects, the printability of 3D scaffolds is limited by factors such as printing fidelity and stability [1]. The additive manufacturing of hydrogel scaffolds presents a unique set of challenges over conventional 3D printing due to the soft nature of the material. Hydrogels typically possess a low phase transition temperature and as a result do not solidify quickly at room temperature as they are extruded onto the build platform [2]. As a result, hydrogel structures are prone to diffusion where line segments spread out as well as fusion where multiple line segments join together [3]. While logistically challenging to print, these soft hydrogels are ideal for mimicking the soft tissues of the human body [4]. 3D printing techniques that are able to produce geometrically accurate scaffolds thus hold significant potential in the tissue engineering field [4].

I.I. Cryogenics

A promising new 3D bioprinting technique is the use of cryogenic temperatures, as introduced by Adamkiewicz et al and continued by Tan et al [2,4]. Cryogenic temperatures allow gel-like bioinks to transform into a solid state as they are deposited onto the print bed. These stable solid structures have a reduced chance of deformation and diffusion, which is ideal for the layer-by-layer 3D printing approach [4]. Recently, Tan et al used cryogenic temperatures to print super soft hydrogels, and successfully replicated the mechanical properties of the softest tissues in the human body. Here we use a Peltier-based cooling system to print hydrogel lattices at -21ºC and room temperature (23ºC). We find that the hydrogel lattices printed at -21ºC display significantly less variation in line width as well as less diffusion and fusion at the outer corners.

II. Material and methods

II.I. Printing Methods

A CELLINK Inkredible+ Bioprinter (Gothenburg, Sweden) was modified to include a custom Peltier-based cooling platform with fine temperature control (± 0.5ºC). The cooling of the build platform was attained after calibration with a K-type thermocouple (Omega SA1XL-K-120), custom analog electronics, and LabView sampled data acquisition system.

The nanocellulose based CELLINK START hydrogel was extruded at 0.0030 ml/s through a 24 gauge nozzle. The nozzle was positioned 0.6 mm above printing platform and moved at a speed of 5 mm/s. The lattices were 24mm x 24mm with nine equally sized rectangular pores. Lattices were printed on a glass coverslip located on the cooling platform at either -21ºC or 23ºC. Temperature was monitored throughout the print to ensure temperature variation less than 0.5ºC.

II.II. Image Collection and Analysis

Digitized uncompressed images (20 x magnification) of each lattice were taken with a microscope camera as shown in Fig 1. NIH ImageJ was used to analyze the images. For the purpose of analysis, the lattices were visually divided into eighteen equal segments where each 8mm side of a rectangular pore was considered to be a segment. The line width was then sampled in three places along each segment and the radius of curvature was sampled at the lower outer corners.

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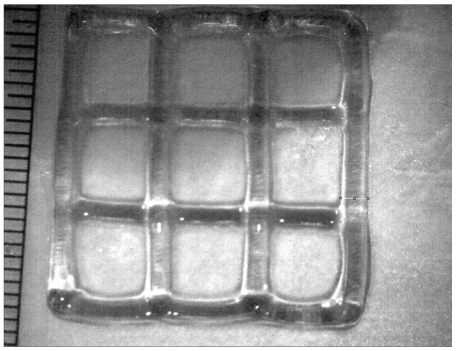

Fig 1. A multilayer 24mm x 24mm hydrogel lattice printed at -21°C. A line used in ImageJ to determine line width is visible on the right hand side and middle row of the lattice.

The total variation between the line widths of segments was compared for the entire lattice at -21ºC and 23ºC. The radius of curvature of the outer corners of the lattices was measured in order to determine the amount of diffusion and fusion occurring at the corners.

III. Results and discussion

The line segments of lattices fabricated at -21 °C displayed significantly less variation in line width than the line segments of lattices printed at 23 °C as shown in Fig 2. A two-sample one-tailed t-test found that the average the line width variation of cryogenic prints was significantly less than the average line width variation of the room temperature prints with a p-value of 8.9556e-12.

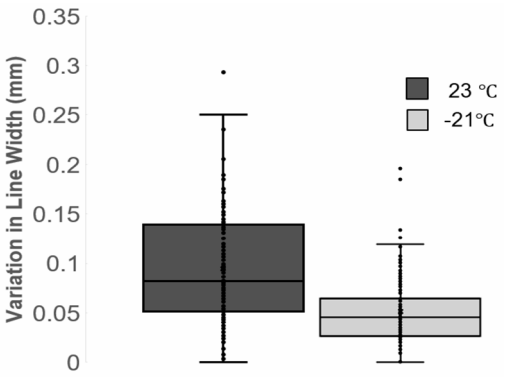

Fig 2. Variation of line width in the segments of lattices printed at 23 °C and -21 °C. Lattices printed at cryogenic temperatures display less variation in line width.

The radius of curvature of the outer corners of the lattices was measured in order to examine the amount of diffusion and fusion occurring at these areas. Fusion of horizontal and vertical line segments at the corner results in a larger radius of curvature. The outer corners of lattices fabricated at -21 °C displayed a significantly smaller radius of curvature than the lattices printed at 23 °C as demonstrated below in Fig 3.

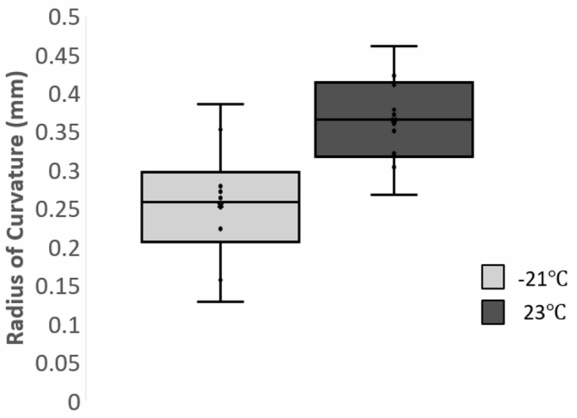

Fig 3. Radius of curvature of the outer corners of lattices printed at 23 °C and -21 °C. Lattices printed at cryogenic temperatures display less corner diffusion and fusion and thus sharper corners.

A two-sample one-tailed t-test was used to compare the average radius of curvature for -21 °C and 23 °C prints. The test found that lattices fabricated at cryogenic temperatures display a significantly smaller radius of curvature, and the p-value was 9.9864e-04.

IV. Conclusions

This report finds that hydrogel lattices printed at cryogenic temperatures display less variation in line width as well as less diffusion and fusion at the outer corners of the lattice. Successful 3D bioprinting requires methods that ensure precision and can easily replicate designs. Future research should examine the range of cryogenic temperatures that are optimal for various types of hydrogel.

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Author's statement

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