Materials Letters 264 (2020) 127382

Contents lists available at ScienceDirect

Materials Letters

journal homepage: www.elsevier.com/locate/mlblue

A comparative study in the printability of a bioink and 3D models across two bioprinting platforms

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ARTICLE INFO

Article history:
Received 30 August 2019
Received in revised form 9 January 2020
Accepted 18 January 2020
Available online 22 January 2020

Keywords:
Alginate
Biomaterials
Bioinks
Bioprinting
Gelatin
Polymers

ABSTRACT

In this study, we used an alginate-gelatin bioink to design and print 3D constructs with lattice, honeycomb and fibrous bundle patterns. These designs were printed using a small-scale laboratory printer at first, and later translated to a larger scale, high throughput-printing platform. A comparative analysis of the structures printed using two dissimilar platforms using gross morphologic evaluation, scanning electron microscopy and swelling assay confirmed our hypothesis that a design printed using a small-scale laboratory bioprinter for optimization of bioink composition and printing parameters can be successfully translated into a large scale-printing platform for high throughput printing of constructs. Since the designs for printing were implemented using a software which was common across both printers, this endpoint was feasible. The only difference in printing parameters resulted from variation in extrusion pressure which was due to a significant difference in barrel size used across both printers (3 ml versus 30 ml), while all other parameters stayed the same. Although the scaffolds were not bioprinted with cells, in future we will investigate how cell viability can be differentially regulated by the variation of extrusion pressure across both platforms.

1. Introduction

Bioprinting is an extension of traditional three-dimensional (3D) printing that can lead to fabrication of living tissues such as bone, cartilage and cardiac tissues eventually paving the way for printing of entire organs for use in the clinic [1,2]. Imminent challenges include complications in mimicking the cellular complexity of the human physiological system which has impeded the progress of clinical 3D bioprinting. Secondly, there is a limitation on the high throughput abilities of the bioprinters used for biofabrication. If these challenges are met, it will help provide opportunities to generate patient-specific tissues for the development of accurate, targeted and completely personalized treatments.

In this study, we used a small-scale laboratory bioprinter (BioX) to optimize the bioink composition and standardize characteristic designs for printing of scaffolds used as building blocks for bone, ligament and cardiac tissues. The bioink composition and designs were then translated to a high throughput printing platform (BioAssembly Bot or BAB) for comparison of printed structures based on morphology, structural fidelity and microstructure. Small-scale printers are compact enough to fit in a biosafety hood, are constrained to move in a three-coordinate rectilinear fashion, have a small barrel volume (~3–10 mL), can utilize up to four different bioinks during a print-job, and usually construct one structure at a time [3]. Large-scale, or industrialized high throughout bioprinters, have more degrees of freedom in motion, can be loaded with high bioink volume (~30–55 mL), can utilize more than four bioinks at a time, and can print multiple constructs in a short period, making them much more versatile [3]. The purpose of this work is a comparative study of printed 3D scaffolds fabricated using a BioX and later translated to a BAB. Prints created from both platforms were expected to mimic structural details included in the design files. Moreover, prints from both platforms should bear morphological and ultrastructural resemblance that classifies as design criteria of a successful translation from a small-scale laboratory printer to a high throughput-printing platform.

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https://doi.org/10.1016/j.matlet.2020.127382
0167-577X/

2. Materials and methods

Three specific patterns including a lattice, a honeycomb structure and a fibrous bundle were designed using CAD platforms and translated onto TSIM software (Advanced Solutions Inc., Louisville, KY) for the BAB (Advanced Solutions, Inc., Louisville, KY) and saved as STL files for the BioX (CELLINK, Gothenburg, Sweden). The lattice design was intended to mimic cardiac tissue, the honeycomb for trabecular bone and the fibrous bundle was for ligament tissue.

Alginic acid sodium salt or alginate (medium viscosity, MP Biomedical, Santa Ana, CA, USA) was employed as a bioink as it is a naturally occurring polymer that is easily crosslinked with multivalent cations. However, alginate does not contain any cell-adhesion moieties, requiring mixing of gelatin (type A, 90–110 bloom from porcine skin, MP Biomedical) into the ink formulation to provide sites of cell attachment on RGD residues along the polymer. The two polymers were mixed into a final composite concentration of 7% alginate-4% gelatin as it has been previously reported to exhibit excellent printability [4]. Gelatin was gradually dissolved in DI water (5 ml, 25 °C) with a magnetic stirrer and then placed in the water bath (10 min, 37 °C). Alginate was then added to the dissolved gelatin solution and the solution was manually stirred (15 min, 37 °C) to ensure homogeneity. This mixture was then loaded into a 3 mL plastic cartridge (CELLINK) and fitted with a precise tip-dispensing needle (22 G, 0.41 mm ID) for printing (100–130 kPa, speed of 3 mm/s). The extrusion capability and uniformity of printed structures was confirmed by printing of structures depicting a straight line and a circle, respectively.

Each sample representing the three varying designs were present as triplicates for this study. The structures printed using the in-house bioink were crosslinked (5 min, 420 mM calcium chloride: ThermoFisher) to retain structural fidelity. Following successful printing of the different structures on the BioX, the bioink was loaded into 30 mL plastic cartridges (Advanced Solutions Inc.) for checking its printing translatability on the BAB. All other printing parameters were maintained constant in comparison with the BioX except extrusion pressure (138–207 kPa for the BAB).

All procedures adopted in this study have been published previously by our group [5]. Gross morphological analysis and scanning-electron microscopic (SEM: S-4800, Hitachi, Japan at voltages of 12 kV at varying magnifications) imaging was applied to comparatively analyze structures printed using both platforms. En-face and cross-sectional SEM micrographs were acquired after air-drying the crosslinked hydrogels and sputter coating with gold (Gatan Model 682 Precision etching coating system, Pleasantown, CA, USA). ImageJ (NIH) was used to analyze cross-sectional SEM images to determine average pore size. A comparison of swelling trends between all crosslinked and non-crosslinked printed structures was performed to determine the degree of swelling for all samples.

3. Results and discussion

The designs were optimized for this study based on analysis of resultant gross morphology of printed structures and inner structural details for each structure (Fig. S1). The printability of the alginate-gelatin bioink was determined by linear and circular patterns deposited and the variances in the uniformity of the constructs was studied, as shown in Fig. 1. Results revealed a relatively uniform extrudability of the bioink, with the mean thickness of the ring structure being 2.06 ± 0.27 mm and the printed line had an average mean uniformity ratio of 1.03 ± 0.02, calculated using published procedures [4].

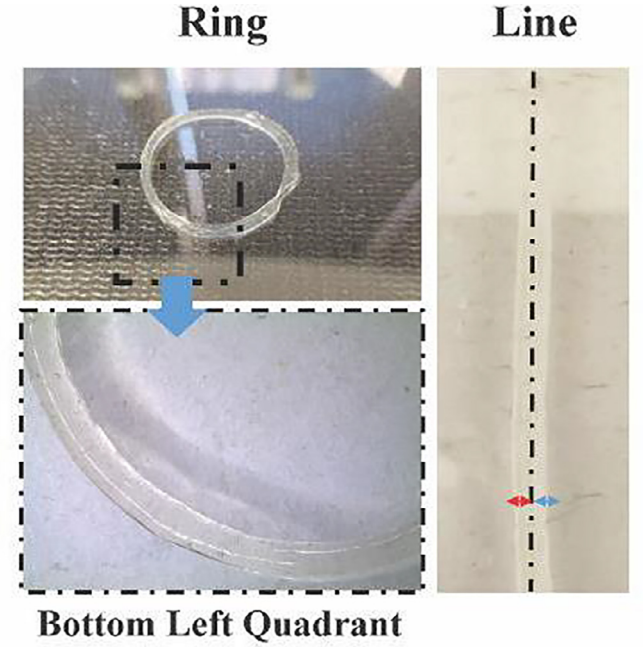

Fig. 1. Characterization of extrusion uniformity of the gelatin-alginate bioink mixture and its printability. Representative images of linear and circular printed patterns are depicted.

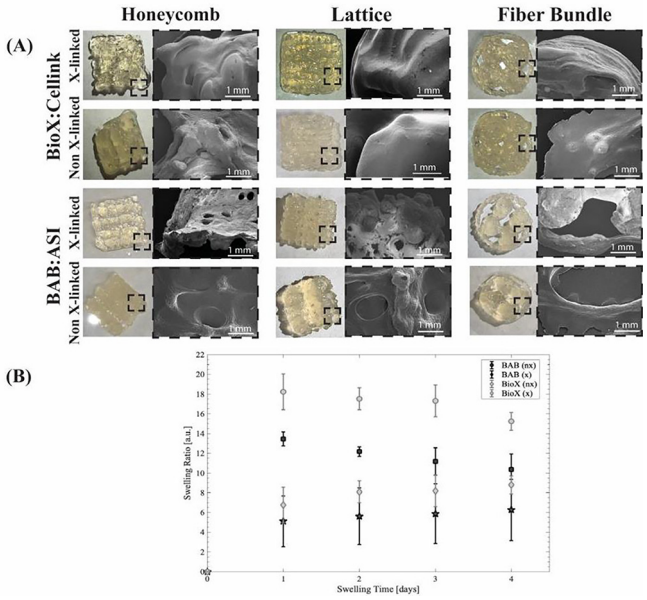

Fig. 2. Comparison of structures printed from BioX and BAB. Shown in (A) are gross morphologies and en-face SEM images of crosslinked and non-crosslinked structures printed. In (B) a comparison of swelling behavior of crosslinked and non-crosslinked structures is shown.

In Fig. 2**,** a comparison of the structures printed using both platforms, and crosslinked after printing or left as is (non-cross-linked) are shown. Although the structures printed using both platforms bore resemblance and macro-structural similarities, prints from the BAB had finer resolution with ultrastructural details (Fig. 2**A**), in comparison with the prints from the BioX.

A comparison of the hydration behavior of crosslinked and non-crosslinked structures (Fig. 2**B**) revealed significant differences in trends between crosslinked ($p = 0.019$) and non-crosslinked ($p = 0.017$) structures, printed using BioX and BAB. Overall, all

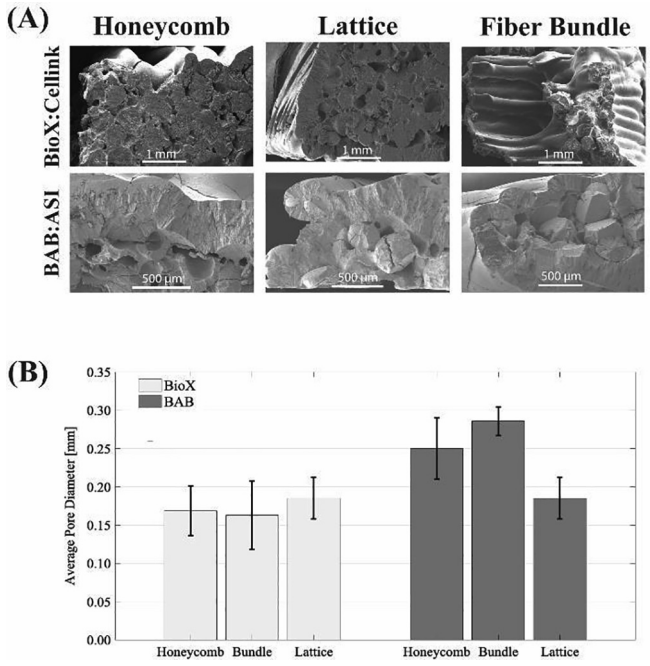

Fig. 3. (A) Cross-sectional SEM images depict the multilayered assembly and homogenous, well distributed porous structure of the printed patterns, from the BioX (top panel) and BAB (bottom panel). (B) Comparison of average pore diameter of the same pattern casted using both printers.

crosslinked structures showed lesser degree of swelling compared to the non-cross-linked structures. The structures printed using the BAB showed enhanced structural stability and lesser extent of swelling compared to the structures printed using the BioX. This implied that scaling up of the printing process from the BioX to the BAB enhanced the degree of resolution of the printed constructs affording improved crosslinking and retention of enhanced structural stability compared to their counterparts printed using the BioX, which is contradictory to our existing knowledge.

A comparative analysis of the SEM cross-sectional images of structures (Fig. 3A) confirmed that pore diameter was conserved although the constructs printed using the BAB showed an overall increase in pore diameter (Fig. 3B, not statistically different) when designs were translated from the BioX. This difference in trend for pore diameter could account for the difference in swelling behavior between constructs printed using both platforms.

4. Conclusion

3D bioprinted scaffolds and hydrogels have an immense translational potential for studying effects of therapeutic agents on cells, as they are known to accurately mimic native tissues and support further relevant cell–cell interactions. However, the development of cost-effective, high-throughput human scale tissue constructs remains challenging. Herein, we presented a proof-of-principle, simple scale-up study where the feasibility of translation of designs depicting increasing structural complexity from a small scale printer to a larger high throughput printing platform was performed for the very first time. These results holds great potential for the translation of development of additive manufacturing based strategies for tissue engineering. Furthermore including the use of cells, biomaterials, and macromolecules to create basic building blocks of tissues and organs, will move forward the field of biofabrication to transform regenerative medicine.

CRediT authorship contribution statement

Matthew Alonzo: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing - original draft, Writing - review & editing. **Erick Dominguez:** Data curation, Investigation, Methodology. **Fabian Alvarez-Primo:** Data curation, Investigation, Methodology. **Amado Quinonez:** Data curation, Investigation, Methodology. **Erik Munoz:** Data curation, Investigation, Methodology. **Jazmin Puebla:** Data curation, Investigation, Methodology. **Antonio Barron:** Data curation, Investigation, Methodology. **Luis Aguirre:** Data curation, Investigation, Methodology. **Ana Vargas:** Data curation, Investigation, Methodology. **Jean M. Ramirez:** Data curation, Investigation, Methodology. **Binata Joddar:** Writing - review & editing, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

M.A. acknowledges the Gates Millennium Scholarship and the Eloise E. and Patrick B. Wieland fellowship. E.D. acknowledges the National Institute of General Medical Sciences of the National Institutes of Health under RL5GM118969, TL4GM118971, and UL1GM118970.

Funding sources

This work was supported by the NSF-PREM (DMR-1827745), NIH 1SC2HL134642-01 and the NSF-MRI (DMR 1826268).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.matlet.2020.127382.

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