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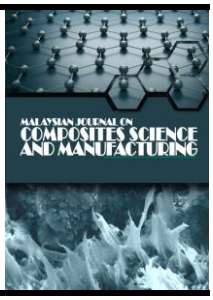
Malaysian Journal on Composites Science and Manufacturing

Journal homepage:
https://www.akademiabaru.com/submit/index.php/mjcsm/
ISSN: 2716-6945



Preparation of Bioink for Hydrogel Printing in Additive Manufacturing

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ARTICLE INFO

Article history:
Received 9 September 2023
Received in revised form 5 October 2023
Accepted 3 November 2023
Available online 30 November 2023

ABSTRACT

Three-dimensional (3D) bioprinting is a significant advancement in tissue engineering as it enables the printing of relevant scaffolds used for tissue repair and treating conditions like organ failure. However, printing soft biomaterials has been a major challenge due to their susceptibility to gravitational collapse. A GelPrint Scaffold method for hydrogel preparation was developed to overcome the obstacle. It provides a solution by holding the soft biomaterials in a gelatin slurry support bath during printing, polymerizing the biomaterial for crosslinking for a well-structured scaffold build-up. The bioprinting process of the alginate scaffold was conducted using a customized liquid extruder attached to a commercial 3D printer. The pores of the crosslinked printed structures were measured to identify the susceptibility of the scaffold for cell culture. The results demonstrated that the approach successfully fabricated 3D printed alginate scaffolds, approximately 90% similar to the Computer-aided design (CAD) design dimension, which has the potential to be applied to various tissue engineering applications. These scaffolds hold great promise for various tissue engineering applications, indicating the potential of the adapted bioink preparation method in advancing the field of regenerative medicine.

Keywords:
Additive Manufacturing, Bioprinting, Hydrogel

1. Introduction

Human tissues contain a supportive structure called the Extracellular Matrix (ECM), which is vital in providing biochemical and biomechanical support to cells [1]. Tissue engineers employ compatible biomaterials, such as natural or synthetic materials, to mimic the ECM in native tissues. These biomaterials enhance tissue function, support tissue repair, and facilitate healing. One notable application of biomaterials lies in three-dimensional (3D) bioprinting. Bioprinting uses a computer-controlled 3D printing device to deposit cells and biomaterials accurately into the models of organs

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https://doi.org/10.37934/mjcsm.12.1.4350

[2]. This cutting-edge technique in tissue engineering involves using a biomaterial and crosslinker to print patient-specific, clinically relevant scaffolds, especially for treating conditions like end-organ failure. The synthesis, bioprinting and crosslinking of bioink significantly influence the mechanical and cellular behavior of the biological structures. Thus, biomaterial is the most important element in 3D bioprinting, influencing the bioprinting technique and selected cells [3].

Hydrogels are commonly used for bioprinting due to their biocompatibility and provide a characteristic of native tissues and the extracellular matrix. Historically, natural hydrogels are commonly used for bioprinting, including alginate [4,5], gelatin [6,7], collagen [8,9], and chitosan [10,11]. In this research, sodium alginate is employed as a biomaterial for the printing process. Alginate hydrogel is an excellent material for imitating the extracellular matrix (ECM), which is the outer environment of the cell [12]. Besides, alginate possesses multiple essential advantages, including non-immunogenic, biodegradable, and biocompatible [13], making it suitable for various biomedical applications, such as wound healing and drug delivery [14]. Alginate stands out as a cost-effective biopolymer due to its wide availability and efficient industrial production [14]. However, natural hydrogels such as alginate are naturally weak, and the obtained filaments spread quite easily [15]. If the printed filaments cannot maintain their shapes, it will further affect the subsequently printed layers and then the whole structure. This is because the first few printed layers can easily collapse or deform under the weight compared to the upper layers. Therefore, printing a natural hydrogel layer-by-layer into a 3D scaffold is very challenging.

A GelPrint Scaffold method was developed to combat this problem. This approach supports soft biomaterials such as alginate in a gelatin slurry support bath during printing and allows subsequent crosslinking for a well-structured printed scaffold. The aim is to apply the GelPrint Scaffold method to 3D print alginate scaffold using the customized liquid extruder attached to a commercial 3D printer. The printability of alginate was evaluated with two different printed designs, and the crosslinked printed structure was analyzed using Scanning Electron Microscopy (SEM). Therefore, the GelPrint Scaffold bioprinting method holds great promise and can be adapted as a valuable teaching technique for bioprinting.

2. Methodology

2.1 Sodium Alginate Preparation

A solution was prepared by adding 2% (w/v) sodium alginate into 100 ml distilled water. The mixture was stirred using a magnetic stirrer until the powder completely dissolved. Subsequently, 0.1% (w/v) blue food coloring was incorporated into the solution. The solution was carefully transferred into an amber bottle, and the lid was securely sealed to prevent contamination. For the 3D bioprinting process, sodium alginate solution was loaded into a syringe to create the desired three-dimensional scaffold.

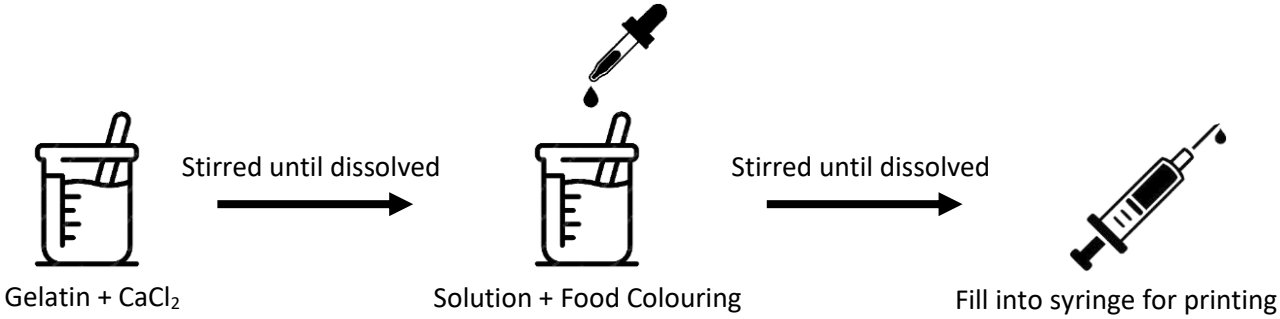

Fig. 1. Preparation of sodium alginate

2.2 Gelatin Slurry Support Bath Preparation

A solution was prepared by mixing 6% (w/v) of food grade gelatin into 150 ml of 11mM $CaCl_2$ and dissolved at 40°C using a magnetic stirrer. Once dissolved, the solution was placed in a 200 ml blender jar at 4°C to form a solid gel. After 12 hours, a cold 11mM $CaCl_2$ was added halfway into the blender jar, and a spatula was used to separate the solid gel from the wall of the blender jar. The blender jar was then filled to the brim with 11mM $CaCl_2$ and placed in a 4°C freezer for 15 min prior to blending to help minimize the risk of overheating. Once removed from the freezer, the solution was blended using "pulse" mode for 120 s to produce gelatin slurry. The blended gelatin slurry was then poured into a 20 ml conical tube and centrifuged for 2 min at 4200 rpm. After centrifugation, the supernatant was removed, and hydrogel slurry was left at the bottom of the conical tube. The centrifuged process was considered complete when no bubbles were present at the top of the supernatant, indicating that most of the soluble gelatin had been removed. At this stage, the gelatin slurry could be stored at 4°C. For 3D bioprinting, the gelatin slurry was transferred into a petri dish to serve as the scaffold's support medium for printing. Figure 2 shows the preparation of gelatin slurry support bath.

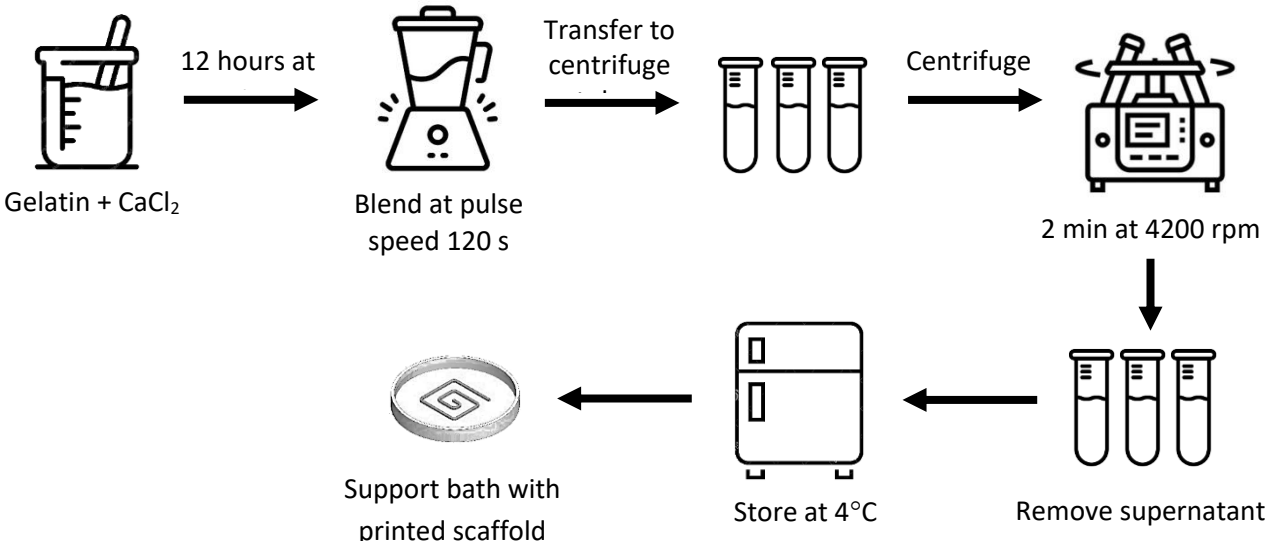

Fig. 2. Preparation of gelatin slurry support bath

2.3 Crosslinked Hydrogel Preparation

5% (w/v) of $CaCl_2$ solution was prepared for crosslinking the printed scaffold. The hydrogel was then submerged in the calcium chloride solution for 12 hours to ensure complete crosslinking. Following the crosslinking process, the hydrated hydrogel was washed with distilled water at least thrice to eliminate any remaining calcium chloride in the printed scaffold.

2.4 3D Bioprinting Structure

The Snapmaker © 3D Printer with a customized extrusion system was used for 3D bioprinting [16]. Two types of scaffolds were designed as illustrated in Figure 3: grid square (35mm × 35mm × 10mm) and blood vein profile using SolidWorks. The printing parameters for this experiment were selected according to the previous study conducted by Xu *et al.* [16] in order to have reproducible and well-structured scaffolds. For the extrusion process of hydrogel, the 3D printer was manually

calibrated in the z-direction by 2 mm from the surface. This adjustment allows the printer to suspend the print within the gelatin slurry support bath while printing. After calibration, the .stl file of the 3D scaffold design was uploaded to the Snapmaker Luban software for printing. Each scaffold was printed out with five layers in the gelatin slurry support bath at 24°C. After printing, the gelatin slurry support bath was melted at 37°C to remove the printed structure. The printed structures were placed in a petri dish containing $CaCl_2$ to crosslink the alginate for about 12 hours.

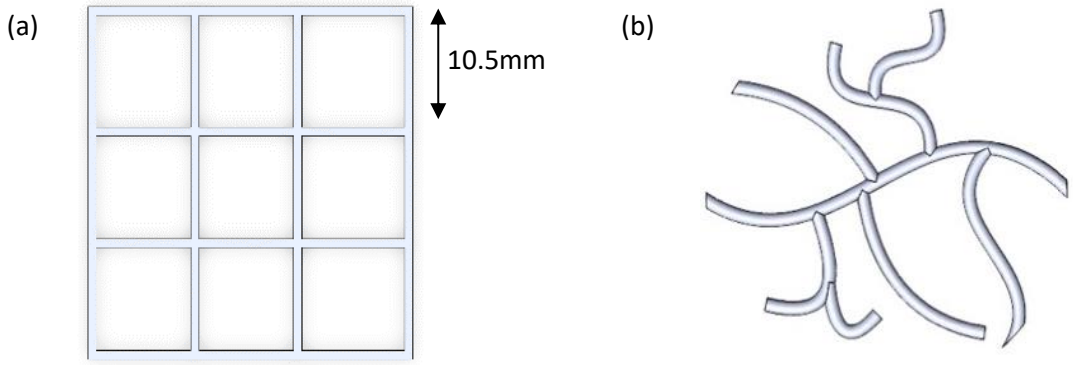

Fig. 3. Scaffold designs: (a) grid square, (b) blood vein profile

2.5 Microstructure Analysis

For the microstructure analysis, the grid square hydrogel was synthesized and printed into a gelatin slurry support bath as described in crosslinked hydrogel preparation. A grid square scaffold was first dried out in a petri dish for about 1 hour at 25°C. The resulting dried grid square scaffold was sliced carefully using a cutter knife and tweezers, and then the cross-section of the grid square was dried again with a high vacuum in Gold Sputter Coater, as shown in Figure 4. After sputter coating, the dried grid square scaffold was analyzed in SU 5000 Field Emission Scanning Electron Microscopy (Hitachi, Germany) with a high vacuum. Four SEM imaging with magnifications of 500x and 2000x were taken on the cross-section of the grid square scaffold (Day 1 and Day 14). ImageJ processing image program was utilized to determine the pore size.

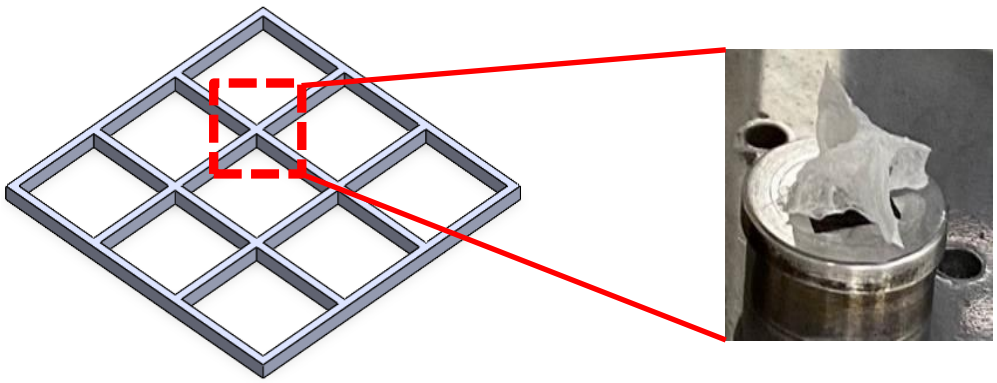

Fig. 4. Cross-section of grid square scaffold

3. Results

3.1 Bioprinting with the GelPrint Scaffold Method

The grid square and blood vein profile were printed using the GelPrint Scaffold method, as shown in Figure 5a. After printing, both scaffolds were immersed in a 5% (w/v) solution of calcium chloride for a complete crosslinking of the alginate. As illustrated in Figure 5b, the results showed both printed scaffolds were successfully printed and well-structured with five layers according to the 3D design scaffold. This observation indicates that the GelPrint Scaffold method with $CaCl_2$ is beneficial for 3D bioprinting of a soft alginate material.

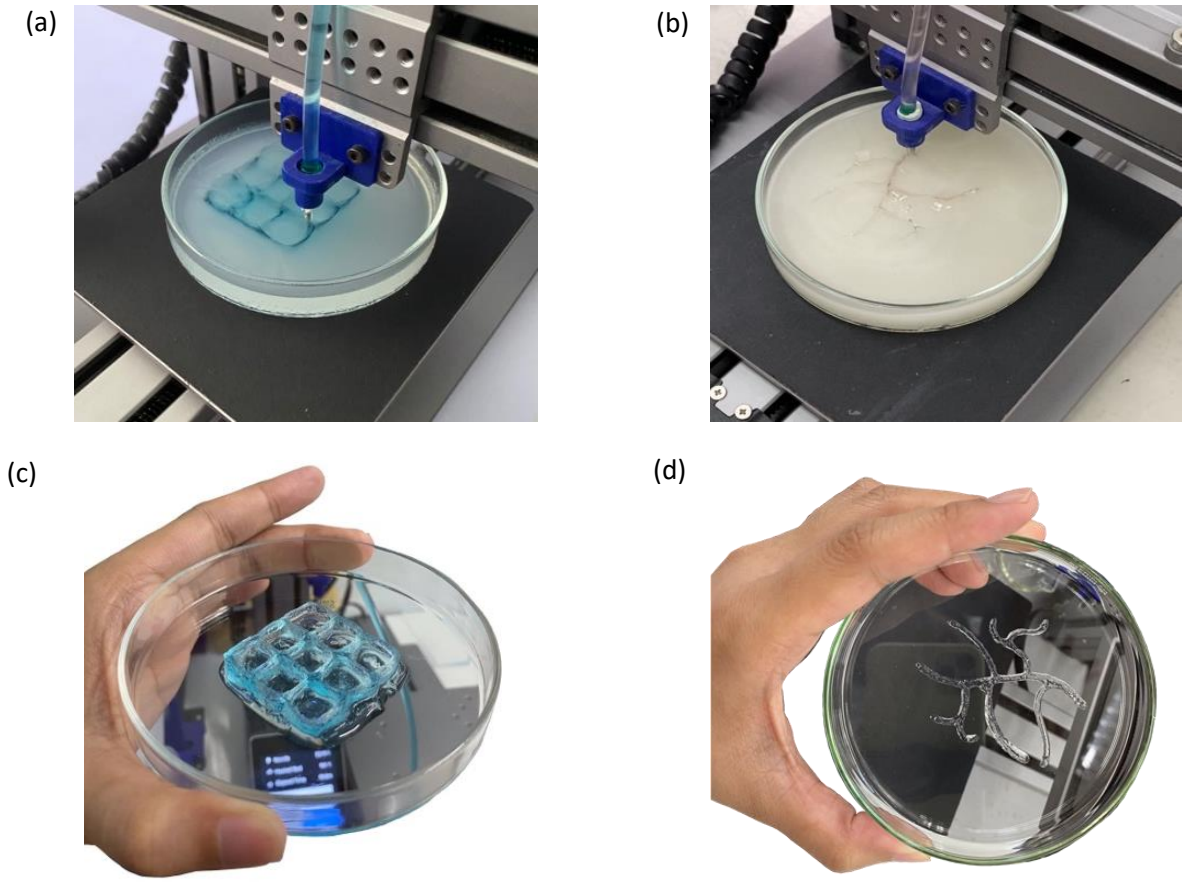

Fig. 5. Bioprinting alginate using GelPrint Scaffold method (a) grid square, (b) blood vein profile, 3D printed scaffolds (c) grid square, and (d) blood vein profile

3.2 Microstructure Analysis

The microstructure of the alginate printed scaffold was observed using Scanning Electron Microscopy (SEM). Figure 6a shows the SEM imaging with magnifications of 500x (left) and 2000x (right) on the cross-section of the grid square. It was observed that the scaffold possesses a highly porous and interconnected pore structure, which resulted from the scaffold drying process and the use of high vacuum during sputter coating. This porous configuration offered significant advantages for cell attachment, proliferation, and tissue growth, facilitating the diffusion of nutrients and exchange of metabolites within the scaffold. As stated in the previous research by Urruela-Barrios *et al.* [17], this honeycomb-like structure provides a structural reinforcement that allows the capsule to possess higher thermal and mechanical resistance. Based on ImageJ, the pore size of the scaffold was

measured to be approximately 25-255 μm, providing an ideal environment for cell attachment and migration. After two weeks of the printed scaffold submerged in $CaCl_2$, the reduction or shrinkage of the porous structure can be observed, as shown in Figure 6b. This change occurred due to the ionic bonding between the excessive Ca2+ ions used and the alginate chains during the crosslinking process, causing the alginate chains to come closer together as a result of the chemical reaction [18]. Unfortunately, this reduction in pore size rendered the printed scaffold unsuitable for tissue culturing, limiting its potential applications.

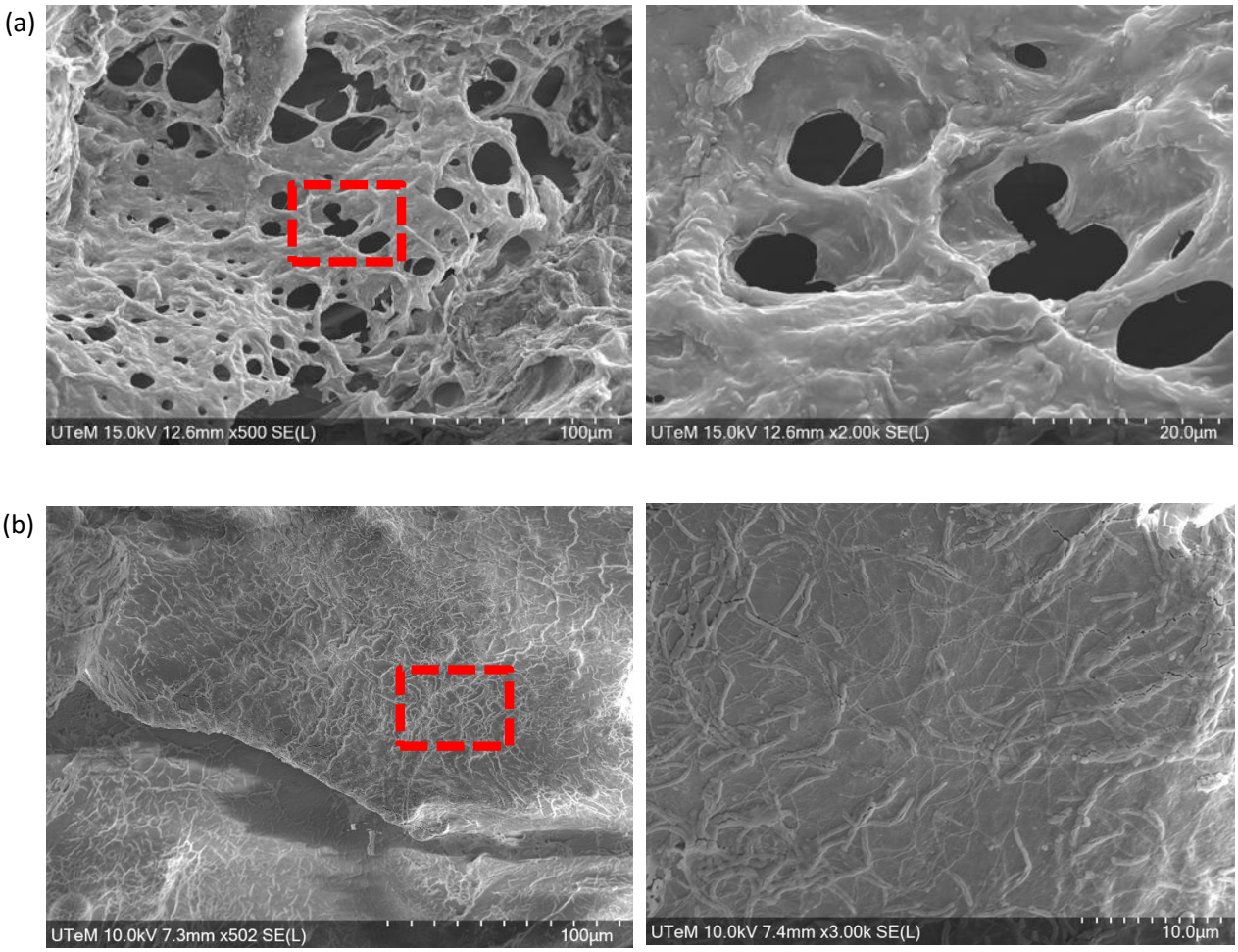

Fig. 6. (a) SEM imaging with magnifications of 500x (left) and 2000x (right) on the cross-section of grid square scaffold with highly porous structure, (b) SEM imaging with magnifications of 500x (left) and 3000x (right) on cross-section with shrinkage structure

4. Conclusions

This study prepared 2% of alginate hydrogel, and their 3D printability was studied. Alginate was considered a soft biomaterial, which was difficult to bioprint more than one layer. The GelPrint Scaffold method on bioprinting proved that a gelatin slurry support bath was a viable way to print a soft biomaterial with a complex design, such as a grid square and blood vein profile. Furthermore, microstructure analysis revealed that the printed scaffold exhibited a highly porous structure, a crucial property desired in tissue engineering for efficient biological delivery. This finding highlights the potential of bioprinting using the GelPrint Scaffold method as a promising technique in the field of tissue engineering. Consequently, it can be concluded that the GelPrint Scaffold bioprinting

method holds great promise and can be adapted as a valuable teaching technique for bioprinting. Its advantages, particularly in the field of tissue engineering, make it a valuable tool for advancing research and applications in the field of regenerative medicine. For future direction, it would be interesting to explore the use of cells in printed scaffolds to determine the viability of scaffolds for cell growth.

Acknowledgment
This research was supported by the Universiti Teknikal Malaysia Melaka (UTeM) through the short-term research grant PJP/2019/FKP(5B)/S01663.

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