



Research Article

Printability of Hydrogel Composites Using Extrusion-Based 3D Printing and Post-Processing with Calcium Chloride

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Abstract

Bioprinting is a promising technique in processing hydrogels to fabricate different matrices with active agents in the pharmaceutical industry. This study investigated the printability of gelatin-alginate hydrogel by extrusion-based 3D printing. 3D printed structures were then post-processed with calcium chloride solution to improve surface smoothness and gel strength. In this study, we aimed to fabricate bioscaffold using natural biopolymers with different ratios of Gelatin and Alginate (G/A) to deliver pharmaceutical or supplemental ingredients. The G/A influenced the rheology properties, which were strongly correlated to the 3D printability and deformability of the materials. There was a shear-thinning behavior for all three materials tested with G/A of 1:4, 1:1, and 4:1. All materials showed the magnitude of G' higher than G'' , and the loss factor $\tan \delta < 1$. Furthermore, the viscoelastic properties of materials with G/A 1:1 and 1:4 were within the range of the loss factor $\tan \delta$ 0.3 to 0.5, which was potentially 3D printable. The best 3D printability and the least deformation were both observed for G/A of 1:1, making it superior than G/A 1:4 and 4:1 for 3D printing purposes. Also, the soaking time for post-processing also affected the surface smoothness and gel strength. When 3D printed matrices were immersed in CaCl_2 solution (0.1M) for a longer time, the surface smoothness was highly improved, but the deformation also increased. Overall, the material with G/A ratio 1:1 and post-processed with CaCl_2 for a shorter length of time should

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have a huge potential to be used for extrusion-based 3D printing for delivering pharmaceutical and supplemental ingredients.

Keywords: 3D printing; Alginate; Gelatin; Hydrogel composite; Post-processing; Rheology

Introduction

3D printing is a technique that prints out materials layer-by-layer to fabricate a 3D formulation by a digital control. The applications of 3D printing technology have been extended from printing plastic parts to the areas of tissue engineering, pharmaceutical, oral devices, food production, and civil engineering [1-3]. The potential of the development in drug delivery systems is unlimited and could provide more flexibility in pharmaceutical processing than the traditional one [4]. By using 3D printing technology, the dosage of active ingredients could be accurately controlled to form personalized tablets for each individual.

Furthermore, precise spatial control of complex-formed drug-releasing profile has been obtained through extrusion-based 3D printing technology [5]. The materials have been used in the area of pharmaceutical and tissue engineering are mainly semi-solid hydrogels made from protein or carbohydrate-based materials, such as alginate, gelatin, cellulose derivatives, and gums [1,6,7]. The rheological properties of those materials are deciders of the printability and deformability of printed scaffold. It is important to formulate the material with appropriate concentration or with added cations, such as Ca^{2+} , to make it suitable for 3D printing purposes.

Alginate, which is salt of alginic acid, is a polysaccharide widely occurred in cell walls of brown algae and has already been used in several biomedical applications due to its hydrophilic properties and the ability to form in much viscous gum [8]. When it comes to bioengineering, biomaterials that come from natural sources are generally being considered. Since alginate's favorable properties, including biocompatibility and ease of gelation, it has been particularly attractive to the bioengineering industry [9]. Sodium alginate has been widely as a 3D printing material, with added cations, such as calcium, to increase the gel strength for 3D printing purpose [10,11]. As alginate is relatively soft and liquid-like in its nature, it is usually combined with other hydrogels, such as cellulose derivatives, starch, or gelatin, to form stronger hydrogels for 3D printing [10,12,13]. Gelatin is a natural protein-based biopolymer derived from collagen hydrolysis. It has already been widely applied in the bioengineering industry as a biomaterial due to its excellent biocompatible and biodegradable ability. However, the rheological properties of gelatin could largely affect the quality of a particular application [14]. Compared to alginate, gelatin is more solid-like and brittle, which limited its usage in 3D printing applications. Alginate and gelatin has been combined to make hydrogel composites to enhance the viscoelastic properties and thermal stability [13]. In previous studies, the composite made of alginate/gelatin has been used in tissue engineering and medical device; however, its usage in food processing area and pharmaceutical is still in its infancy [15,16].

In this study, the 3D printing materials were prepared by using different ratios of gelatin and alginate to obtain the optimal formula of the biopolymer matrix for printing purposes. To solidify the printed scaffold, the printed parts were immersed into calcium chloride solution as a post-processing treatment. As alginate consists of α -L-Guluronic acid (G) and β -D-Mannuronic acid (M) it could be formed into the gel matrix by binding a divalent cation such as Ca^{2+} on to the G sequences of alginate (Agulhon et al., 2011) [11]. Additionally, by immersing the gelatin-alginate matrix into the calcium chloride solution, it could also help prevent the hydrogel from degradation in pharmaceuticals [17]. However, the gel strength is influenced by the interaction between cations and G sequences and also by the concentration of alginate, and the gelling or hardening time [18]. Therefore, this study aims to investigate the optimal gelatin and alginate ratio of the 3D printing material and the gelling time for post-processing.

Materials and Methods

Materials

Sodium alginate (TICA-algin® 400 Powder) was provided by TIC Gum (White Marsh, MD). Bovine gelatin (Gelatin from bovine skin, Type B) was provided by Sigma-Aldrich (St. Louis, MO). Hydrogel composites were prepared by dissolving gelatin and alginate powder in 47.5g of deionized water with three different ratios of gelatin and alginate to create a 5% (w/w) of total solid. The three ratios for gelatin and alginate were designed as 1:4, 1:1 and 4:1(w/w). Table 1 shows the formula for 5% of the gelatin-alginate mixture with three gelatin/alginate ratios. By continuous stirring while heating at 40°C, gelatin and alginate were completely dissolved in DI water. After all gelatin-alginate hydrogels were cooled down, they were centrifuged at 3000rpm for 10 minutes to eliminate air bubbles, which were formed during stirring. For studying the printability of the gelatin-alginate materials, a portion of the samples were further transferred into a sterile syringe of the extrusion-based 3D printer.

Rheological Properties

The rheology properties were conducted by the use of Discovery HR-2Rheometer (TA Instruments, New Castle, DE) with parallel geometry at a gap size of 1mm under ambient temperature. To determine the printability of material, two rheology tests were conducted. The flow ramp test was carried out to evaluate the apparent viscosity under an increasing shear rate ranging from 0.1 to 30.0 1/s. In order to characterize the dynamic modulus of a material, the oscillation frequency sweep test was conducted under a small strain of 0.1 with increased frequency from 0.1 to 1000 rad/s throughout the test.

3D Printing Process and Post-Processing

The printing of gelatin-alginate composites was performed using a customized extrusion-based 3D printer configured on the platform of the Velleman K8200 3D printer (Velleman Inc., Fort Worth, Texas).

All samples were printed out one day after material preparation to make sure the polymers were fully hydrated. Figure 1(a) shows the configuration of the customized 3D printer and figure 1(b) is the conceptual sketch of the extrusion-based 3D printer. The geometric was designed in the shape of a cuboid with a rectilinear infilled pattern. The designed shape was sliced into layers and translated into G-code using the open-source Slic3r software. The gelatin-alginate composite in the syringe was extruded out through a nozzle at a speed of 8.0mm/s. The objects were printed into twelve layers using a syringe with a nozzle diameter of 0.636mm. Post processing were conducted by either applying 0.1M CaCl_2 solution on top of the surface of the printed objects, or by soaking the printed objects into 0.1M CaCl_2 solution for a different time, i.e.10, 20, 30 and 60 minutes, respectively.

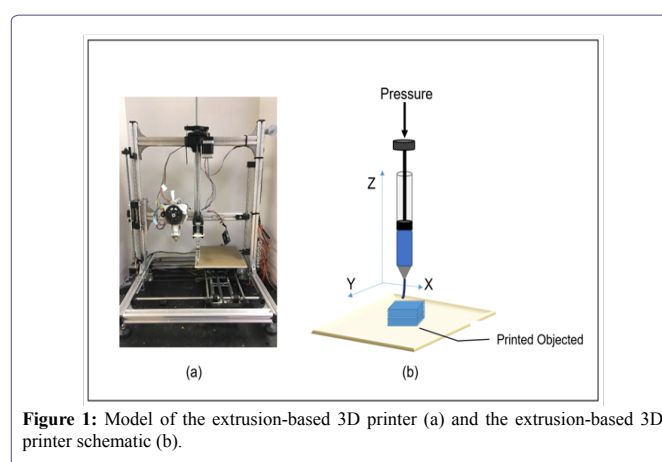


Figure 1: Model of the extrusion-based 3D printer (a) and the extrusion-based 3D printer schematic (b).

Statistical Analysis

All experiments were performed in triplicates and representative data were presented in mean values. All 3D printed objects were printed in duplicates or triplicates but only representative images were selected.

Results and Discussion

Rheological properties

Flow ramp was conducted to investigate the flow behavior and viscosity of materials. According to figure 2(a), as the shear rate increased, the apparent viscosity decreased. The shape of curves indicated that there was a shear-thinning behavior for all three materials. This shear-thinning behavior suggested that when there is shear stress during the 3D printing process, the material would flow easier with reduced viscosity. This is probably due to the network of molecules were disrupted under shear stress. Due to this property, these materials are considered to be 3D printable through the extrusion-based printer once the stress higher than the yield stress is applied.

	Polymer (w/w)%	G/A Ratio	Total polymer(g)	Gelatin(g)	Alginate(g)	Water(g)	Total Weight(g)
1	5%	1:4	2.5	0.500	2.000	47.5	50
2	5%	1:1	2.5	1.250	1.250	47.5	50
3	5%	4:1	2.5	2.000	0.500	47.5	50

Table 1: The formula for 5% of gelatin-alginate hydrogel with three Gelatin/Alginate (G/A) ratios, 1:4, 1:1 and 4:1.

The viscoelastic properties of the composite gelatin-alginate hydrogels were determined under a frequency sweeps at constant stress by comparing the magnitude of loss modulus (G'') and storage modulus (G'). Also, the relation between G'' and G' were presented by the loss factor ($\tan \delta = G''/G'$). A value of $\tan \delta > 1$ indicated that the material is predominant with liquid-like behavior, while a value of $\tan \delta < 1$ indicates that the material is predominant with solid-like behavior [19,20]. Figure 2(b) shows that all materials showed the magnitude of G' higher than G'' and the loss factor $\tan \delta < 1$ within the frequency range applied, which means that all materials were more solid-like [19,21]. The values of $\tan \delta$ for materials with gelatin/alginate ratio 4:1 were apparently lower than those of 1:1 and 1:4 (Figure 2b). This means that the rheological property for gelatin-alginate 4:1 is more solid-like with poor fluidity. According to our preliminary study, gelatin behaved more like a brittle solid compared to alginate with a concentration of 3-5%. This could further explain the rheological property of the hydrogel of G/A 4:1, which had a higher portion of gelatin within the material. In order to be potentially 3D printable using the current 3D printer, the $\tan \delta$ values should fall into the range of 0.3 to 0.5 during the frequency sweep of 0.1 to 1000 rad/s [2]. We can see from figure 2(b) that both G/A 1:1 and 1:4 are within this range, indicating the potential of using these two materials for 3D printing.

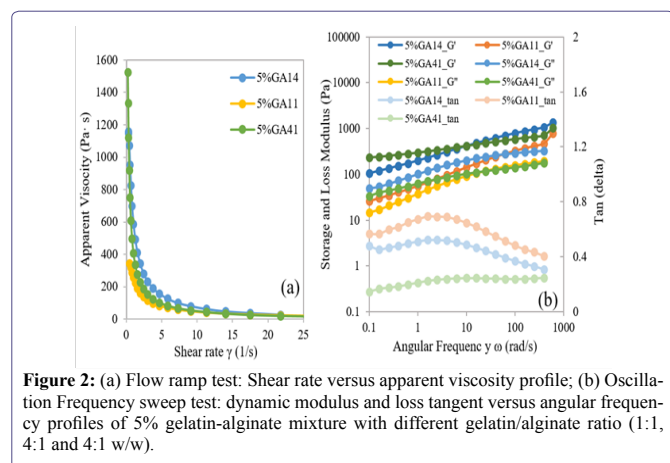


Figure 2: (a) Flow ramp test: Shear rate versus apparent viscosity profile; (b) Oscillation Frequency sweep test: dynamic modulus and loss tangent versus angular frequency profiles of 5% gelatin-alginate mixture with different gelatin/alginate ratio (1:1, 4:1 and 4:1 w/w).

3D printing of hybrid composite

According to our preliminary studies not reported here, using gelatin or alginate alone for 3D printing is not feasible. Hydrogels made of gelatin is too brittle to be 3D printed, and alginate is too liquid-like to hold its shape after 3D printing. Thus, we used a hybrid composite made of different ratios of gelatin and alginate for 3D printing purposes. All samples were printed out one day after material preparation to ensure the polymers were fully hydrated. After the rest time, the hydrogels with different ratios of gelatin and alginate were 3D printed into the designed shape of a cuboid. As shown in figure 3 (a and b), when increasing the ratio of alginate, the sample would be more in a liquid form with higher flow ability. Therefore, when printing out with the material with a 1:4 gelatin/alginate ratio, the 3D printed cuboid structure could not hold its structure but spread out instead. On the other hand, when increasing the gelation portion, the material with a 4:1 gelatin/alginate ratio would be a brittle mass. Therefore, the upcoming layer was fully integrated with the existing layer. The layers would not stick to each other as good as the one with a ratio of 1:1. From the three types of materials we printed, it showed that

the gelatin/alginate ratio 1:1 has the optimal quality considering its printability, resistance to deformation, and surface smoothness.

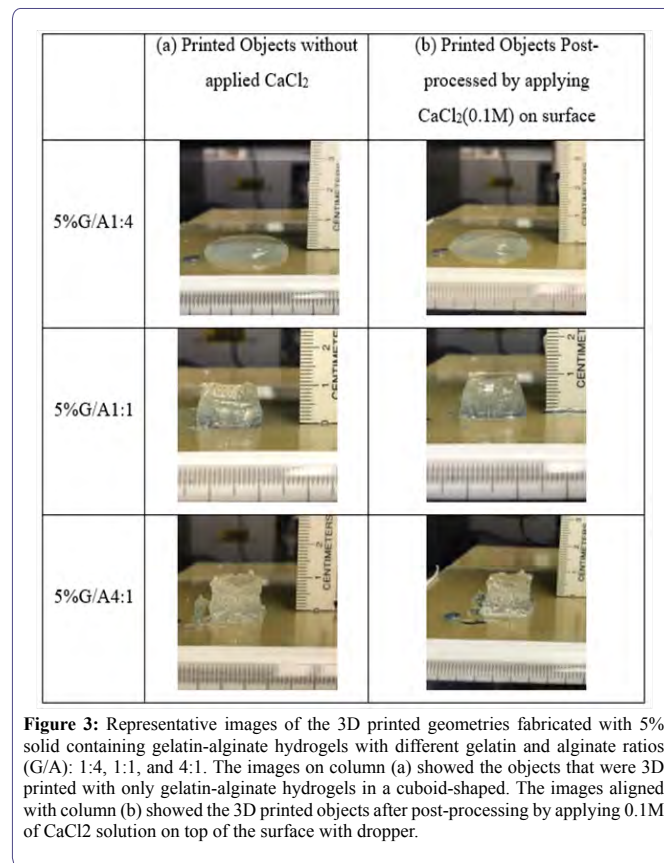


Figure 3: Representative images of the 3D printed geometries fabricated with 5% solid containing gelatin-alginate hydrogels with different gelatin and alginate ratios (G/A): 1:4, 1:1, and 4:1. The images on column (a) showed the objects that were 3D printed with only gelatin-alginate hydrogels in a cuboid-shaped. The images aligned with column (b) showed the 3D printed objects after post-processing by applying 0.1M of CaCl₂ solution on top of the surface with dropper.

Post processing of 3D printed matrix

To increase the surface smoothness and gel strength, the 3D printed geometries were fully immersed into the aqueous solutions of 0.1 M CaCl₂ for different length of time, which is 10, 20, 30 and 60 minutes, respectively. By comparing figure 3a and figure 4, we found that by immersing 3D printed matrices in CaCl₂ solution, the surface smoothness was highly improved.

The hypothesis is that with the presence of Ca²⁺, the degree of cross linking between Ca²⁺ and alginate also increased, thus stronger gels were formed. The gel strength was not quantified at this stage, but texture analysis will be conducted to measure the effect of soaking time on gel strength in our future studies. The change of intermolecular networking may also change the intermolecular forces and surface tension of the printed matrix, resulting in contraction and shrinkage of the cuboid shape into a more spherical shape. With the increase of soaking time, the degree of shape contraction also increased. This result is consistent with previous studies showing that increasing the gelling time would increase the degree of cross linking between Ca²⁺ and alginate. The increased degree of cross-linking also caused the contraction of gels [13]. For 3D printing purposes, a smooth surface of the printed objects is usually preferred, but shape deformation is not. Further study is needed to achieve the balance of making a smoother surface with better shape retention capacity. This may be achieved by employing shorter soaking time and use lower concentrations of CaCl₂ solution.

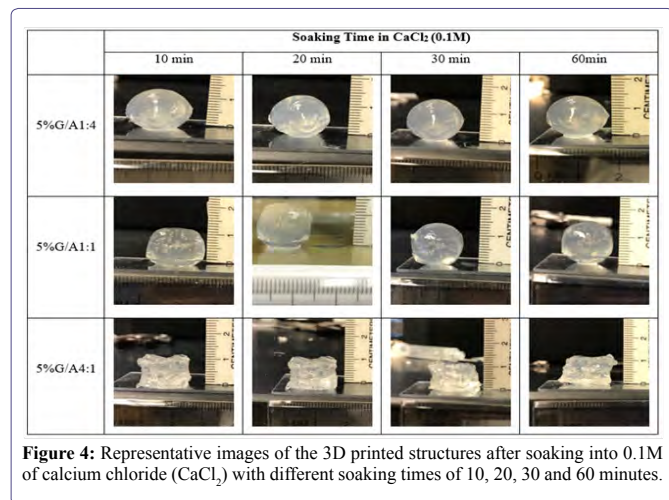


Figure 4: Representative images of the 3D printed structures after soaking into 0.1M of calcium chloride (CaCl₂) with different soaking times of 10, 20, 30 and 60 minutes.

Conclusion

In this study, the rheology test was conducted to evaluate the viscosity and viscoelastic properties of gelatin-alginate hydrogels. According to the findings from the flow ramp test, these gelatin-alginate hydrogels tested are considered to be printable due to the shear-thinning behavior. Additionally, based on the viscoelastic properties shown in the oscillation frequency test, gelatin/alginate hydrogels with ratio 1:1 and 4:1 shows a better printability potential. Based on the rheological properties and the 3D printing results, we can conclude that the optimal ratio for the gelatin and alginate is 1:1. In addition, the post-processing treatment with different soaking time in calcium chloride solution indicated that longer soaking time would result in a stronger gel; however, further tests should be done to determine the effects of soaking time on gel strength. The most important finding of this study is that the gel strength and surface smoothness can be significantly improved by soaking the 3D printed geometries in the calcium chloride solution. The problem to address in the next step is to improve shape retention during the post-processing.

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