A Study of Variable Stiffness Alginate Printing for Medical Applications

Adaleena Mookerjee¹, Daniel L. Cohen¹, David H. Peng², Lawrence J. Bonassar¹,², Hod Lipson¹,³

¹Department of Mechanical and Aerospace Engineering
²Department of Biomedical Engineering
³Department of Computer and Information Science
Cornell University, Ithaca, NY

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Abstract

Technologies for multi-material 3D-printing of anatomical shapes are useful both for fabrication of heterogeneous cell-seeded implants as well as for fabrication of synthetic models for surgical planning and training. For both these applications, it would be desirable to print directly with biological materials to best emulate the target’s properties. Using a novel material platform, we describe a series of experiments attempting to print variable-stiffness hydrogels. We vary compliances by alternating 2% alginate hydrogel and a Dextran-infused calcium chloride post-crosslinker. Stiffness throughout the construct ranged from 4 kPa to 20 kPa as a function of post-crosslinker concentration, which was spatially specified by the user.

Introduction

Technologies for multi-material 3D-printing of anatomical shapes are useful both for fabrication of heterogeneous cell-seeded implants as well as for fabrication of synthetic models for surgical planning and training [9]. Currently, printed implants typically consist of single compliance, high stiffness materials such as metals and polymers. These materials are not suitable for emulating heterogeneous tissue such as a heart valve, where a wide range of mechanical stiffnesses are required [11]. Additionally, many of these materials cannot act as tissue engineering constructs because they are not biocompatible [13, 19, 22].

At the same time, soft materials have yet to be extensively applied in SFF for the purpose of surgical planning. In order for a practitioner to plan an approach to the target site, a comprehensive understanding of the spatial orientations of structures is critical. In normal anatomies, experience alone can be relied upon. However, in complicated cases including trauma and rare pathologies, anatomies are often highly abnormal, and, therefore, difficult for surgeons to navigate in-situ without prior advanced study [6]. Consequently, it is highly beneficial to provide real 3D models for a surgeon to analyze, and practice upon, prior to surgery [8, 22].

Rapid prototyping using alginate hydrogels is a method for fabricating bioimplants and surgical planning. Complex models can be produced relatively quickly where time is critical [5, 6, 7, 10]. Up until now, single compliance models are not only unsuitable to use for printing multi-compliance parts (e.g., heart valves), but also insufficient for re-creating key tactile cues required for surgical planning [10, 20]. Furthermore, since these models are rather stiff, it is difficult to practice procedures such as incisions and suturing [10, 20, 22]. Lastly, since many of these materials are not biocompatible, cells cannot be seeded into the material to fabricate tissue engineering scaffolds [13, 21, 22, 24, 25].
Problem Statement
Our research aims to address the current lack of an effective platform for producing multi-compliance, low stiffness models for medical applications. Consequently, we propose a novel platform which would enable the production of multi-compliance, low-stiffness biocompatible implants and other medical models for patients [2, 13]. This material involves an alginate hydrogel printing approach and alternating layers of Dextran-infused calcium chloride crosslinker to selectively post crosslink specific regions.

Background
Alginate is a viscous gum extracted from the cell walls of brown algae. It consists of a linear copolymer with mannuronate and guluronate blocks in a long chain. These are joined together to produce a linear copolymer. There are some open regions where ions can be added. Thus, crosslinker can be added to form a gel. Alginate gels can be used for medical applications such as bioimplants, and potentially for surgical planning, due to the material’s biocompatibility and low-compliance, respectively [17, 20, 22, 24].

Previous attempts have been made to enhance the efficacy of surgical planning. Ahn et al. [1] analyzed the effects of rapid prototyping and its contribution to orthopedic surgeries. The group prepared the part using a single photopolymer and an Objet Quanda Polyjet printer. They incorporated this printing at a local hospital and used it for planning. The process was used to print a distal tibia, proximal tibia and iliac fracture wing of a pelvis. The printing time was recorded and the actual duration of the surgery was noted. From the group’s observations, on average, it took approximately eight hours to print the tibia fracture and more than a day to print the pelvis fracture. They found that incorporation of rapid prototyping in surgical planning cut the duration of the surgery by about half the time expected by conventional planning.

Potamianos et al. [3] performed another case study examining a woman who fell off a horse and injured her right shoulder, with possible fractures in her clavicle and scapula. This kind of injury would require a quite lengthy and complicated surgery, not only for the surgeons performing the surgery, but for the patient as well with quite a lot of scarring. From analyzing her CT scans, it appeared inevitable that surgery was the only solution to fix her injuries. However, through careful analysis via rapid prototyping, it was determined that the fracture was not as bad as demonstrated by the CT scan and the scapula still remained attached to the shoulder. This spared the patient of intense surgery and the surgeons from wasting resources and time to perform the surgery.

Mahaisavariya et al. [4] looked at rapid prototyping for surgical planning as well. This group studied child patients with a cubitus varus deformity resulting from complications of supracondylar fractures in the humerus. They looked at two patients with similar fractures. From analyzing the patients’ arms, they were able to obtain a print of their elbows and compare their elbows to that of normal patients and make the necessary angular adjustments. They were also able to modify the post operative treatment for the patients, allowing for a smoother recovery for the patient.

Similarly, alginate hydrogels have also been found to be successful in other research outside of surgical models. Augst et al. [17] looked at the applications of alginate hydrogels and found that it can be used for other tissue engineering applications such as serving as a bulking agent. If the gel is biocompatible and maintains a consistent shape in the body, it would form a bulking agent. Additionally, these gels could also be
used for drug delivery through the encapsulation and release of proteins, helping with effectiveness and targeting. Lastly, gels were also found to be used for cell transplantation in tissue formation and regeneration, a long term goal for this study.

Kuo et al. [16] used calcium sulfate and calcium carbonate mixed with glucono-delta-lactone (GDL) to produce homogeneous hydrogels. From their results, they found that calcium carbonate mixed with GDL produced very strong, three dimensional gels. Over the course of 48 hours, they found that their calcium sulfate gels diffuse. Additionally, the calcium sulfate based hydrogel was produced too quickly to produce uniform gels. This, however, was resolved from the research done by Cohen et al. [14].

An alternate goal in using alginate hydrogels is for printing a heart valve via photocrosslinking [23]. Currently, heart valves are being modeled using a checkerboard print. Some prints were also made using the Fab@Home at Cornell University.

Wan et al. [18] used an alcohol based hydrogel to print a heart valve via injection molding. These gels were mechanically tested to obtain a stress-strain curve and creep. They were successful in printing the heart valve and found that their primary limitations would be in the deformation resulting from the polymer creep. They did find their gel to be biocompatible and thus, would be worthwhile in the medical field [18, 22].

Another body of work that has been completed is the development of the dual syringe Fab@Home printer, which can be used to deposit materials such as alginate hydrogels varying in stiffness [15]. This approach allowed for three dimensional complex objects printed layer by layer varying in stiffness. However, this printer had never been used with a material set that was relevant to the production of medical models. The work discussed herein focuses on developing a useful material set to produce multi-compliance, low-stiffness models [15].

**Materials and Methods**

For this study, alginate hydrogels were selected because of their clinical relevance and generally low-stiffness [17, 25]. Furthermore, the stiffness of alginate can be granularly varied as a function of calcium crosslinker concentration [12]. In addition, we used a calcium based crosslinker due to the ease it provided in cell encapsulation and biocompatibility [17, 19, 22, 25].

We attempted two different approaches to crosslink alginate to produce multi-compliance hydrogels. The first method consists of printing the crosslinker into the alginate with varying crosslinker concentrations. The second method involved pre-mixing the crosslinker with the alginate and selectively post-crosslinking within each layer. Each of these methods is further elaborated upon below.

**Hydrogel Preparation: Method 1-Printing Crosslinker into Alginate**

In this process, we, first, prepared gels using 2% or 4% LF 10-60 mixed with Dulbecco’s Phosphate Buffered Saline (DPBS). Thereafter, we experimented with three primary crosslinker solutions which are calcium sulfate, calcium chloride, and calcium carbonate with glucono-delta-lactone (GDL). The preparation of the solution varied depending on its concentration. The powder was mixed accordingly with PBS and left spinning on a stirring plate.

The crosslinker was injected into the pool of alginate upon deposition using the printer’s syringe-based deposition tool. Various tips diameters and traverse speeds were experimented with as detailed below.
Hydrogel Preparation: Method 2-Selectively Post-Crosslinking Pre-mixing Alginate Hydrogel

This method consisted of pre-mixing 1% calcium sulfate with alginate. These gels were prepared using 2% LF 10-60 mixed 200 times with 1% calcium sulfate crosslinker in a reciprocating [14] fashion using two syringes (Figure 1). Thus, we filled the two syringes with calcium sulfate and the alginate mixture respectively, and then pressed on its piston alternately to mix them and produce the gel.

Figure 1: Reciprocating Motion. This reciprocating motion, adopted from Cohen et al. [14] was implemented to mix the 1% calcium sulfate with the 2% alginate solution to produce a single stiffness hydrogel.

Upon deposition of the alginate hydrogel, we selectively post-crosslinked each layer with a Dextran-infused calcium chloride solution. The preparation of the post-crosslinker solution varied depending on its concentration. Based on the desired concentration of the solution, we mixed the calcium chloride powder with PBS and left it spinning on a vortex spinner for approximately five minutes. Then, we added 2.5 tablespoons of Dextran per 100 mL of calcium chloride while the solution was spinning. The solution was left to mix for another five minutes and then inserted in the syringe for printing.

Hydrogel Deposition: Multi-Compliant Gels

Hydrogels were deposited using the Fab@Home SFF system (Figure 2). For our purposes, we selected a syringe with tip diameter of 0.84 mm. The printer planned tool paths for deposition with each layer being 0.3 mm thick. Since the post-crosslinker solution had Dextran added to it, the viscosity of the post-crosslinker matched quite closely to the hydrogel. This similar material behavior led to minimal changes to the printing parameters between the two materials. In order to add the post-crosslinker in between layers of the hydrogel, a pause setting was used so the user could alternate between the syringes of alginate hydrogel and the post-crosslinker.

The alginate hydrogel was printed layer-by-layer. However, the Dextran post-crosslinker was selectively added upon certain regions where further stiffening was desired. This enabled us to vary and control the spatial distribution of stiffnesses.
Results

Printing Crosslinker into Alginate
The gels produced by the above methods along with the printing of the hydrogel alginate were tested and the results analyzed for quality and ease of printing. In the first method of printing, i.e., printing cross-linker into alginate, a robust gel (Figure 3a) was initially produced using calcium sulfate powder. While the mechanical integrity of the resultant parts was desirable, the direct addition of calcium sulfate powder (by hand) proved difficult to reliably deposit (in powder form) by machine. To overcome this deposition challenge, we explored putting the calcium into solution. The first attempt was suspending the calcium sulfate in a DPBS solvent. However, the calcium very quickly fell out of solution leading to variable amounts of calcium being deposited, and ultimately, heterogeneous printing properties (Figure 3b).

Next, we attempted to use calcium chloride to overcome the issue of the solvent fallout out of solution. However, the next issue that arose was the difficult-to-control diffusion of the calcium upon deposition. This lead to road widths that were 2-3 times wider than the deposition tip. Moreover, the road width was variable and difficult to reliably control. In our effort to resolve the problem of crosslinker diffusion, we considered trying calcium carbonate mixed with glucono-delta-lactone (GDL). This solution was more effective in that it did not rampantly diffusion throughout the alginate, and thus, produced very fine lines. We tested the material platform by printing a cube, which still failed due to the crosslinker falling out of solution during the print and yielding heterogeneous printing properties. (Figure 3g). Consequently, all the approaches tried thus far were rejected in favor of more successful ones to follow.

It may be mentioned in this context that although this method was not implemented in the actual production of the multi-compliant gels, the experiments in this method were extremely critical in understanding the printing qualities of the material set. It helped in the selection of the post crosslinker and led to a clearer understanding of what constitutes a suitable post-crosslinking solution, i.e., one that stayed in solution for at least 20 minutes and didn’t uncontrollably diffuse upon deposition.
Figure 3: Printing Crosslinker into Alginate. (a) Calcium sulfate powder was tapped into areas of alginate. (b) Calcium sulfate powder dissolved in a PBS solution and printed onto a bed of alginate using the Fab@Home. Only selected areas of the gel crosslinked. (c),(d) Calcium chloride lines printed in a bed of alginate (0.2” average width). (e) Calcium carbonate with GDL lines were printed in a bed of alginate. Very fine lines resulted. (f) An alginate solid, 1.691” by 1.798” by 0.525”, was printed using a calcium carbonate/GDL crosslinker solution. (g) Calcium carbonate/GDL crosslinker solution printed on alginate using the Fab@Home.

Premixing Alginate with Crosslinker
The results of pre-mixing alginate with crosslinker which involved the process of mixing crosslinker and alginate 200 times in a reciprocating fashion [14] were analyzed. The resultant print of a hydrogel cube (Figure 4) was found to be more rigid and firm showing...
little dissociation or diffusion. However, this being a single stiffness print, it did not satisfy the goal of a multi-stiffness and multi-compliant model.

**Figure 4: Single Stiffness Hydrogel via Premixing.** A single stiffness, 1” by 1”, alginate hydrogel cube made of 20 layers was successfully printed using the Fab@Home.

In order to test the printing of gels with varying stiffnesses, we took several gel prints. One such print was a rectangularly shaped gel, 50 mm long, 31 inches wide and 2.5 mm tall. The shape was drawn in CAD and output as an STL for the Fab@Home to print.

**Figure 5: CAD Model and Corresponding Hydrogel Prints.** (a) A manual print was made first to test the effectiveness of the method. (b) This proved successful and thus, a Fab@Home print was also made (1” by 2” by ~0.04”).

Both tactile testing and mechanical testing were performed on these prints, and results confirmed multi-stiffness in both cases. The location of maximum stiffness was approximately 0.62 inches from the end of the part, while the spread of the post-crosslinker was 0.40 inches. The mechanical testing provided a quantitative measure of the varying stiffness hydrogels. Table 1 show the variations along with the corresponding quantitative moduli.
Table 1: Mechanical Test Data. Mechanical testing (n=3 for each concentration of post-crosslinker) was done for three selected areas in the regions where the post crosslinker was added and where it was in absence. There are some variations in stiffness of the gel.

<table>
<thead>
<tr>
<th>Concentration</th>
<th>10% CaCl₂ (un-post-crosslinked region)</th>
<th>10% CaCl₂ (post-crosslinked region)</th>
<th>20% CaCl₂ (un-post-crosslinked region)</th>
<th>20% CaCl₂ (post-crosslinked region)</th>
<th>30% CaCl₂ (un-post-crosslinked region)</th>
<th>30% CaCl₂ (post-crosslinked region)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Modulus Mean (kPa)</td>
<td>5.1</td>
<td>5.0</td>
<td>8.9</td>
<td>21.3</td>
<td>3.8</td>
<td>8.9</td>
</tr>
<tr>
<td>Modulus S.D. (kPa)</td>
<td>2.7</td>
<td>0.6</td>
<td>1.3</td>
<td>1.5</td>
<td>1.3</td>
<td>6.6</td>
</tr>
</tbody>
</table>

While the mechanical testing did give a quantitative measure of the stiffness variation, we found certain limitations in its reliability when compared with that of tactile testing. Normally, due to effects of dehydration in the hydrogel, the stiffness tends to vary significantly over the course of a few hours. Additionally, in order to prevent degradation of the gel, it is necessary to place the hydrogel in the refrigerator which quickens the dehydration process. In an attempt to keep the gel hydrated, we added excess PBS to the petri dish. This caused the calcium chloride post-crosslinker solution to spread, causing unwanted regions of the gel to stiffen. Thus, we observed that in order to obtain the best results, testing had to be done immediately, which was not always possible due to the complexity and duration of the printing process along with the process of mechanical testing. Thus, tactile tests proved more effective in determining differences in stiffness of the gels. To summarize, the following table illustrates the advantages and disadvantages of each of the experiments previously discussed.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
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<tbody>
<tr>
<td>Printing Crosslinker into a Bed of Alginate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcium sulfate powder</td>
<td>Selective crosslinking produced a robust gel</td>
<td>Solid powders cannot be printed using the Fab@Home</td>
</tr>
<tr>
<td>Calcium sulfate solutions</td>
<td>Thin, fine lines results in the gel</td>
<td>Quick dissociation rate of the solution since calcium sulfate is insoluble with PBS</td>
</tr>
<tr>
<td>Calcium chloride solutions</td>
<td>Very slow dissociation rate (dissociation only resulted in very concentrated solutions)</td>
<td>Crosslinker dissipated to nearly twice its width upon being printed in the gel</td>
</tr>
<tr>
<td>Calcium carbonate + GDL solution</td>
<td>Thin, fine lines and very strong crosslinking between layers produced a robust gel</td>
<td>Quick dissociation rate of the solution (faster than that of calcium chloride, slower than that of calcium sulfate)</td>
</tr>
<tr>
<td>Adding a Calcium Chloride Post Crosslinker</td>
<td>Multiple stiffness gel resulted</td>
<td>Gel stiffens over the course of time in the refrigerator; calcium chloride spreads over time</td>
</tr>
</tbody>
</table>

Table 2: Summary of the Advantages and Disadvantages of the Methods. The advantages and disadvantages of producing the gels using the methods discussed above.
Conclusions and Future Work
In this paper, we proposed a method for printing an object which can be used for medical applications such as multi-compliant, biocompatible implants and surgical planning. This technique can represent objects with differing stiffnesses which can give a more accurate depiction of the body parts within the patient [9]. Additionally, multi-compliant gels would also assist in producing implants due to its biocompatibility and cell encapsulation [13]. Using the Fab@Home printer, we were successful in printing a soft hydrogel which comprises multiple stiffnesses.

While these prints do produce a single object with varying densities, other combinations of prints could also be used, going forward [10]. Additionally, other gels or crosslinking methods could be implemented which could produce objects of significantly different stiffnesses or give better results than the ones obtained thus far. Cells also need to be added to these implants to test the biocompatibility of this particular material platform.

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References


