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Localized Immunotherapy Delivery Using Injectable in situ Forming Chitosan Hydrogel

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Localized Immunotherapy Delivery Using Injectable in situ Forming Chitosan Hydrogel

Undergraduate Honors College Thesis

in the

Department of Biomedical Engineering

College of Engineering

University of Arkansas

Fayetteville, AR

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Abstract

Cytokine-based cancer immunotherapies stimulate a host's immune system to fight cancer. In particular, interleukin-12 (IL-12), a potent pro-inflammatory cytokine, has demonstrated the ability to eliminate tumors in a number of preclinical models. Toxicities associated with the systemic delivery of IL-12 have precluded its use in the clinic. We are developing a novel chitosan-based hydrogel to maintain high local concentrations of cytokines, such as IL-12, in the tumor while minimizing its systemic dissemination. This hydrogel was found to form spontaneously within ten seconds of mixing two proprietary components. To increase the usefulness of the hydrogel, an efficient mixing and delivery system is needed. We designed and evaluated a device capable of mixing two solutions from two syringes during injection. A total of eight prototypes were created using three-dimensional printers; six were printed on an Object30; one was printed on a MakerBot; another was printed on an uPrint SE Plus. Three tests were used to determine the effectiveness of the device. The first test was a dimensional test to check for fitting of the syringes and needle. After passing this test, the fluid dynamics were tested using distilled water. If the device passed the previous tests, the third test determined the mixing ability of the device using the novel hydrogel. After success in all three tests, the sterility of the device became the main goal. Hydrogel formation was achieved but a better material for the device is still under investigation.

1. Introduction

With the rise of cancer, investigation of cures has come to the forefront of biomedical sciences research. One method to fight off the disease is overstimulation of the immune system. Previous research demonstrated cytokine-based cancer immunotherapies are able to fight cancer. Interleukin-12 (IL-12) is a potent pro-inflammatory cytokine with the ability to eliminate tumors. IL-12 activates T lymphocytes and natural killer (NK) cells to cause recession of the tumors. A byproduct of the immune stimulation is the production of interferon (IFN)- γ . This byproduct increases the toxicity of IL-12 treatments. Phase I and II clinical trials determined a need for safer dosages calling for a wider therapeutic range. For example, the treatment for renal carcinoma using 500 ng/kg/day of rhIL-12 caused two deaths. Toxicities associated with the systemic delivery of IL-12 cause grade 3 and 4 adverse events. These events have precluded its use in the clinic.^{1,2}

Hydrogels are hydrophilic, cross linked single chain polymers or co-polymers; the cross linking causes them to be insoluble in water but allows them to intake 10-20 times their molecular weight while retaining their three dimensional structure. The aqueous uptake causes the hydrogel to mimic tissue physiology increasing patient compliance. The integrity of the structure enables the protection of encapsulated drugs such as proteins and peptides. Hydrogels often display biocompatible capabilities with minimal immunogenicity for synthetic hydrogels. The biocompatibility and physical similarities cause hydrogels to be considered the closest synthetic biomaterial replica of human tissue. These properties make hydrogels applicable in many biomedical fields including drug delivery.^{3,4,5}

Hydrogel drug delivery is favorable because it allows time controlled drug release and targeted delivery. The porosity of a hydrogel dictates the rate of drug release through either diffusion or convection. Porosity can be controlled through the cross-link ratio of polymers. A greater ratio decreases the porosity causing a lengthier drug release. Its adhesive property allows the hydrogel to anchor in one place. With the hydrogel in one location, the release of the drug is targeted toward local tissue. Some hydrogels are deformable allowing injection but most need surgical implantation. For ease of use, noninvasive hydrogels are desirable.^{2, 3, 4}

There are two different mechanisms for hydrogel formation: physical and chemical. Physical hydrogel formation is caused by molecular entanglement; a polyelectrolyte interacts with a multivalent ion of opposite charge to cause the cross-link network. Because entanglement is the main mechanism, these hydrogels are reversible. Chemical hydrogel formation creates cross-linked networks through covalent bonds. These bonds are irreversible causing permanent hydrogel formation. Both hydrogels are heterogeneous in regard to the non-uniform density of cross-links throughout the hydrogel.⁶

Chitosan, a natural derivative of chitin (shrimp exoskeleton), is biocompatible, biodegradable, and mucoadhesive. This biomolecule has shown to increase bioactivity and local retention of cytokines such as IL-12. Along with increased potency of the cytokine, the chitosan/IL-12 mixture is capable of generating immunological memory to control metastasis and prevent tumor reoccurrence.^{2, 7}

A novel chitosan-based hydrogel was developed to maintain high local concentrations of cytokines, such as IL-12, in the tumor while minimizing its systemic dissemination. This

hydrogel was found to form spontaneously within ten seconds of mixing two proprietary components. To increase the usefulness of the hydrogel, an efficient mixing and delivery system is needed. In this project, a device capable of mixing two solutions from two syringes during injection was designed and evaluated.⁷

2. Methods and Materials

2.1 Materials. The 3mL BD Luer-Lok tip disposable syringe (BD 309657) was purchased from Fisher Scientific (Pittsburgh, PA). A 20-gage BD needle (BD 305179) was purchased from Fisher Scientific (Pittsburgh, PA). An 18-gage BD needle (BD 305199) was purchased from Fisher Scientific (Pittsburgh, PA). The proprietary hydrogel was created in the Laboratory of Vaccine and Immunotherapy Delivery (Fayetteville, AR).

2.2 Three Dimensional Printers. Three printers were used to create prototypes. The first prototypes were printed on an Object30 printer using VeroWhite, a material similar to polypropylene (PP) created by Stratasys. Once the dimensions were correct, the prototypes were printed with two other materials, polylactide (PLA) and acrylonitrile butadiene styrene (ABS). The MakerBot printer was used to print the PLA prototype; the uPrint SE Plus printed the ABS prototype.

2.3 Creation of Prototypes. The computer aided design (CAD) software, SolidWorks, was used to create the prototypes. The prototypes consist of two male luer locks allowing two 3mL syringes to attach to the top of the device. Channels from the 3mL syringes allowed the two solutions to mix. The newly mixed solution exits from a needle. The device has a female connector at the bottom where an 18- or 20-gage needle is able to

attach. Once the design was printed, the prototype was evaluated for fit by connecting the syringes and needle while the fluid dynamics of the device were tested using distilled water. If the water flowed from both syringes through the device and out of the needle consistently, an instant forming chitosan-based hydrogel, with a proprietary formulation, was used to test the mixing efficiency of the device.

3. Results

3.1 Fitting the Syringes and Needle.

3.1.1 Prototype One. After printing, the fit of the syringes and needle was tested. The first prototype was 85.52mm wide and 124.06mm tall with 99mm separation between the two syringe connectors (see Figure 1a). The mixing portion included 15 rotations within 29.30mm to insure a homogenous solution (see Figure 1b). The separation and height were too large for practical use; the syringes were too far apart to simultaneously inject into the device (see Figure 1c).

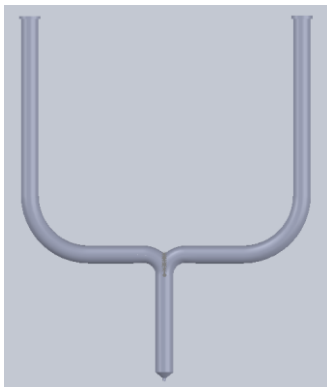


Figure 1a. This figure is the front view of prototype one. The syringes connect to the top and the needle connects to the bottom.

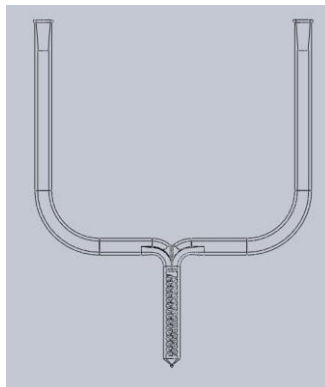


Figure 1b. This figure is the translucent front view of prototype one. The mixing portion of the syringe contained 15 rotations in 29.30mm.

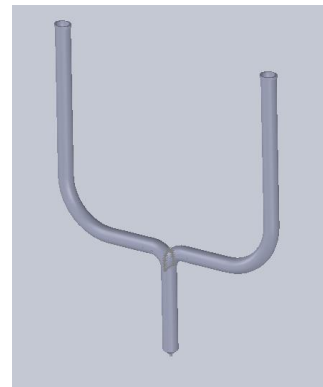


Figure 1c. This figure is the trimetric view of prototype one. The syringes were too far apart and the device was too tall.

3.1.2 Prototype Two. A more practical design was created for the second prototype (see Figure 2a). This design was much smaller at 40.66mm wide, 46.00mm tall, and 9.23mm separation between the syringe connectors. The thickness of the walls was 3.00mm while the syringe connector diameter was 16.00mm. The previous mixing component included in the first prototype was eliminated because the fluid dynamics of the small tube would cause natural mixing. Although the second prototype featured a more ergonomic design, the connectors were too large to secure the syringes and needle. The thickness of the device walls also needed modifications (see Figure 2c).

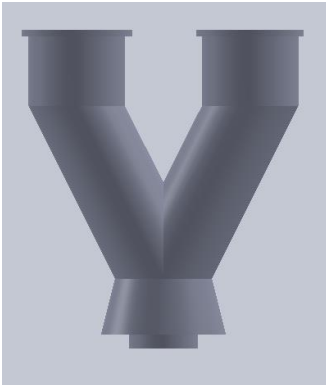


Figure 2a. This figure is the front view of prototype two. The connectors for the syringes and the needle were too large.

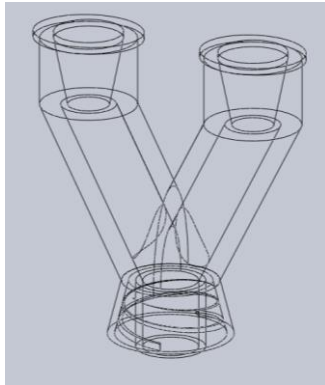


Figure 2b. This figure is the translucent trimetric view of prototype two. There is no mixing system: enough mixing will occur in the needle.

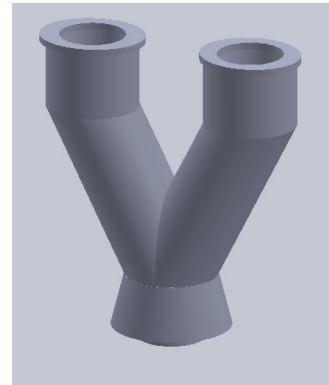


Figure 2c. This figure is the trimetric view of prototype two. The thickness of device walls needs to be decreased.

3.1.3 Prototype Three. The ergonomic separation of connectors and height of the device were established in the second prototype and used throughout the rest of the prototypes; all future prototypes also eliminated the mixing component (see Figure 3b). The third prototype reduced the wall thickness to 0.40mm and connector diameter to 4.20mm (see

Figure 3a). This design also failed to connect properly to the syringes and needle; the connectors for this prototype were too small (see Figure 3c).

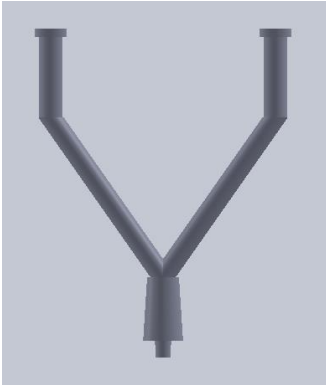


Figure 3a. This figure is the front view of prototype three. The connectors for the syringes and the needle were too small.

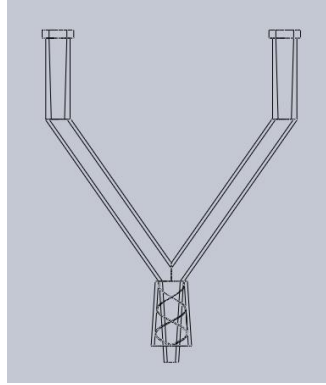


Figure 3b. This figure is the translucent front view of prototype three. The small opening causes enough mixing.

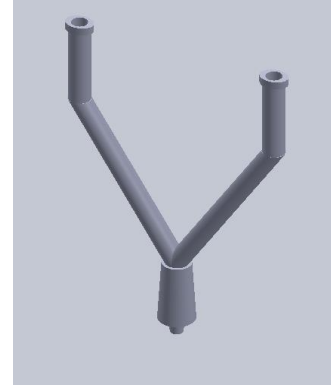


Figure 3c. This figure is the trimetric view of prototype three. The device wall thickness was fixed from the previous prototype.

3.1.4 Prototype Four. The fourth prototype kept the same wall thickness but increased the connector diameter to 8.10mm (see Figure 4c). The geometry of the needle connector changed from an angular connector to a vertical connector (see Figure 4b); this change relieved SolidWorks design difficulties associated with the angular geometry. Two different syringe connectors were designed to test the assembly of the syringe and the device (see Figure 4c). The needle locked into the connector and the syringe locked into the right connector; this connector had an additional angular piece to help secure the locking mechanism. The left connector was straight making the syringe screw into the connector continuously thus interfering with the syringe locking into the device (see Figure 4a).

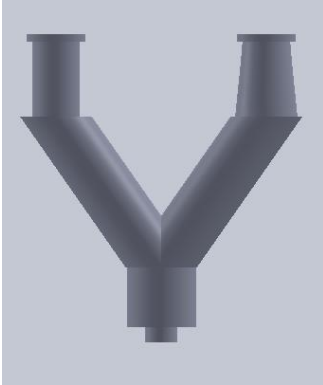


Figure 4a. This figure is the front view of prototype four. The right connectors for the syringes and the needle fit best.

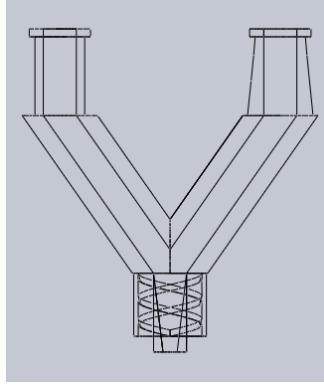


Figure 4b. This figure is the translucent front view of prototype four. The needle connector changed from angular to straight.

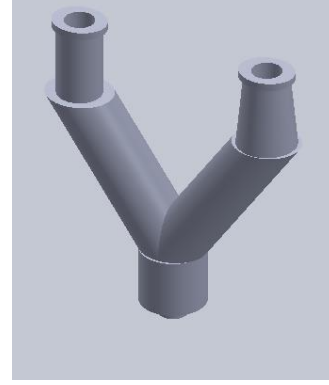


Figure 3c. This figure is the trimetric view of prototype four. To decrease prototype iterations, two different connector shapes were printed.

3.2 Reinforcing the Needle Connector

3.2.1 Prototype Five. The fifth prototype was the same as the fourth design, but both syringe connectors resembled the right (angular) connector (see Figure 5a). After multiple demonstrations of the connectors and a fluid dynamic study with distilled water, the inside needle connector component broke. The thickness of the inside wall was weak at the interface of the two channels to one channel (see Figure 5b).

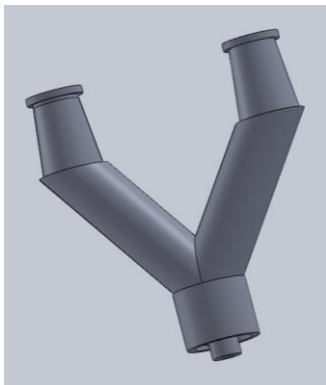


Figure 5a. This figure is the front view of prototype five. The syringe connectors are the same as the right syringe connector in prototype four.

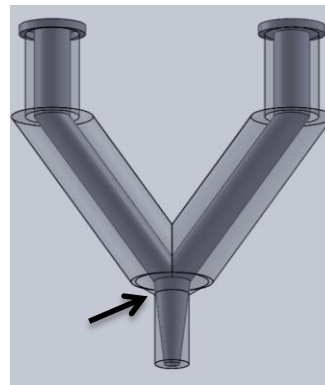


Figure 5b. This figure is the translucent front view of prototype five. The clear part represents the wall thickness. The arrow points to the limited wall thickness.

3.2.2 Prototype Six. The thickness of the inside needle connector could not simply be increased without creating a completely new design. By making the two channels connect more quickly, the thickness of the inside connector was increased (See Figure 6). The increase was enough to last through multiple fluid dynamic tests with distilled water. The instant forming hydrogel was tested using the device. With pressure from the table, the solutions were mixed enough to form the hydrogel. After a few uses, again the inside needle connector broke. Instead of changing the device dimensions, the following prototypes tested the strength using different printing materials.

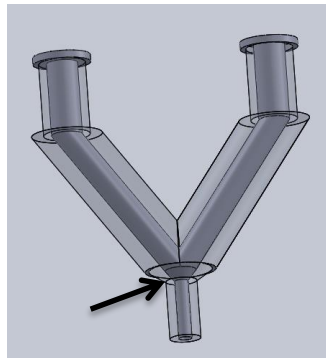


Figure 6. This figure is the front view of prototype six. The clear part represents the wall thickness. The arrow points to the increased wall thickness.

3.3 Material Testing

3.3.1 Prototype Seven. The first six prototypes were printed on an Object30 printer using VeroWhite material (similar to polypropylene (PP)). The dimensional limit of the printer is 0.1mm. In an attempt to reduce printing costs, the MakerBot Replicator 2X (from now on referred to as the MakerBot) printer was used; this printer uses polylactide (PLA).

With a dimensional limit of 0.8mm, the seventh prototype was unusable because the device was not properly printed (see Figure 7).



Figure 7. This figure is a photo of prototype seven. The dimensional limit of the MakerBot was not small enough causing inaccurate printing.

3.3.2 Prototype Eight. Because the sixth prototype was not strong enough for multiple uses, a stronger material was needed. For preclinical uses, a multiple-use device must be easily sterilized. VeroWhite cannot be sterilized by either ethylene-oxide or autoclaved, both available in the lab. Acrylonitrile butadiene styrene (ABS) can be sterilized by ethylene-oxide. An uPrint SE Plus printer was used to create the ABS prototype. Along with its sterility, the prototype was considerably stronger allowing many uses before failure (see Figure 8). The prototype was rougher than the VeroWhite prototype. The increased roughness led to increased pressure; the solutions no longer mixed into a single channel but overflowed at the site of the syringe connector.



Figure 8. This figure is a photo of prototype eight. The uPrint SE Plus printer created a prototype made of ABS. The material was too rough and caused overflowing of the hydrogel mixture.

4. Discussion

4.1 Fitting the Syringes and Needle. Except for the first prototype, the first four prototypes were iterations of differing connector diameter and wall thickness. The device was created for easy use in a laboratory setting and eventually a clinical setting; therefore the ergonomics of the device was a high priority. The trouble with connector fitting was an issue with measurements. With no prior SolidWorks experience, I learned how to use the software through trial and error. For the first prototype, my dimensions were not easily visualized causing a large and hardly usable device. The second and third prototype dimensional incorrectness arose from measuring errors of the needle and syringe and mistakes in distinguishing the radius and diameter of the connectors.

By the fourth prototype, I had hours of exposure to SolidWorks keeping me from making novice mistakes. After using a caliber to measure the syringe and needle and confidence in my design ability, I chose to decrease the prototype iterations by utilizing the two

syringe connectors. There were two proposed designs for this connector. Designing the connectors with the two different designs allowed for increased feedback because multiple prototypes did not need printing. The angled connector gave more support and was more appealing; the straight design on the other connector was simpler. The straight design was strong enough, but it made the syringe continuously screw onto the connector. This continuous screwing decreased stability. The angled connector increased the stability of the syringe device connection because a locking affect occurred between the female and male connector. Because proper fitting was not established for both syringes and the needle, no fluid dynamic studies ensued.

4.2 Reinforcing the Needle Connector. At the time, I thought the fifth prototype would be the last iteration. All parts were able to connect, and the first fluid dynamics test with distilled water was successful. The water was able to flow from both syringes out of the needle. After multiple tries, the inside needle connector broke. The SolidWorks drawing gave insight to the broken connector; the wall at the interface to one connector was meager. The inside connector thickness was critical for the needle connection; an innovative modification was needed to add strength without directly changing the thickness. By decreasing the distance to combine the syringe channels into one channel, thickness was indirectly added to the interface.

The sixth prototype used this design modification. The fluid dynamics were tested again with distilled water. With successful testing, the two components needed to create the instant forming hydrogel were tested. When pressure from the table was applied to the needle opening, the hydrogel formed as it came out of the needle tip. After a few more uses of the device, it broke again. With successful creation of the hydrogel from the

device, printing materials became the focus of strengthening the device and making the device sterile.

4.3 Material Testing. The first six prototypes were printed with VeroWhite on an Object30 printer. Accessibility of the printer was the main reason the first prototypes were printed using the Object30. Once the material of the prototype became a factor, different printers were explored.

The MakerBot printer used PLA, a more cost effective material. This printer was also easily accessible. One concern was the decreased dimensional limit of the printer.

Because the prototype was small and precise, the dimensional limit of the printer needed to be on the order of a tenth of a millimeter. The seventh prototype was created using the MakerBot and was unusable. Without even trying to connect the syringe or needle, it was obvious the device could not be utilized.

After investigating different materials, ABS was an accessible material with greater strength and the ability to be sterilized with ethylene-oxide. The first time the prototype was printed one of the connectors was filled with ABS. After changing the printer orientation from vertical to horizontal, both connectors were printed hollow. The promising strength and sterility of the material became useless after testing the hydrogel solutions. Both solutions overflowed from the syringe connection. We assumed the increased roughness of the ABS prototype caused the overflow. ABS was not an ideal material for this device.

5. Conclusion

This study proposed creating a new medical device for the use of an injectable instant forming hydrogel. Through this investigation, the dimensions of a mixing device for two solutions into one mixture with immediate injection abilities were created. In the process, eight prototypes were created. Six of the prototypes were concerned with the dimensions of the device while the other two focused on the materials of the device.

For multiple uses, the device needs sterility capabilities. With the hydrogel forming instantly, some hydrogel would form in the device. The sterilizing process would clean the device of any leftover materials and keep disease from spreading. The roughness of ABS was found to be cause problems with mixing. Another material is needed for the device to be used.

Further studies could be conducted to determine a material suitable for the device and the feasibility of mass-producing the device. While determining the feasibility, the market value of the device could be determined. To increase the impact of the device, further research could determine other applications of the mixing device in medicine.

Dimensional experimenting could lead to an optimal length of existing parts for hydrogel formation. Because changes in the two solutions impact the hydrogel formation time, the device could test the characteristics of different formulation times.

6. Acknowledgements

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