Bioprinting Methodology Using a Pectin Based Bioink

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Abstract

Bioprinting is a form of additive manufacturing using biological materials to create a physical three dimensional (3-D) model. Two major drawbacks for bioprinting are printing vascularized tissues/organs and developing a viable bioink to act as an extracellular matrix. The extracellular matrix is crucial for cell growth and carrying out critical functions. This study investigated a new methodology for bioprinting using a pectin based bioink. Pectin is a linear polysaccharide found in the peels of apples and oranges, making it biocompatible, an ideal characteristic for tissue engineering. Pectin can act as an extracellular matrix allowing for cells to survive, proliferate, migrate, and carry out specific functions. Pluronic F-127 was incorporated into the pectin based bioink to obtain the desired shape during the bioprinting process because of its ability to gel above 27 degrees Celsius. The Fab@Home M3 bioprinter was used to create the desired hydrogel scaffold shape. The printer operates using Seraph software which was used to create the precise dimensions for printing objects to test the feasibility of a pectin matrix. Once an object was printed, it was treated with Ca^{2+} or oligiochitosan (pectin cross linker) to create a final tissue or organs structure, allowing the object to sustain a stable shape during storage and at body temperature. The results indicate that pectin mixed with Pluronic® F-127 is a potential bioink for tissue engineering. This methodology provides a novel and fast approach for bioprinting.

Keywords: Bioprinting Methodology, Bioink, Pectin Hydrogel

Introduction

With an increase in life expectancy of the population, there has been a dramatic rise in the need for organ and tissue transplants in the United States over the last two decades. This demand for organs and tissues has increased rapidly each year, while the number of donors has remained fairly consistent (Figure 1). Every day 22 people die waiting for an organ, and every 10 minutes a new person is added to the list of patients in need of a transplant [1]. Bioprinting provides a new method of engineering organs and tissues that uses the patient’s own cells; this would not only save lives, but potentially decrease the overall rejection rates of transplants.
Bioprinting is a type of additive manufacturing that uses biological materials. Currently, there are several approaches for printing biological materials, such as inkjet bioprinting, microextrusion bioprinting, and laser-assisted bioprinting. Inkjet bioprinting delivers the bioink to specific locations and is the most commonly used method available. The print time is very fast, and also maintains minimal costs and an average cell viability. Microextrusion printing uses a mechanical force to dispense bioink. It is an affordable process with a slow print speed and a low to average cell viability. Laser-assisted bioprinting uses a laser to push certain cell materials from a substrate onto a collector. This printer type is expensive, and typically features an average print speed and a low cell viability. While methods currently exist for bioprinting tissues and organs, significant drawbacks continue to exist in terms of vascularization of tissues and developing a bioink to act as a cellular scaffold. Without a vascularized network, tissues and organs cannot obtain the vital nutrients needed to carry out cellular functions, ultimately leading to the death of the organs and tissues when placed in vivo. During the bioprinting process, the bioink is essentially used to fabricate scaffolds that act as an extracellular matrix (ECM). The ECM provides structural support to the surrounding cells. It allows proliferation, migration, and communication between cells to occur. Creating a viable bioink that can carry out basic functions of the ECM while remaining biocompatible is important for the success of a bioprinted tissue or organ. One polymer that displays these vital characteristics is pectin.

Pectin is a linear polysaccharide found in the peels of apples and oranges. The numerous hydroxyl groups found in its structure contribute to pectin’s hydrophilic nature. When treated with Calcium ions ($\text{Ca}^{2+}$), the carboxyl groups in pectin combine with the $\text{Ca}^{2+}$ creating a stable hydrogel. Overall, the goal of this research is to print a viable hydrogel shape using pectin bioink that can then be used to print vascularized tissues/organs. Similar studies have been performed to bioprint hydrogel materials using alginate rather than pectin. In one study, sodium alginate was used to print human adipose derived stem cells (hADSCs) which were crosslinked with calcium chloride, sodium chloride, and porcine gelatin to create hydrogels. This study tested the optimal viscosity and cell density for the highest possible cell viability. In addition, different oxidation percentages and cell proliferation over a short period of time were tested. This study found that cell viability was greater in a middle viscosity solution as opposed to a high viscosity solution. Furthermore, the hADSCs proliferated and spread in certain solutions with optimal oxidation concentrations as well as cell concentrations. This study gives a detailed analysis of alginate bioink capability. Considering the similarities between alginate and pectin, it was hypothesized that pectin would also be a viable bioink for bioprinting.
Materials and Methods

2.1 materials

The pectin used in this study was obtained from Willpowder (Miami Beach, FL) and the Pluronic® F-127 was obtained from Sigma-Aldrich (St. Louis, MO). Pharmaceutical grade oligochitosan was purchased from Zhejiang Golden-Shell Pharmaceutical Co. (Zhejiang, China).

2.2 software and printer model

A Fab@home M3 bioprinter was used for this research (Figure 2). This bioprinter operates on seraph software that is composed of Seraph Studio and Seraph Print. An STL file of an object was generated using a 3-D Computer Aided Design (CAD) Software and uploaded into the Seraph Studio software. When placed in the software, adjustments were made to properties of the object to modify the size, position, scaling, orientation, and material. Once completed, the program generated an XML file that contained geometric information for the object file, such as print location, slice height, material deposition rate, etc. Seraph Print then uploaded the XML file and controlled the printer head start location for all three axes. Once the printer head was in the correct location and the files were ready, the object is was printed in successive two dimensional (2-D) layers to eventually create a physical 3-D model.

Figure 2. Bioprinter Set-Up

2.3 bioprinting process

A bioink composed of 3% pectin (w/v) and 20% (w/v) Pluronic® F-127 solution was prepared. The solutions were placed inside a plastic syringe and the desired nozzle tip was attached. The syringe was sealed using a white stopper. Then the syringe was placed into the printer and a metal rod was twisted into place to allow for deposition of the material. The material was printed onto a petri dish heated by an Amscope Microscope Temperature Control Stage Slide Warmer (TCS-100; Amscope, Irvine, CA). This was done to keep the printed bioink at 37 °C in order to allow the pluronic® F-127 in the bioink to gel the mixture as the object printed.

2.4 post printing process
While printing into a petri dish, pre-warmed Ca\(^{2+}\) (150 mmol) or 5\% oligochitosan (w/v) was slowly added into the dish to maintain the temperature of the bioink at 37 °C. This process gelled the bottom layers during the printing process and continued to gel each layered as they printed. During the bioprinting process, warm Ca\(^{2+}\) or oligochitosan solution was added to gel both Pluronic® F-127 (heat-induced) and pectin (cross-linking) molecules to form permanent structures. Once the print job was complete, the entire object was treated with Ca\(^{2+}\) /oligochitosan to permanently gel the object so it could keep its structure in storage as well as in vivo. This process is shown in Figure 3.

**Results and Discussion**

First the minimum concentration of pluronic needed for gelation was found to be 20\% (w/v). Figure 4 shows the images of the pectin 3\%/pluronic 20\% solution and gel. The first image on the left shows the solution maintained at room temperature. The image on the right shows the gelled pectin and pluronic solution (shortly after incubation at 37 °C water bath). It can be seen that in the image on the left there is a slant in the showing it is still in the liquid state. The image on the right does not have this slant showing that the solution is gelled.

Many parameters including nozzle size, deposition rate, printer speed, and slice height were investigated in this study. A 22, 24, 30 and 32 gauge syringe tip were used during testing. The 30 and 32 gauge nozzles were too small to allow pectin to flow. When used during printing, this nozzle created too much pressure and the pectin was forced out of the top of the syringe rather than through the nozzle opening. Hence, 22 and 24 gauge nozzles were chosen for the rest of the study. When printing, the layers needed to gel by the heating plate. The path speed of 5 mm/s allowed this to occur while still moving fast enough to ensure an even spread of the liquid pectin bioink. A deposition rate of .003 mm/mm (millimeters of plunger motion/millimeters of tool travel) was found to be optimal.
Simple structured hydrogel scaffolds, shown in Figure 5, were printed. Figure 5 B (side view of the cross section) and C (top view) show the same structure. This confirmed that the different layers anneal together rather than separately during the bioprinting process.

![Image of printed ear scaffold](image)

**Figure 6. Printed Pectin Hydrogel Ear Scaffolds**

To show the printing capability of an organ or tissue shape structure an ear was chosen. Both Ca\(^{2+}\) and oligochitosan were used as cross-linkers, however, oligochitosan failed to create a stable scaffold (data not shown). Figure 6 shows a more detailed image of a printed scaffold using Ca\(^{2+}\) as a cross-linker. In Figure 6A, the modeled ear in Seraph software is shown \(^{[7]}\). Figure 6B shows a side view of the ear and Figure 6C shows a top view of the printed ear scaffold.

**Conclusion**

Both simple and complex structures were successfully printed by using the pectin/Pluronic® F-127 solution as the viable bioink. Adding warm Ca\(^{2+}\) or oligochitosan solution during bioprinting process is a critical step to allow both Pluronic® F-127 (heat-induced) and pectin (cross-linking) molecules to gel, forming permanent structures. However, oligochitosan failed to act as a cross-linker during this study. Oligochitosan treated scaffolds were soft and fragile, which was most likely caused by a different molecule crosslinking process than that of the Ca\(^{2+}\)\(^{[8,9]}\).

To summarize, this study found that with the optimal bioprinting parameters as well as this printing methodology, the pectin/Pluronic® F-127 solution, was a viable bioink for printing scaffold structures. This methodology provides a novel and fast approach for bioprinting. Further investigation into bioprinters with high precision could be done to test the viability of this ink with complex structures.

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**References**


