Printable Biodegradable Hydrogel with Self-Crosslinking Agents for Wound Dressings

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Abstract

The printed Biomaterial Laboratory at UTEP does research a printable hydrogel which can have appropriate properties for tissue engineering of the skin. Skin is the largest organ in our body which protects us from the environment and pathogens. Skin can be affected by burns and also by diabetic foot ulcers. The current tissue engineered skin substitutes for treatment of diabetic foot ulcers have many shortcomings including difficulty of handling, little if any host integration and not being customizable. The goal of this research is to create a wound care material that helps by integrating with the host tissue. We have been investigating a biodegradable hydrogel which is derived from natural proteins and carbohydrates creating a scaffold to use as a substrate to grow cells. The main components of this hydrogel are gelatin and alginate, both materials with very high biocompatibility and promoting cell proliferation and vascularization. Here we have been studying the oxidation of sodium alginate to generate aldehyde groups that can crosslink the amino group of gelatin and form the biodegradable hydrogel. We also have been investigating the viscosity, gelling time and degree of crosslinking of alginate as a function of pH, degree of oxidation, concentration and temperature. Viscosities for 10% alginate solutions in the range of 5-10cp are obtained, making this material printable. For printable testing we modified an inkjet printer to control the temperature of the cartridge and of the deposition plate. In general, control over the concentrations of alginate as well as the spatial dispensing via printing in a temperature-controlled environment should allow us to generate wound dressings of tunable properties. For future work we will include testing viscosity and printability of alginate adding different types of cells, as fibroblast, keratinocytes and endothelial cell, varying cell concentration. We will also include testing the wound dressing in a small animal model on healing and wound contraction.

Introduction

Many people suffer from burns and also diabetic foot ulcers. According to the American Burn Association (ABA), in the United States 450,000 people receive medical treatment when they are burned and the survival rate is 94.8% which means that 23,400 people die by burned injuries [1] . The majority of deaths are due to the massive fluid losses and microbial infections [2]. Diabetic foot ulcers are the most common complications of Diabetes mellitus. These kinds of injuries are caused by arterial abnormalities which are too difficult to treat because many complications and infections can be developed by these injuries. These wound are the cause of many foot and leg amputations.

Alginate is a carbohydrate extracted from brown algae. It shows good biocompatibility and low toxicity [3]. The oxidation of sodium alginate has been previously studied. It is known that sodium periodate generates aldehyde groups in the modified alginate (MA) which can crosslink with the amino groups of the gelatin [4] with no addition of other chemical agents (like $CaCl_2$). Additionally, the MA shows a good biodegradability which is not observed in the unmodified alginate [5]. The viscosity in the MA decreases and makes it printable (printable biological ink). Gelatin is a polymer derived from collagen which is the principal protein of the skin, and it has been used in medical applications because it shows good biocompatibility and biodegradability [6]. Both alginate and gelatin have many applications in drug delivery, cell therapy, wound dressing, and tissue engineering [7] [8].

Inject printer is the most popular printer because it has a high quality printing at low cost. Inject printing is a contactless technique. The printer receives computer information and reproduces on a substrate [9]. Ink is ejected out of the nozzle as is required, and the amount of printed ink can be controlled [10]. Currently, inkjet printing has been used in biomedical engineering applications (drug screening, biosensors and genomics) [9]. Recently, it was researched cell viability during inkjet printing on biological substrates [11].

The main goal of this research is to create a low cost wound care material that promotes the regeneration of the skin by integration with host tissue using inkjet printing technology. In this research we modified a Hewlett-Packard Deskjet 340 printer and created a biological ink to disperse different types of cells, as fibroblast, keratinocytes, and endothelial to create a biodegradable hydrogel with different cell layers.

Materials and Methods

Alginic acid sodium salt (medium viscosity) and sodium periodate ACS reagent grade from MP biomedical, gelatin from porcine skin (type A 300 bloom) from Sigma Aldrich, sodium tetraborate decahydrate (Borax) from Fhisher scientific, phosphate buffered saline (PBS), 2,4,6-trinitrobenzenesulfonic acid (TNBS) from thermo scientific, ethyl alcohol from Pharmco AAPER, Spectra/Por 6 membrane tubing MWCO 2000 from Spectrum laboratories.

Alginate Oxidation

The alginate was oxidized with sodium periodate to create aldehyde groups in the alginate [12] [13]. 10 g of sodium alginate were dispersed in 25 ml of ethanol and 25 ml of 0.4 M sodium periodate were added. The solution was stirred at room temperature in the dark for 6 h. Water was added to the solution

reaching 1 L with distilled water, and the solution was kept in the dark for 72 h. The precipitate solution was purified in dialysis tubing for 48 h changing water twice per day. The presence of periodate was determined taking 0.5 ml of the dialyzed water and adding 0.5 ml of 1% silver nitrate solution until no more precipitate was present. The purified modified alginate (MA) was lyophilized.

Alginate Characterization

1. Aldehyde Group

The lyophilized MA was dissolved in water and 200µL were put on the polytetrafluoroethylene (PTFE) card. The samples were dried in a desiccator for 24 h. The presence of aldehyde group in the MA was measured in Perkin Elmer Spectrum 100 Fourier transform infrared spectrometer (FTIR) in the spectral range between 4000 and 400 cm^{-1}, but the range to analyze is between 2500 and 500 cm^{-1}.

2. Viscosity

Different solutions of MA were prepared (5%, 10%, and 15%) in PBS and in 0.1M borax. The viscosity of the solutions was measured in a Brookfield DV-E Digital Viscometer using a small sample adapter with a spindle number 18. The viscosity was measured varying the speed and at room temperature in most of the solutions, but in some cases the viscosity was measured at different temperatures with the purpose to analyze the behavior of MA solutions (Data not shown).

3. Gelling time

Different concentrations of gelatin and MA were prepared. 0.5 ml of MA solution and 0.5 ml of gelatin solution were mixed using a magnetic stirring hot plate and a stir bar (0.5 cm in diameter and 1 cm in length). The solution was mixed at 37 °C and 250 RPM. Gelling time was observed when the stir bar stopped [14].

4. Degree of crosslinking

The degree of crosslinking (DC) was determined by TNBS assay [12]. The gels were frozen and lyophilized. 5 mg of the lyophilized powder was reacted with 1ml of 0.5% TNBS and 1ml of 4% $CHNaO_3$ at 60˚C for 4 h. 1 ml of this solution was reacted with 3 ml of 6N HCl at 40˚C for 1.5 h. TNBS solution reacts with the unreacted gelatin and forms a soluble solution. The absorbance (ABS) was measured at 334nm in a Biomate 3 Thermo Scientific Spectrophotometer, and determined by equation 1.

$$\%\ \mathrm{DC} = \left[1 - \left(\frac{\text{ABS of crosslinked gel}}{\text{ABS of non-crosslinked gel}}\right)\right] * 100 \qquad (1)$$

Inkjet Printer Modification

A HP Deskjet 340 Printer and HP 33 cartridges were modified to control the temperature of the cartridge and the deposition plate. An aluminum plate was adapted to the printer using the paper feeding sensor and two switches along the y-axis. This plate has a serpentine inside, which has an inlet and outlet. Water flows inside and a heat interchange occurs between the water and the plate, which allows us to control the temperature of the plate. The water is heated or cooled by a Thermomix/Frigomix 1460/1495 Braun chilled/heater. The z-axis is regulated by hand with an adjustable manual stand allowing us to regulate the distance between the cartridge and the plate (see ***Figure 1***).

Biodegradable Hydrogel Fabrication

The biological ink mentioned above was added into the modified HP 33 cartridge. 10% gelatin in 0.1M borax solution was used as bio-paper on a microscope slide. The biological ink was printed on the gelatin. ***Figure 2*** shows the biodegradable hydrogel with 10% MA and 10% gelatin, both solutions in 0.1M borax.

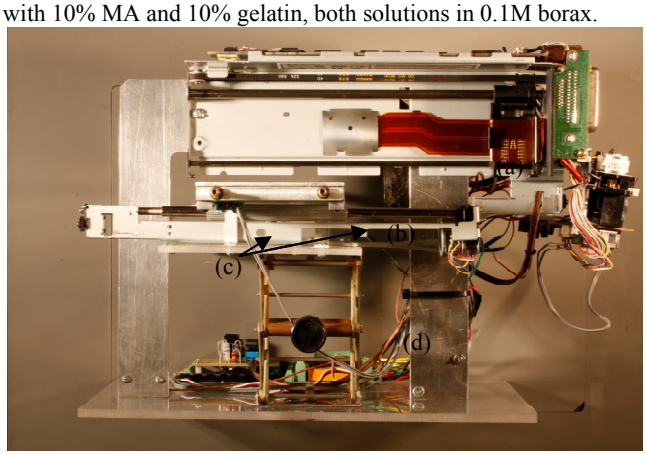

Figure 1. Modified HP Deskjet 340 Printer a) cartridge holder, b) deposition plate (y-axis), c)the arrows indicate the inlet and outlet of the deposition plate through the liquid flows.

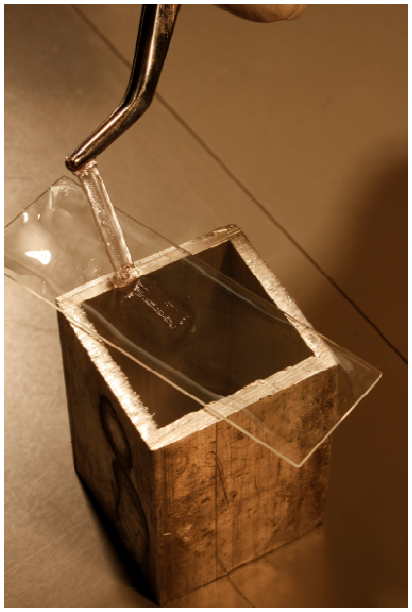

Figure 2. Printed hydrogel (10% MA and 10% gelatin, both solutions in 0.1M borax) using a modified HP Deskjet 340 Printer.

Results and Discussion

Sodium alginate is a heteropolysaccharide in which resides the 4-linked β-mannuronic acid and its C-5 epimer, α-guluronic acid [15]. The hydroxyl groups of the guluronic acid in the alginate are oxidized with sodium periodate, by the rupture of the carbon-

carbon bond, and the formation of aldehyde groups occurs in each oxidized monomeric unit.

As seen in ***Figure 3*** the graph shows the FTIR spectra of alginate and modified alginate. The characteristic peak of aldehyde group is observed at 1739 1/cm and with this peak we proved the existence of aldehyde group in the modified alginate. The aldehyde group makes possible the crosslink between the MA and the amino acid group of the gelatin, creating a biodegradable hydrogel non-thermo-reversible.

The viscosity was measured at different concentrations of MA (5%, 10%, and 15%) and as the concentration increases, the viscosity increases. The speed of the spindle was varied at room temperature (~25˚C). The viscosity average for the different solutions were 1.675, 4.13, and 6.235 cP, for 5%, 10%, and 15% MA in 0.1M borax, respectively (see ***Figure 4***). Additionally, we determined the viscosity of 10% MA in PBS at different temperatures (25, 30, 35, and 40 ˚C), and noticed that as the temperature increased, the viscosity decreased (data not shown for 30, 35 and 40˚C). The viscosity for the 10% MA in PBS at different temperatures was 23, 19.89, 17.36, and 15.13 cP, respectively.

The gelling time was determined for different solutions of gelatin and MA varying the concentration in both solutions. ***Figure 5*** shows the gelling time of 10% gelatin in 0.1M borax solution at different concentrations of MA in 0.1M borax solution. It is shown that as the concentration of MA decreases, there is a decrease in the gelling time, however the gelling time between 10 and 15% MA in 0.1M borax is not significant. ***Figure 6*** shows the gelling time when MA is constant (10% MA in 01.M borax solution) and the concentration of gelatin solutions was varied. Additionally, the gelling time of gelatin and MA hydrogels was compared when 0.08M $CaCl_2$ was added to the gelatin solutions. It was observed there was a faster gelling time when there was no addition of $CaCl_2$. This could be because all MA reacts with the gelatin to form the hydrogel, and when $CaCl_2$ is presented, part of MA reacts with the gelatin and the other part reacts with the $CaCl_2$.

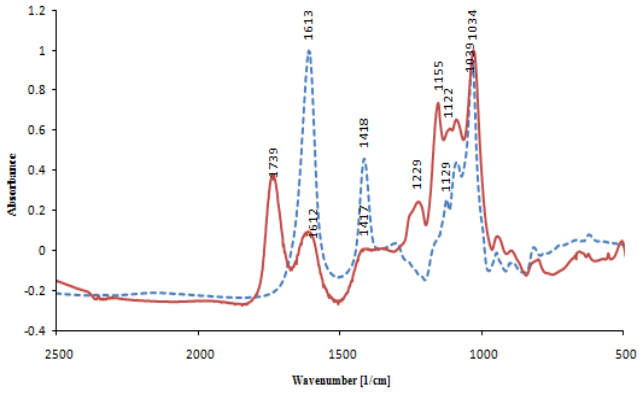

Figure 3*. The dashed line spectra shows the characteristic peaks for alginate, and the solid line spectra shows the characteristic peaks of MA. The arrow shows the C–O region (general), but in this case the characteristic peak of aldehyde group is at 1739 1/cm.*

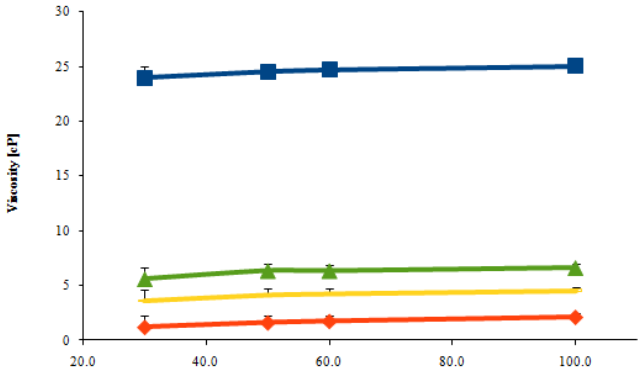

Figure 4*. 10% MA in PBS (■) , 15% MA in 0.1M Borax (▲) , 10% MA in 0.1M Borax (▭) ,and 5% MA in 0.1M Borax (◆) .*

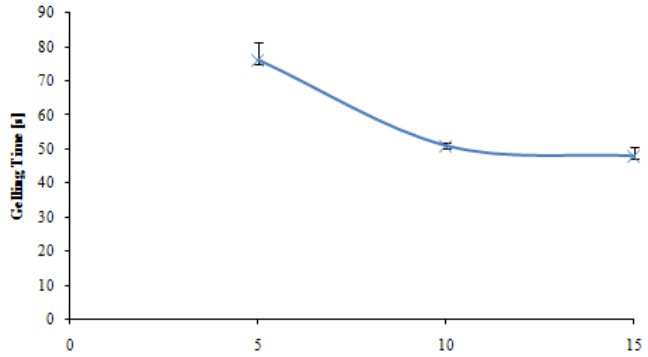

Figure 5*. Gelatin solution was constant (10% gelatin in 0.1M borax solution), and the concentration of MA was varied (MA was dissolved in 0.1M borax solution).*

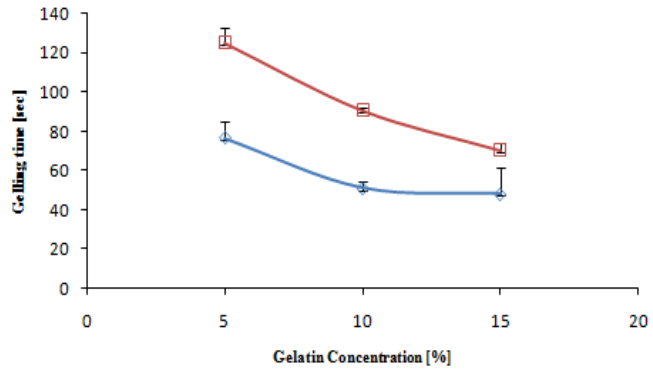

Figure 6*. The concentration of MA was constant (10% MA in 0.1M borax solution). Gelatin in 0.1M borax solution (◇), and gelatin in 0.1M borax solution + 0.08M $CaCl_2$ (□).*

The degree of crosslink was determined by TNBS assay. ***Figure 7*** shows the degree of crosslink when varying the concentration of gelatin in 0.1M borax solution (5%, 10%, and 15%), and maintaining constant the concentration of MA (10% in 0.1M borax solution). It was observed that as the concentration of gelatin increases, the degree of crosslink decreases.

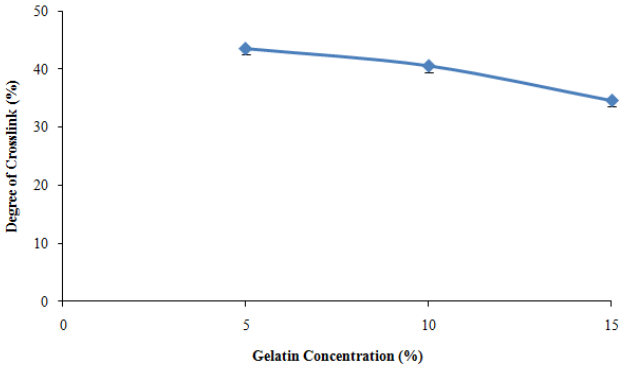

Figure 7.*The concentration of MA was constant (10% MA in 0.1M borax solution), and the gelatin concentration was varied.*

Conclusions

The chemical modification of sodium alginate with sodium periodate allows us to create a printable biological ink where we can disperse cells with the purpose to obtain a biodegradable wound care material with tunable properties.

In summary, control over the concentration of MA as well as the spatial dispensing via printing, should allow us to generate a wound dressing of tunable properties. The use of a portable device makes MA solution attractive to low resource setting.

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