3D INKJETTING DROPLET FORMATION OF BACTERIAL CELLULOSIC EXOPOLYSACCHARIDE GEL

Daniel Aguiar*, Amanda Albuquerque†, and Bingbing Li*

*Department of Manufacturing Systems Engineering & Management, California State University Northridge, Northridge, CA 91330
†Universidade Federal de Pernambuco - UFPE, Pernambuco, Brazil

Abstract

On-demand 3D printing of scaffolds and cell-laden structures has shown promising results that can significantly impact human welfare. The objective is to fully understand the behavior of bacterial cellulosic exopolysaccharide gel (BCEG) as a new bioink with low toxicity and high biocompatibility for regenerative medicine. Its possible application is to construct scaffolds that can be used for several biomedical applications, especially tissue engineering and treatment of critical bone defects. By using a MicroFab inkjet micro dispenser, BCEG was dispersed to create drops on demand that can be used to fabricate scaffolds. In order to fully understand the material's behavior and droplet formation, we analyzed the physical and mechanical properties of the BCEG in different concentrations (0.1% 0.5% and 1%) and characterized it by its macroscopy, microscopy, rheology and particle size distribution.

1. Introduction

Within the past few years, the manufacturing world has undergone several changes, including the creation of new materials and new manufacturing techniques. Among them, we can highlight the field of additive manufacturing, also known as 3D printing, which has advanced continuously through the past few decades[1]. 3D printing is widely applied in various industries, including the biotechnology field, and scientists are constantly improving it. 3D bioprinting in particular has shown promising results and it is being studied extensively, in order to further refine its application and biomimicry accuracy.

Currently, there are few 3D bioprinting techniques in practice and each of them have their advantages and disadvantages. Among them all, there are three types that have been used extensively in studies: micro extrusion, inkjetting and laser-assisted[2]. Inkjetting is one of the preferred methods for dispensing cells because of its high cell viability. This is due to the lower shear stress caused during the dispensing of bioinks, when compared to other methods such as micro extrusion[1].

The objective of this study is to analyze and understand the droplet formation process when dispensing the cellulosic exopolysaccharide gel (BCEG) through a micro nozzle dispensing system, using one of the inkjet approaches called Drop on Demand (DOD). Ultimately, the motivation of this experiment is to decide whether it is a viable method for scaffold construction, which can be used in several biomedical applications. These uses include tissue engineering and treatment of critical bone defects, as bone is the second most transplanted tissue worldwide.[3]

This is a promising technique for in situ fabrication of scaffolds that can be customized to patient needs and specific geometry. The goal is to create a highly compatible scaffold by using the cellulosic exopolysaccharide gel. This would ultimately result in reduced surgery time and decreased immune rejection, while presenting a viable solution for the current problem of organ donor shortage. This use of novel renewable material is very promising and manufacturing

technique approaches shall be studied in order to achieve an effective, repeatable and scalable fabrication.

2. Materials and Methods

2.1 Hydrogel Fabrication

The proprietary Bacterial Cellulosic gel, POLYGEL®, is a hydrogel produced by POLISA, a startup originated at the Biopolimero de Cana-de-Açúcar Group at the Universidade Federal Rural de Pernambuco. This gel is a natural product composed of bacterial cellulosic exopolysaccharide obtained from sugarcane molasses by Zoogloea sp4. This polymer was refined in PA grade and was presented as a hydrogel in three concentrations: 0.1%, 0.5% and 1%. w/v. The solutions were solely composed of dry bacterial cellulose and deionized surgical grade water, and sterilized by gamma-ray irradiation under ANVISA standards, for use in this study.

This bacterial cellulosic exopolysaccharide biopolymer has been used for different biologic applications and studies have demonstrated its high biocompatibility for biomedical applications, including grafts for wounds and bone defect repair in animal studies[5, 6] and in humans[7, 8, 9]. The biodegradability, sustainability and wide availability of its raw materials are also very interesting properties of this novelty material. When compared to materials already available on the market, such as ePTFE, the BCEG is a cheaper yet more effective material to construct films and grafts[10]. Due to the fact that hydrogels have high water content, they are ideal for absorbing high levels of nutrients and oxygen[11]. Its high biocompatibility and bio integration make hydrogels a strong material candidate that can be applied to a wide range of medical applications.

2.2 Experiment Setup

The inkjet printing process in this study was implemented using a MicroFab PH-46 (MicroFab, Plano, TX) micro dispensing subsystem. The bioink was ejected using a MicroFab 80 μm micro nozzle dispenser. The DOD pulse was controlled using a MicroFab Jet Driver, and a MicroFab pneumatic controller was used to adjust the backpressure of the fluid reservoir, to obtain an ideal meniscus for good droplet formation. An imaging system camera STC-MB33USB (SENTech America, Carrolton, TX) was used to observe the droplet formation process. The setup schematic is shown in **Figure 1.** The parameters were adjusted for each of the gel concentrations, in order to obtain the droplets by increasing or reducing the back pressure and pulse. Initial arbitrary values were used, in order to set a range for the polymeric gel used in the study, then were fine-tuned.

2.3 Hydrogel Characterization

Macroscopic observations were made and the hydrogel's visual, olfactory and tactile characteristics were recorded. Microscopic analysis was performed by Scanning Electron Microscopy Zeiss Supra 40VP (Zeiss, Oberkochen, Germany) and utilized to obtain images of the dried polymer samples' morphology and organization. Optical Microscopy (Olympus, Tokyo, Japan) imaging was also performed, in order to observe the arrangement of the hydrated fibers in the hydrogel. A Microtac Zeta Potential analysis (MicrotracBel, Osaka, Japan) was performed and the particle size distribution was observed for the biopolymer solution concentrations. Rheology of the hydrogel was characterized using a TA AR200 Rheometer (TA Instruments, New Castle, DE), using a 20mm parallel plate geometry with a measuring gap of 1 mm, and experimental conditions were kept constant. Temperature was kept constant at 25 degrees Celsius, as viscosity of the hydrogel can vary depending on temperature[12].

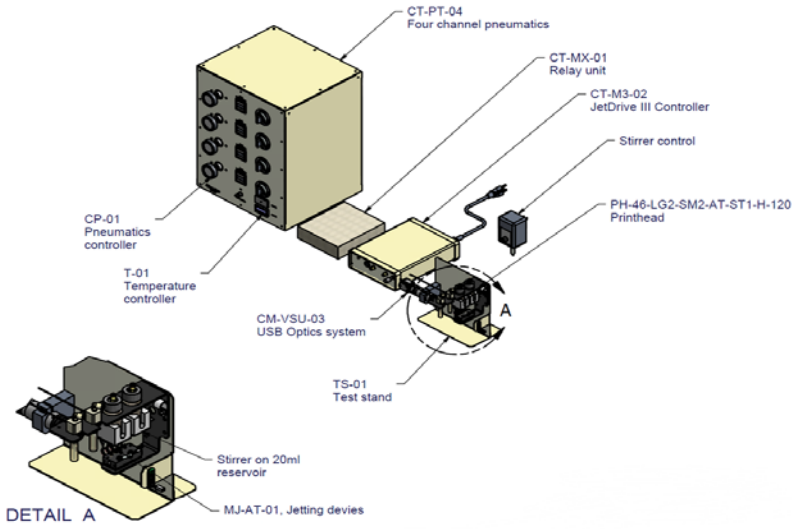

Figure 1 Microfab dispensing experiment setup.

3. Results

3.1 Macroscopic Examination

During the visual macroscopic examination of the polymer at room temperature, it presented a homogeneous, semi-transparent, viscous aspect. Its olfactory properties presented no smell and the gel was soft to the touch, with a gel-like texture.

3.2 Microscopy Analysis

3.2.1 Optical

Widefield microscopy was conducted and the hydrated fibers in the solution were observed. They presented a uniform morphology of elongated fibers that were clumped together in clusters. On **Figure 2**, a fibril cluster is observed in a 1% BCEG sample.

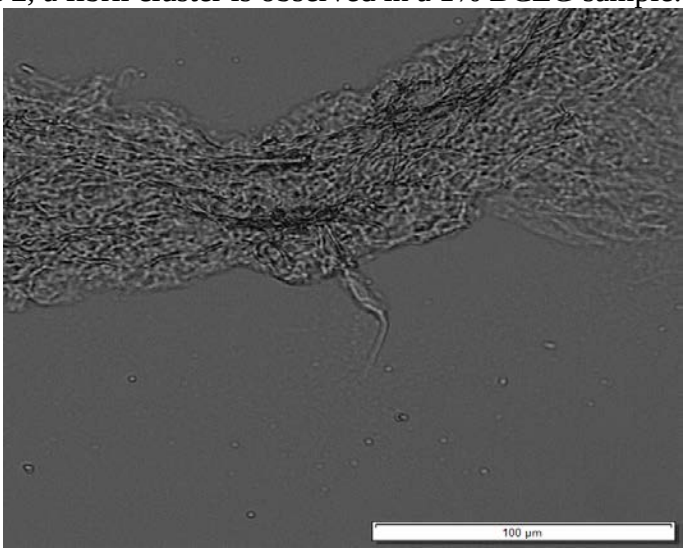

Figure 2 Fibril cluster of the Bacterial Cellulosic Exopolysaccharide at 1% concentration

3.2.2 SEM

The BCEG samples were dried for 24 hours over silicon wafers on a heat plate set to 45 degrees Celsius. It was then gold coated and taken to SEM imaging in **Figure 3**. The images show the morphology and arrangement of the polymer fibrils when dried. Their size reduced due to shrinkage during the drying process where the water content was evaporated.

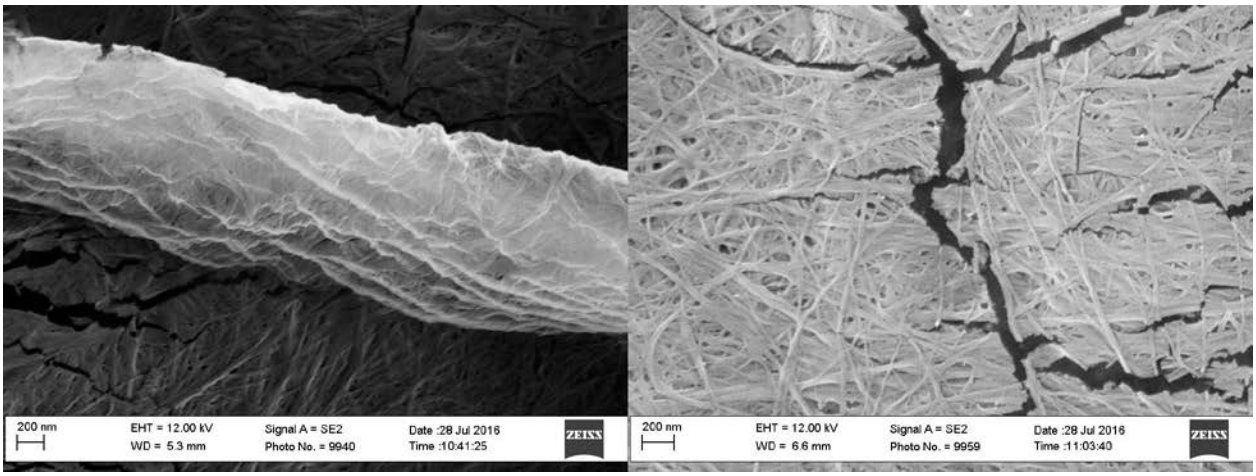

Figure 3 SEM imaging of dry samples of the polymer showing the morphology and arrangement of its fibers.

3.3 Zeta Potential

The zeta potential test shows that the particle size range (0.05 - 6.54 um) was the same in all samples as the only variable changed was the concentration of the polymer. The particle size distribution is somewhat uniform in each polymer concentration in the hydrogel form.

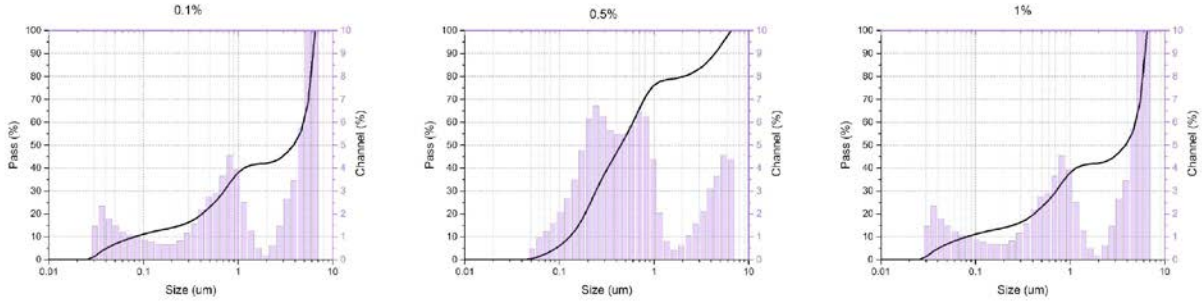

Figure 4 Zeta potential particle size distribution in 0.1%, 0.5% and 1% concentrations of the biopolymer

3.4 Rheology

The Viscosity as a function of shear rate for the BCEG in 0.1%, 0.5% and 1% concentrations recorded using the rheometer in **Figure 5**.

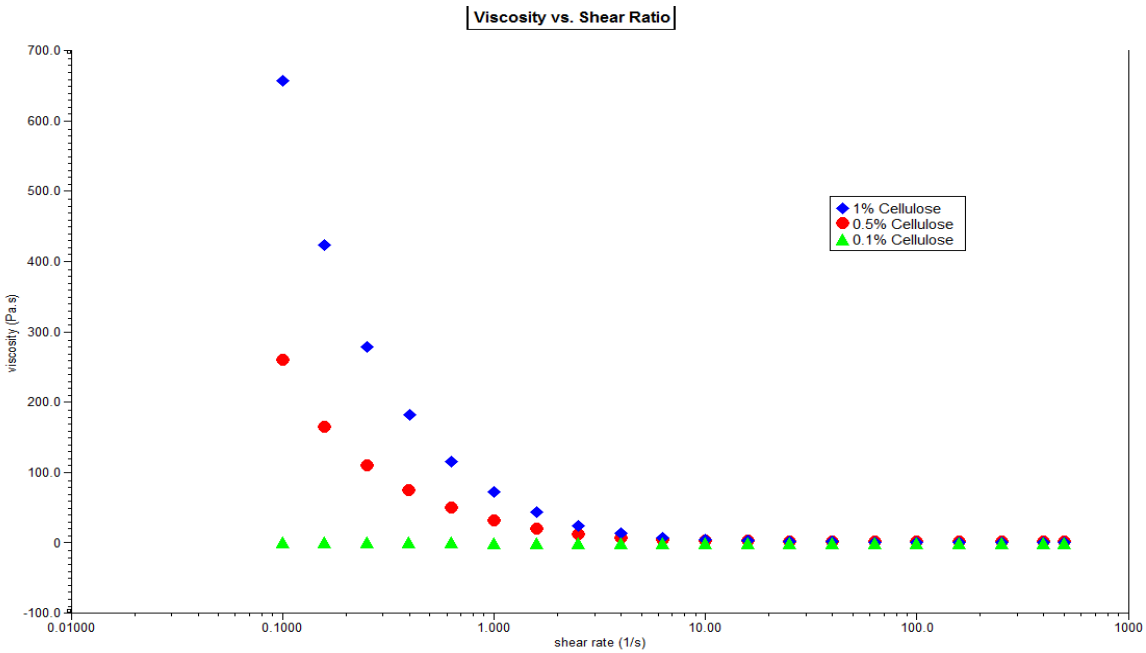

Figure 5 Viscosity and shear ratio of the BCEG in 0.1%, 0.5% and 1% concentrations

3.5 Ideal Dispensing

When the drops that are formed fall in a controlled fashion, as it is observed for the 0.1% concentration BCEG gel in **Figure 6**.

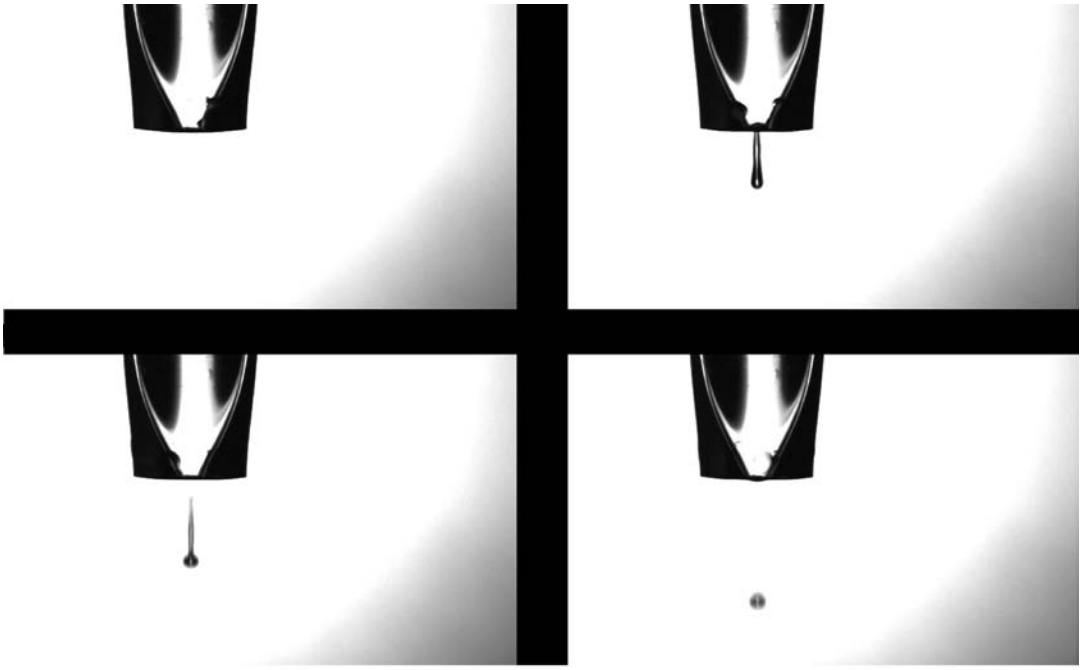

Figure 6 - An ideal droplet sequence formation of BCEG in 0.1% concentration

3.6. Non-Ideal Dispensing

Non-ideal droplet formation is observed, with the incorrect droplet dispensing formation resulting in inaccurate geometry, in **Figure 7.**

Figure 7 Non-ideal droplet formation in 0.5% BCEG concentration

4. Discussion

As expected, the gel presented higher viscosity proportional to the concentration of exopolysaccharide of the polymer. **Figure 5** illustrates the viscosity of the gel and its shear thinning properties for concentrations of 0.1%, 0.5% and 1%, respectively. The polymer with 0.1% concentration behaves like water, with very low density. The viscosity will affect the how the gel is dispersed through the micro dispensing nozzle.

When ideally formed, the drops will fall in a controlled fashion. Each drop's size and rate of falling are both uniform, and these properties can be controlled by changing the dispensing parameter, which is a combination of back pressure, microcontroller and pulse control. In **Figure 6,** the sequential formation of uniform droplets is observed for the 0.1% concentration BCEG gel, which has the lowest viscosity among the samples used in this study. The higher the concentration of the gel, the more difficult it becomes to achieve an ideal droplet formation.

As presented in another study[1] non-ideal droplet formation is frequently observed. With the incorrect droplet dispensing formation, the accuracy and precision of the drop fall is compromised, so the drops will not stack or align with the previous droplet, resulting in inaccurate geometry. During the dispensing process, the polymer's micro fibrils are partially ejected through the nozzle attaching to the tip, which disturbs the trajectory and formation of the drops. This was clearly observed when dispensing the hydrogel in 1% concentration as the accumulation of particles in the nozzle is directly proportional to the viscosity of the polymer solutions. The viscosity and the fibrous particle size, as well as the accumulation in the nozzle tip, are also responsible for the disturbance and can be observed in **Figure 7**. Wetting of the nozzle tip is another problem that commonly occurs while inkjet printing, since the back pressure must be carefully adjusted before dispensing, contributing to this issue.

Based on the collected data, the droplet formation is relatable to its rheological properties; the higher the viscosity of the hydrogel, the more difficult it is to dispense it[3]. The hydrogel in the concentration of 0.5% presented very inconsistent droplet formation and the ideal formation was not observed in the concentration of 1% either. The polymer micro fibrils tended to accumulate in

the tip of the dispensing nozzle, making it more difficult to dispense the material. In addition to that, the fibrils passing through the nozzle changed the shape of the droplets. The SEM and optical imaging confirms the assumption that the fibrils are elongated and tangled. Analyzing the viscosity of the three concentrations shows a much higher value for the 0.5% and 1% concentrations, when compared to the 0.1%. This information alone can justify the DOD behavior and uniform formation when dispensing the polymer in lower concentrations which has a much lower viscosity and fluidity.

5. Conclusion and Future Work

Knowing the rheological properties and inkjetting behavior of the BCEG polymer was crucial in order to fully understand and design an effective dispensing mechanism to fabricate 3D structures for medical applications. With a uniform and on-demand dispensing system, a repeatable and scalable automated system could be designed and constructed to fabricate scaffolds.

Unfortunately, the inkjet Drop on Demand system might not be the best alternative to disperse the biopolymer gel, due to its high viscosity variation. The size of the fibrous microstructure and the difficulty of configuring the inkjet parameters for each different concentration also complicated the process in which ideal dispensing was only reliably achieved when dispensing the polymer at 0.1%. A pneumatic extrusion method shall be tested and validated, due to its better handling of gels in several viscosities and the fact that it is easier to control the dispensing parameters and exchange dispensing tips. A study of the relation between the biopolymer concentration and tissue regeneration is in progress and will be published later this year. That will be crucial to determine the ideal gel concentration for dispersion, based on cell viability, organism assimilation and regeneration factors.

6. Acknowledgements

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7. References

1. Xu, Changxue, Meng Zhang, Yong Huang, Amod Ogale, Jianzhong Fu, and Roger R Markwald. "Study of Droplet Formation Process During Drop-on-demand Inkjetting of Living Cell-laden Bioink." Langmuir : The ACS Journal of Surfaces and Colloids, 30.30 (2014): 9130.
2. Murphy, Sean V, and Anthony Atala. "3D Bioprinting of Tissues and Organs." Nature Biotechnology, 32.8 (2014): 773
3. Liu, Yuchun, Jing Lim, and Swee-Hin Teoh. "Review: Development of Clinically Relevant Scaffolds for Vascularised Bone Tissue Engineering." Biotechnology Advances, 31.5 (2013): 688-705.
4. Paterson-Beedle, M, J.F Kennedy, F.A.D Melo, L.L Lloyd, and V Medeiros. "A Cellulosic Exopolysaccharide Produced from Sugarcane Molasses by a Zoogloea Sp." Carbohydrate Polymers, 42.4 (2000): 375-383.

5. Barros-Marques, Silvio Romero De, Esdras Marques-Lins, Maria Cláudia Sodré De Albuquerque, and José Lamartine De Andrade-Aguiar. "Sugarcane Biopolymer Patch in Femoral Vein Angioplasty on Dogs." *Journal of Vascular Surgery* 55.2 (2012): 517-21. Web.
6. Cordeiro-Barbosa, Francisco de Assis, José Lamartine de Andrade Aguiar, Mariana Montenegro de Melo Lira, Nicodemos Teles de Pontes Filho, and Sidcley Bernardino-Araújo. "Use of a Gel Biopolymer for the Treatment of Eviscerated Eyes: Experimental Model in Rabbits." Arquivos Brasileiros De Oftalmologia, 75.4 (2012): 267-272
7. Martins, Ana Gabriela Santos, Salvador Vilar Correia Lima, Luiz Alberto Pereira de Araújo, Fábio de Oliveira Vilar, and Niedson Thiago Pereira Cavalcante. "A Wet Dressing for Hypospadias Surgery." International Braz J Urol : Official Journal of the Brazilian Society of Urology, 39.3 (2013): 408
8. Silveira, Fábio Coelho Alves, Flávia Cristina Morone Pinto, Sílvio Da Silva Caldas Neto, Mariana De Carvalho Leal, Jéssica Cesário, and José Lamartine De Andrade Aguiar. "Treatment of Tympanic Membrane Perforation Using Bacterial Cellulose: A Randomized Controlled Trial." Brazilian Journal of Otorhinolaryngology 82.2 (2016): 203-08. Web
9. Silveira, Fábio Coelho Alves, Pinto, Flávia Cristina Morone, Caldas Neto, Sílvio da Silva, Leal, Mariana de Carvalho, Cesário, Jéssica, & Aguiar, José Lamartine de Andrade. (2016). Tratamento do tímpano perfurado com enxerto de celulose bacteriana: ensaio clínico controlado e randomizado. Brazilian Journal of Otorhinolaryngology, 82(2), 203-208. https://dx.doi.org/10.1016/j.bjorl.2015.03.015
10. Marques, Silvio Romero de Barros, Lins, Esdras Marques, Aguiar, José Lamartine de Andrade, Albuquerque, Maria Claudia Sodré, Rossiter, Renata de Oliveira, Montenegro, Luciano Tavares, & Vieira, Roberto José. (2007). A new vascular substitute: femoral artery angioplasty in dogs using sugarcane biopolymer membrane patch - hemodynamic and histopathologic evaluation. Jornal Vascular Brasileiro, 6(4), 309-315. https://dx.doi.org/10.1590/S1677-54492007000400003
11. Ng, Wei Long, Wai Yee Yeong, & May Win Naing. "Polyelectrolyte gelatin-chitosan hydrogel optimized for 3D bioprinting in skin tissue engineering." International Journal of Bioprinting [Online], 2.1 (2016): n. pag. Web. 31 Aug. 2016
12. Chang, Jung Yun, Yu-Kyoung Oh, Han-Gon Choi, Yang Bae Kim, and Chong-Kook Kim. "Rheological Evaluation of Thermosensitive and Mucoadhesive Vaginal Gels in Physiological Conditions." *International Journal of Pharmaceutics* 241.1 (2002): 155-63. Web.