3D Bioprinting and Characterization of Bioinks with Different Concentrations of Hyaluronic Acid Methacrylate (AHMA)

Caio Athayde de Oliva[1*], Arthur João Reis Lima Rodovalho[2], Leonardo Santana Ramos Oliveira[3], Lucca Ribeiro Alves[3], Willams Teles Barbosa[2], Ana Paula Bispo Gonçalves[4], Jaqueline Leite Vieira[4], Paulo Romano Cruz Correia[2], Milena Botelho Pereira Soares[4], Josiane Dantas Viana Barbosa[2]

[1]Graduating in Mechanical Engineering; Scientific Initiation - CNPQ; [2]SENAI CIMATEC University Center; [3]Bahiana School of Medicine and Public Health; [4]Gonçalo Moniz Institute, Oswaldo Cruz Foundation, FIOCRUZ; Salvador, Bahia, Brazil

The 3D Bioprinting technique for tissue engineering has been constantly developed and generating different results due to the range of applicability of the technique. It is necessary to study and analyze matrices to use this technology, as an example of hydrogels, which are viable for use in conjunction with cells to create the bioink used in bioprinting, verifying their physicochemical properties, cellular reviews, and properties mechanics for the desired application. Based on this, this work aims to analyze these properties, focusing on the hyaluronic acid (HA) matrix in different concentrations during the manufacture of bioink for 3D bioprinting for future application in tissue engineering.

Keywords: Hyaluronic Acid. Bioprinting. Bioink. Tissue Engineering.

Introduction

In tissue engineering, a technique that has been gaining prominence with its recent advances and several studies is the implementation of 3D bioprinting [1]. This technology allows variation of parameters for the most diverse applications in health and regeneration and tissue mimicry with the help of hydrogels capable of simulating a viable environment for cells that can differentiate into living tissue. This technique generates products that must undergo chemical, mechanical, and biological studies and analyses to verify various aspects to find out whether the application of a certain hydrogel with cells (called bioink) is compatible with the tissue that is desired to be regenerated or mimic so that both the correct parameters and the correct raw material are used to produce the hydrogel and bioink [2].

In tissue engineering, a technique that has been gaining prominence with its recent advances and several studies is the implementation of 3D bioprinting [1]. This technology allows variation in the production parameters of scaffolds, structures used for cell growth, and the most diverse applications in health and regenerative medicine. The molds produced using this technique aim to mimic tissues, using thermoplastic biopolymers and hydrogels capable of simulating the cells' natural environment so that they can later even differentiate into tissue similar to that *in vivo*. The materials used to synthesize the products of this technique must undergo physical-chemical, mechanical, and biological studies and analyses to verify various aspects to know whether the application of a given biomaterial is compatible with the tissue desired to regenerate or mimic. Furthermore, in the case of hydrogels used for printing together with cells (bioinks), a rigorous adjustment of parameters is necessary so that, in the end, a viable scaffold is obtained [2].

Hyaluronic acid (HA) has been widely studied due to its viscoelasticity, ease of gelation, and biological properties for various applications in biomedicine[3]. This study aimed to produce scaffolds with different concentrations of HA by 3D bioprinting to evaluate the printability of these hydrogels, analyzing their physicochemical, mechanical, and biological properties, verifying

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Address for correspondence: Caio Athayde de Oliva. Rua Castro Neves, Edf Diana 394, Apt 1101 - Matatu. Zipcode: 40255-020 Salvador, Bahia, Brazil. E-mail: olivathayde.jr@gmail.com.

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how the concentration of HA interferes with these properties and their capacity to produce scaffolds with viable cells for application in future tissue engineering studies.

Materials and Methods

The method of this work was divided into four stages: preparation of the hydrogel, preparation of the bioink, printing of the scaffolds by 3D bioprinting, and finally, the characterization of the biomaterials.

The hydrogel was prepared using HA dissolved in ultrapure water under constant stirring until complete dissolution. Then, methacrylate was added to the solution, and its pH was adjusted to 8 by dripping a 5 M NaOH solution. With the pH adjusted, the solution was left in a shaker, shaken overnight at 4ºC, and removed for washing with ethyl alcohol, using a centrifuge to remove the unreacted methacrylate from the solution. After washing, the precipitate formed was frozen at -80ºC for at least 2 hours and then taken to a freeze dryer for 10 hours to complete the production of HA methacrylate (AHMA).

The lyophilized AHMA was weighed to different concentrations (1.5, 3; 4.5, and 6% w/v) and dissolved in Phosphate-Buffered Saline (PBS) in a sterile environment inside a syringe to create a compatible and viable environment for cells to prepare the bioink. Subsequently, these were suspended in Dulbecco's Modified Eagle Medium (DMEM) mixed with the hydrogels, giving rise to bioinks. 0.05% of the photoinitiator Lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP) was used for each bioink concentration and was then taken to bioprinting to obtain the scaffolds.

The printer used in this study was the Octopus from 3D Biotechnology Solutions (3DBS) company. Cylindrical scaffolds measuring 13 mm in diameter, 4 mm in height, and 80% filled were printed. These were printed on Petri dishes and sterile 6-well plates through a 0.7 mm needle and exposed to UV light for 5 minutes for photocuring. After the end of UV light exposure, the scaffolds were subjected to mechanical compression rheological and biological testing.

For mechanical analysis, the behavior of the stress x strain curve of each scaffold at different concentrations was analyzed using a Brookfield CT3 texturometer to evaluate how increasing the concentration of AHMA could change this parameter. This test used a 1000g load cell and 0.01 mm/s speed. The rheological analysis was carried out using a Haake Rheotest rheometer (Medingen 2.1) to check the viscosity of the bioink with the variation in its concentration using the Ostwald-de Waele model. For biological analysis, 5x104 cells/mL GFP murine stem cells were analyzed by microscopy 10 days after printing.

Results and Discussion

Figure 1 shows the stress x strain curve obtained by the compression test for each printed scaffold. The increase in tension required to rupture the scaffold is notable with the increase in concentration of each AHMA made. Therefore, the 6% AHMA concentration was the one that presented the highest stress value due to the increased HA content in the formulation. These curves are characterized by a linear elastic and plastic region caused by an increase in tension without a significant increase in deformation.

Figure 2 shows the viscosity x shear rate curves of the different formulations studied. It is noted that the higher the concentration, the more viscous the hydrogel tends to become, maintaining its shear rate similar for all concentrations.

Figure 3 shows the cells (light points) distributed in the bioink, demonstrating its viability for concentrations of 1.5 to 6% as bioinks for application in 3D bioprinting.

Final Considerations

The results generated were favorable for future studies on the viability and applicability of AHMA in tissue engineering due to the increase in compressive strength and cell viability at higher concentrations, as shown in the results. From

Figure 1. Stress x strain curves of scaffolds with different concentrations of AHMA.

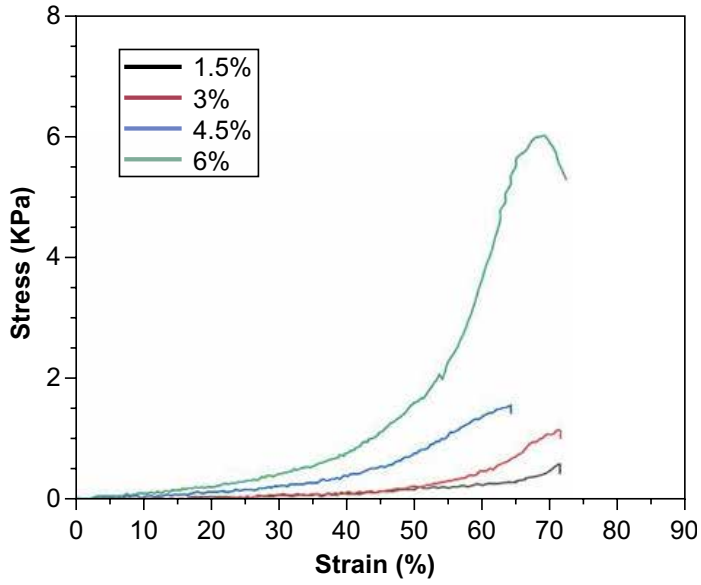


Figure 2. Viscosity x shear rate curves of hydrogels with different concentrations of AHMA.

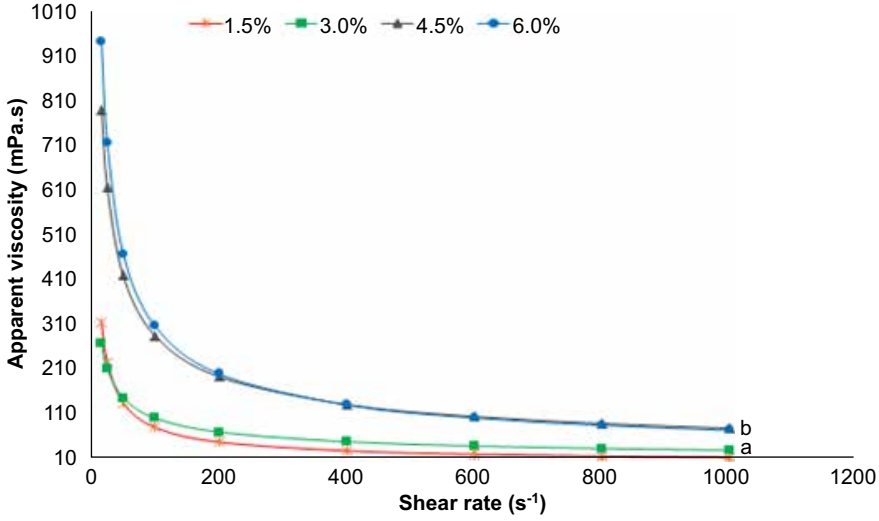
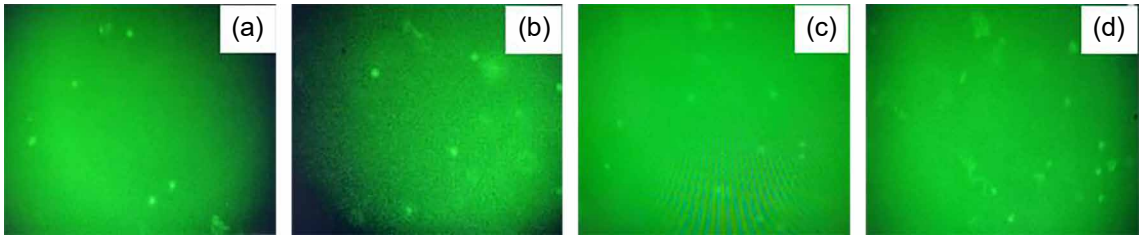


Figure 3. Visualization of L929 within AHMA at concentrations 1.5% (a), 3% (b), 4.5% (c) and 6% (d).



this, it is possible to extend the level of the study further, increasing both the number of days for the biological test and the concentration to find a viability limit that increases mechanical properties but only makes cell growth feasible within bioink.

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