Nanochem Res 5(2): 120-127, Summer and Autumn 2020

RESEARCH PAPER

An Artificial Soft Tissue Made of Nano-Alginate Polymer Using Bioxfab 3D Bioprinter for Treatment of Injuries

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ARTICLE INFO

Article History:
Received 03 July 2020
Accepted 28 September 2020
Published 15 October 2020

Keywords:
Biological Properties
Bioprinter
Cardiovascular Obstruction
Tissue Engineering

ABSTRACT

Some pulsed tissues are replaced with non-pulsed damaged tissues that may endanger the heart function after a heart attack. The restoration is performed by a patch tissue to repair defective tissues. It is supposed to attach to the outside of the heart and connect to the wounded area. The patch is made of a conductive polymer on which a separate electrical polymer called "alginate" through a process called 3D bioprinter was fabricated. The mechanism of the prepared patch for biological and cell behavior needs to be investigated. Besides, we explain the results of the combination of these polymers with natural and synthetic polymer composites. As a natural and biological soft patch for cardiovascular disease (CVD), the adhesion of cells to patch is more efficient and important. In this study, we used a novel technique to print sodium alginate for CVD problems with a soft hydrogel patch loaded by a restorative drug. The mechanical and biological properties and severity of degradability of the patch can be controlled using a specific polymer. In other words, by producing soft tissue patches, researchers and clinical surgeons can obtain more desirable properties made of natural and synthetic polymer composites for the treatment of heart disease. In this study, four CVD patches are fabricated using 3D bioprinter X4bioFab with various amounts of drug on their surfaces containing 2%, 4%, 6%, and 8%. The obtained values for mechanical and biological performance present proper features for the sample containing 6% drug. The results indicated that the prepared patch can be a suitable candidate for heart disease with sufficient cell attachment after a while.

How to cite this article
Salimi K., Eghbali S., Jasemi A., Shokrani Foroushani R., Joneidi Yekta H., Latifi M., Saber-Samandari S., Khandan A.S.. An Artificial Soft Tissue Made of Nano-Alginate Polymer Using Bioxfab 3D Bioprinter for Treatment of Injuries . Nanochem Res, 2020; 5(2):120-127. DOI: 10.22036/ncr.2020.02.002

INTRODUCTION

The heart is one of the most important muscular organs of the human body and is considered as one of the strongest ones that delivers oxygen and nutrients to other parts of the body [1-5]. The heartbeats begin during development in the uterus before birth [2-6]. During our lifetime, the heart may suffer from diseases caused by many modifiable risk factors, such as unhealthy diet, smoking, overweight and obesity, inactivity, high

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blood pressure, diabetes, and unfavorably old age [6]. Loss of myocardial tissue may cause irregular heartbeats, heart failure, myocardial disruption, and even sudden death [7-8]. These problems have been treated with coronary bypass surgery, balloon angioplasty and inserting stents, and heart transplants; however, nanotechnology and soft-tissue engineering can easily solve complicated problems using high-technology [9-15]. There are some challenges in cardiac tissue engineering including cell adhesion and alignment, electrical impulses, supplying arteries, the thickness of cardiac structures, regular cardiac cycles, and tissue integration [16-21]. Various types of scaffold-based three-dimensional structures have been studied by researchers. They inserted/injected iPSC-CM or cardiac sample cells into the prefabricated three-dimensional scaffolds [21-28]. As shown previously, the hiPSCs are derived from cardiac fibroblasts which are better than skin fibroblasts, due to their effectiveness in treating myocardial damages. Moreover, cardiac fibroblasts have more access to Ca^{2+} ions, which is a crucial cation for myocardial contraction [29-35]. Recently, genetic engineers, biologists, and soft-tissue engineers have developed a type of polymer patch that can pick up electrical signals from surrounding cells and transmit those signals between wounded slits, contract, and expand with the heart which all are crucial for cardiac muscle functions [36-41]. Patches are automatically glued after printing and can be used for cardiac disease. Experimental studies on the arteries of mice revealed that these patches can work efficiently after being implanted/transplanted in the myocardium [42-57]. This study aimed to investigate and create an artificial patch for the damaged myocardial tissues made with the 3-D bioprinter that can be attached to the outer layer of the cardiac tissue. We aimed to create a patch that can detect atherosclerotic plaques, is able to deliver therapeutic biomolecules to the site of blocked arteries, and eliminate or decrease coronary atherosclerotic plaques.

MATERIALS AND METHODS

The 3D printed path was fabricated by OMID-AFARINAN company with a highly printable hydrogel and created a suitable environment similar to the extracellular matrix for cell growth and differentiation [2, 23]. To print this sample, the BIOFABX2 3D printer was used with two printing modules that allow the printing of a variety of biological and cellular materials simultaneously. To monitor the morphology of the patch, the scanning electron microscopy (SEM) was used. The alginate polymer (bioink) was prepared according to the protocol explained by OMID-AFARINAN company. Fig. 1 shows schematically how the designed bioprinted patch is implemented to the outer layer of the heart. Fig. 2 shows the preparation process of the patch for treating the cardiac scars after cardiovascular disease (CVD). The following patch could be evaluated by its biological and mechanical properties in a biological environment such as phosphate saline for several days. The drug was purchased from the Merck Company and dissolved in the distilled water and stirred for 4 h using a magnetic stirrer. The tensile strength and elastic modulus were measured using the electronic mechanical machine. The cell growth and cell viability of the bioprinted patch were investigated after three days of incubation.

RESULTS AND DISCUSSION

The special patch had 4 various drug content. According to the observed tensile strength and biological features, the patch had satisfactory mechanical and biological properties, indicating that that we may use similar products for cardiac applications. During the use of these cells, the number of capillaries in the part of the heart modeled as a cardiac arrest was increased. Fig. 3 shows the tensile strength of the fabricated patch made by the bio3Dprinter BIOFABX2 model. It can be seen that the coated drug on the surface of the patch increased the tensile stress until the third sample. As the amount of drug increases by more than 6%, the tensile strength decreases regarding the amount of drug and stress-strain diagram. Fig. 3 also shows a line chart as an independent variable of the mass of sample. The graph shows the tensile strength value in the range of 34 KPa to 32 KPa acts as a hyperviscoelastic properties. Fig. 4 shows a decreasing trend of the samples' weight after soaking for three days in PBS. The graphs show that as the sample coating amount increases, the weight loss decreases, that is corresponding to the functional group of coated drugs [11-27]. Fig. 5 (a-b) illustrates the morphology of the printed patch with SEM. The porous sizes ranged from 200 to 300 microns. The shape of the porosity is cubic that enables cardiac stem cells to enter the holes and regenerate the defective tissue. Fig. 6 indicates the MTT assay of the sample incubated

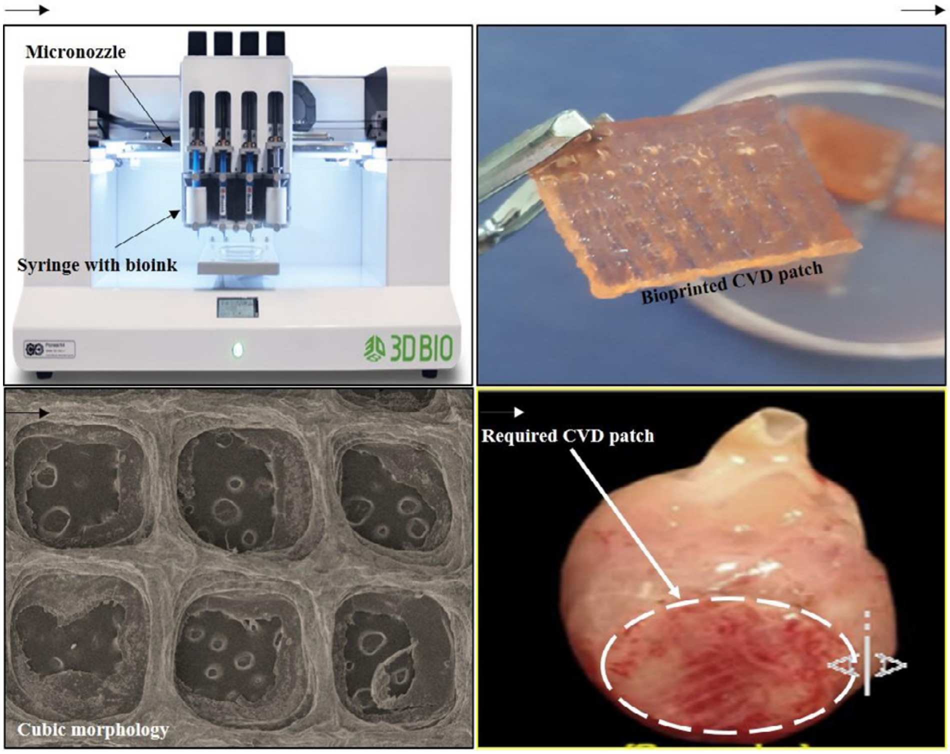

Fig. 1. BioXfab 3D bioprinter machine, fabricated 3D patch, SEM images of the fabricated patch, and application of the prepared patch for the cardiac application using alginate hydrogels and hyaluronic acid

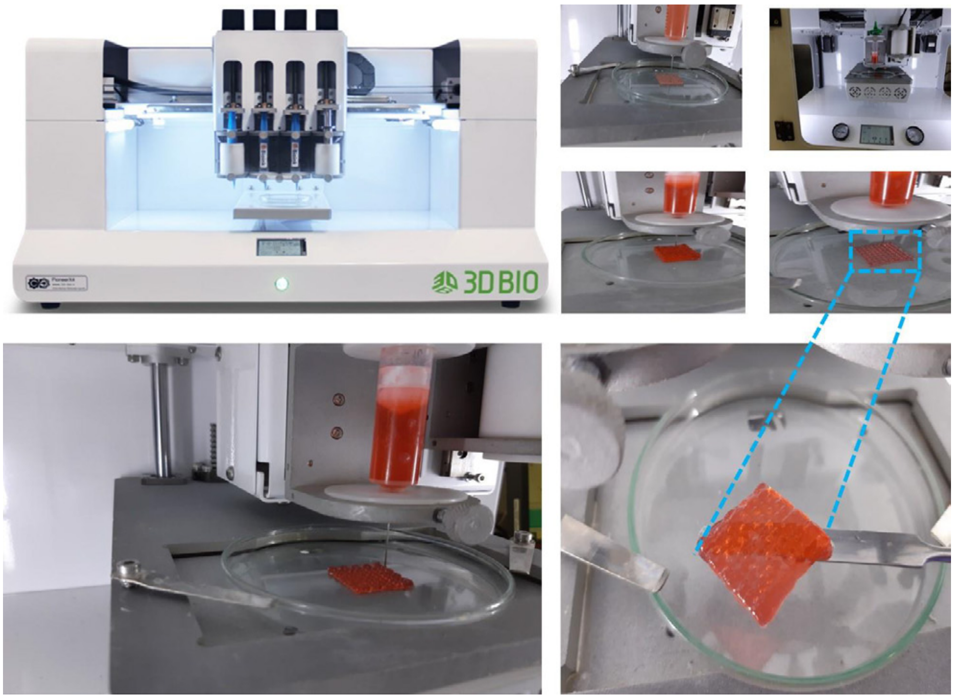

Fig. 2. Schematic of the preparation of polymeric filler for CVD application using the bioprinter

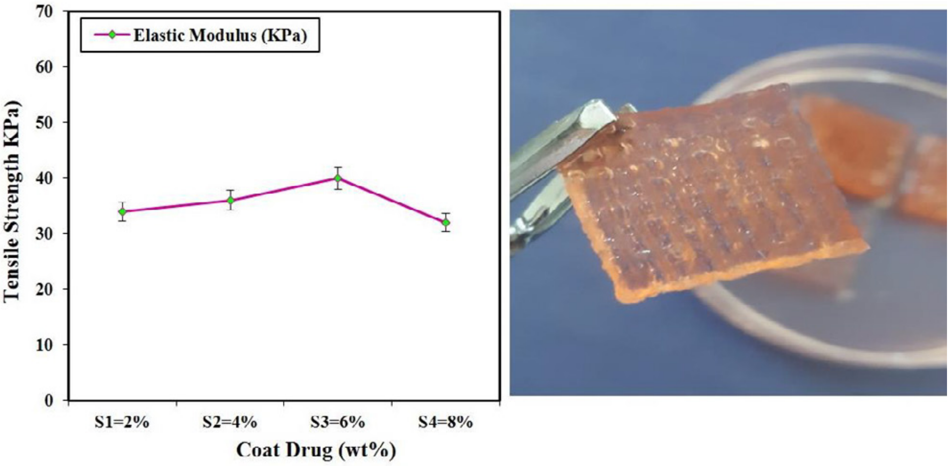

Fig. 3. Tensile strength of polymeric patch for CVD application using the bioprinter

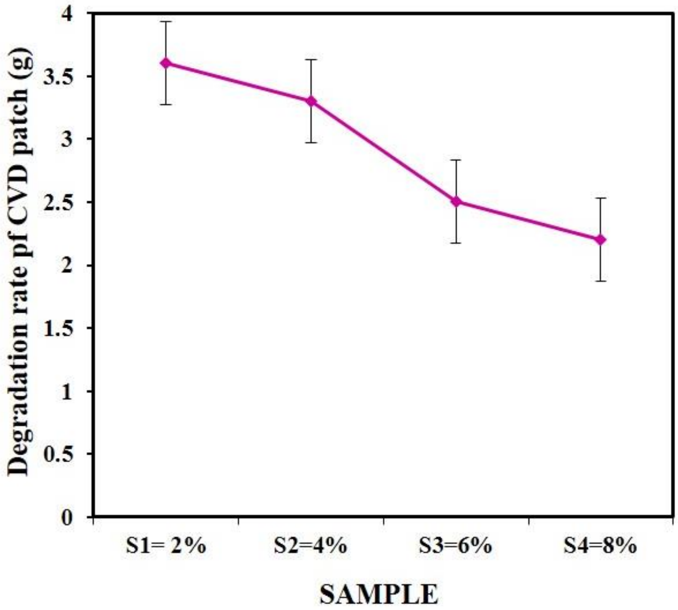

Fig. 4. Amount of degradation and weight loss of the four samples in the phosphate buffer saline after three days of soaking

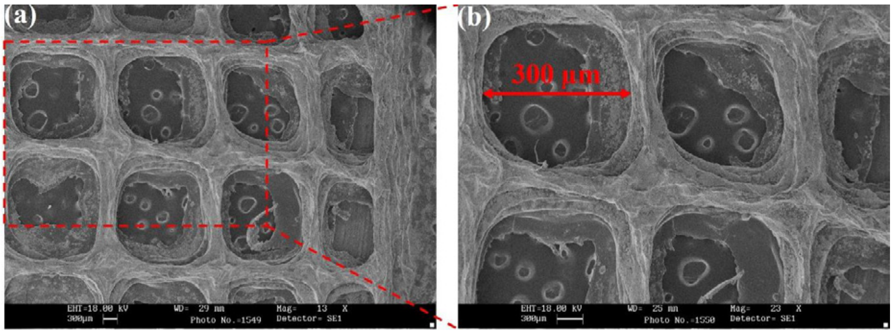

Fig. 5. SEM images of (a) bioprinted patch with cubic shape, and (b) magnified patch with cubic shape

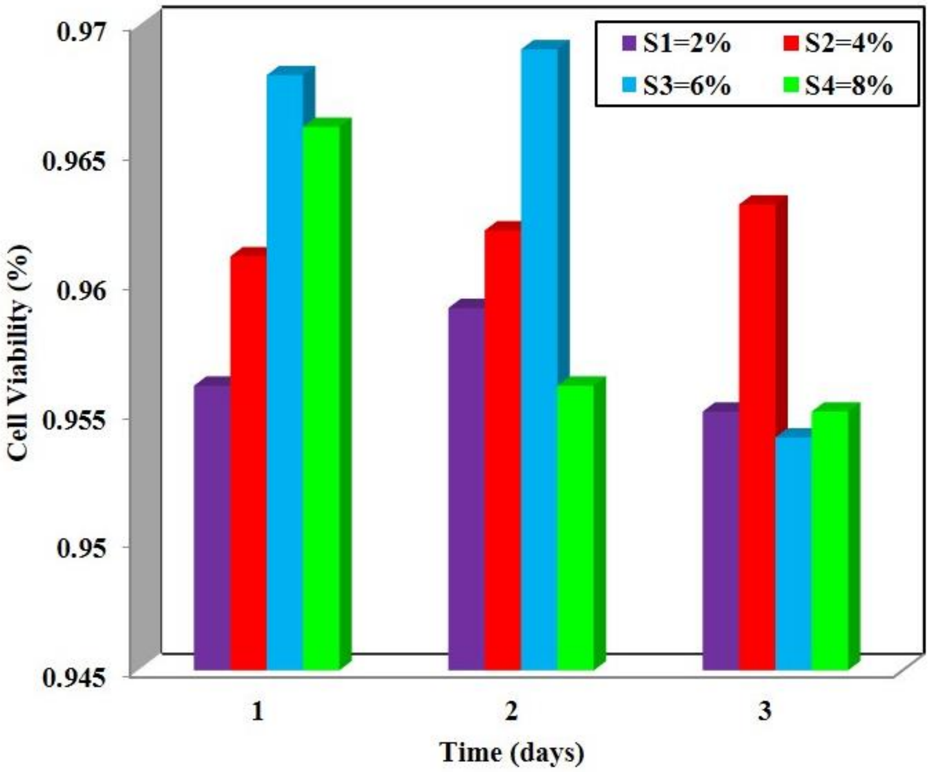

Fig. 6. MTT assay result of the strength of polymeric patch for CVD application using bioprinter

Table 1: Tensile strength, degradation rate, and MTT assay of the bioprinted patch for CVD disease.

Sample Name	Tensile strength (KPa)	Degradation rate (%)	MTT assay
Sample 1	34	3.6	0.998
Sample 2	36	3.3	0.986
Sample 3	40	2.5	0.998
Sample 4	32	2.2	0.984

for three days in the cell culture medium. The obtained results indicated that the sample with 6% coated drug have a proper and sufficient chemical and biological response compared to the other specimens. The heart patch is an important agenda in cardiovascular failure regarding the myocardial infarction that several researchers have worked on that [28-38]. The mechanical calculations show the micromechanical properties of the patches with and without cells. The mechanical and biological values are presented in Table 1. Based on the results, the effective elastic modulus is increased and the overall mechanical properties are relatively improved. This increase in mechanical properties may cause cardiac tissue dysfunction and also may lead to patient death [39-42]. In this study, a patch for soft-tissue implementation was fabricated using BioFabX4. Alginate polymer was used as a water-soluble material with potential modification on its crosslinking procedure. The samples were coated with 2%, 4%, 6%, and 8% of the drug to determine the effect of the drug on sample degradation and mechanical performance. The drug and other elements were used to enhance the mechanical properties and biodegradability of the final products. Recently, the 3D printer has been used to enhance the treatment of soft tissue using the new generation of biomimetic materials to complete the regeneration approaches [27, 43-49]. The physical and mechanical properties of the printed patches need to be investigated. Cardiac stem cells are also extracted from heart tissue as multipotent stem cells. Due to their cardiac origin, the possibility of mechanical and electrical compatibility with surrounding cells is high. These cells can produce myocytes, endothelial cells, and smooth muscle in the extracorporeal environment. The low production efficiency and sensitive sampling method are among the limitations in using these typical cells.

CONCLUSION

The mechanical performance of the designed patch was improved in a sample containing 6 wt% drug, while the sample with 8 wt% drug may have a downward trend compared to the pure sample. The degradation of the patch decreases with the addition of the drug to the bioink after soaking for three days in the PBS solution. Regarding the morphological behavior of the bioprinted patch, it has a cubic shape with 300-micron pores and a homogenized shape. The main advantages of using these biopolymers bioink are that their porosity, density, structure, and composition can be controlled and they can be designed for cells and various cardiac applications.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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