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Research Paper

AN ARTIFICIAL SOFT TISSUE MADE OF NANO-ALGINATE POLYMER USING BIOXFAB 3D BIOPRINTER FOR TREATMENT OF INJURIES

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INTRODUCTION

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 X_4bioFab 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 sufficientcell attachment after a while.

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 oxygenand 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 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 softtissue 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 Ca2+ 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.

3D Bioprinting (3DBP) technologies open many possibilities for the generation of highly complex cellularized constructs. Nano-biomaterials have been largely used in tissue engineering and regenerative medicine (TERM) for different purposes and functions depending on their intrinsic properties and how they have been presented in the biologic environment. Combination of bioprinting and nano-biomaterials paves the way for unexpected opportunities in the biofabrication scenario, by improving critical weakness of these manufacturing processes while enhancing their efficiency by spatially arranging nano-features. 3D organization of cells is fundamental for a successful design and maturation of native tissues. A critical challenge for the production of biological constructs is to support and guide cell growth toward their natural microenvironment ensuring a harmonious presence of specific biochemical and biophysical cues to directcell behavior. Also, precise arrays of stimuli need to be designed to induce stem cell differentiation toward specific tissues. Introducing nano-sized bioactive material can direct cell fate, playing a role in the differentiation process and leading to the biofabrication of functional structures. Nano-composite bio-ink can be used to generate cell instructive scaffolds or either directly printed with cells.

The nano-scale size allows these materials to be active at the cellular and sub cellular levels, therefore they have been used also for instruct and guide cells, for example CNT array configuration modified osteoblasts orientation (Giannona et al., 2007). Moreover, the hierarchical structure of micro- and nano-environments of biologic tissues can be mimic by use of nano-biomaterials. Typically, the nano hydroxyapatite (nHA) and tricalcium phosphate nano-composite (nTCP) have shown the ability to be manufactured into bone's hierarchical like structure, with relevant microenvironment and mechanical properties (Yang et al., 2011). Furthermore, nano-biomaterials interaction directly with the cell or from the cell's internal

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environment, made them perfect candidate for local drug delivery. Sustained release lowers the systemic drug dose and aims for direct targeting. Specifically, polymer nano-particles (NPs) can be tuned for sustaining release of drugs in a controlled manner (McCarron et al., 2008), but also ceramic NPs, which present complementary physical properties have been used as well in drug delivery (Yang et al., 2010). Additionally, nano-biomaterials can be remotely controlled and stimulate with different energy sources such as near-infrared, ultrasound and magnetic fields (Marino et al., 2018). Therefore, they can act as nano-transducers at the cellular or subcellular levels allowing for remote modulation of cells functions as biochemical signaling pathways (Bonnemay et al., 2015), gene/protein expression (Stanley et al., 2012), electrochemical reactions (Xu et al., 2017), neural firing (Yong et al., 2014), and muscle contraction (Marino et al., 2017).

In **Figure 1** we schematically represent the focus of this work. We review how 3DBP have been used in synergy with nano-technology for applications in TERM, analyzing what are the benefits to combine them for biofabrication strategies. Initially we discuss the cell-laden nano-composite bio-inks used in 3DBP, focusing on the applications of biomolecule release, biomimicking of the extracellular matrix structure, triggering cell response and cell instructive remote control. Next, cell-free nano-composite bio-inks for 3DBP are discussed. Finally, new technological applications in bionic tissues and soft robotics for TERM are introduced.

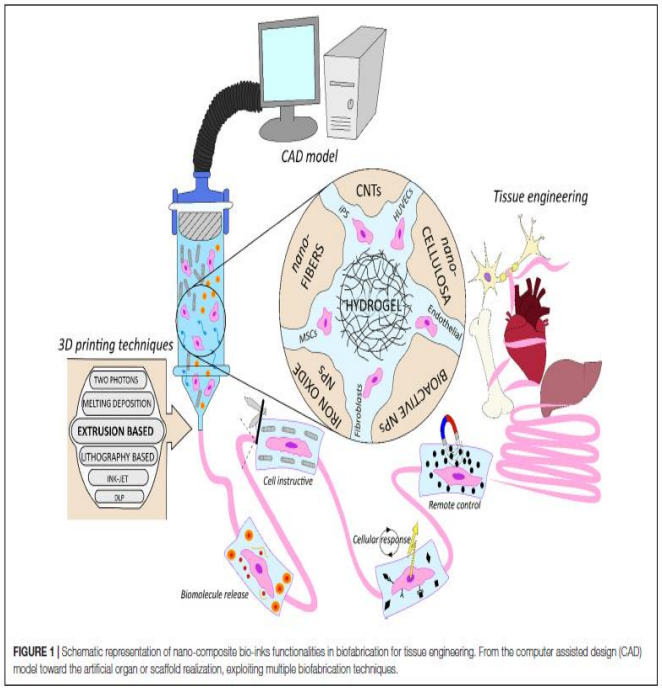

FIGURE 1 | Schematic representation of nano-composite bio-inks functionalities in biofabrication for tissue engineering. From the computer assisted design (CAD) model toward the artificial organ or scaffold realization, exploiting multiple biofabrication techniques.

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MATERIALS AND METHODS

The 3D printed path was fabricated by OMIDAFARINAN 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 ofbiological 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.2.1 shows schematically how the designed bioprinted patch is implemented to the outer layer of the heart. Fig. 2.2 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 forseveral 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.

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.2.3 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

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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.2.3 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. 2.4 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.2.5 (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.2.6 indicates the MTT assay of the sample incubated

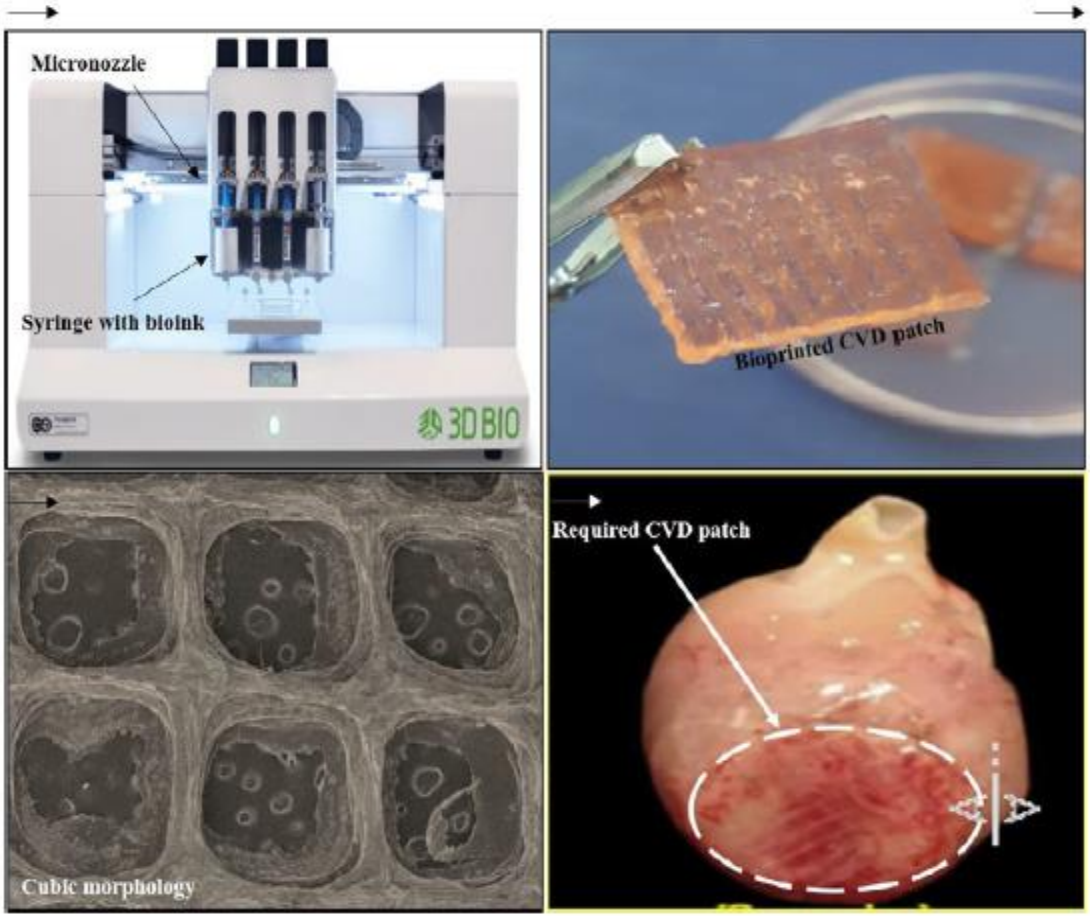

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Fig. 2.1. BioXfab 3D bioprinter machine, fabricated 3D patch, SEM images of the fabricated patch, and application of the prepared patch for the cardiac application using alginate hydrogels and hyaluronic acid

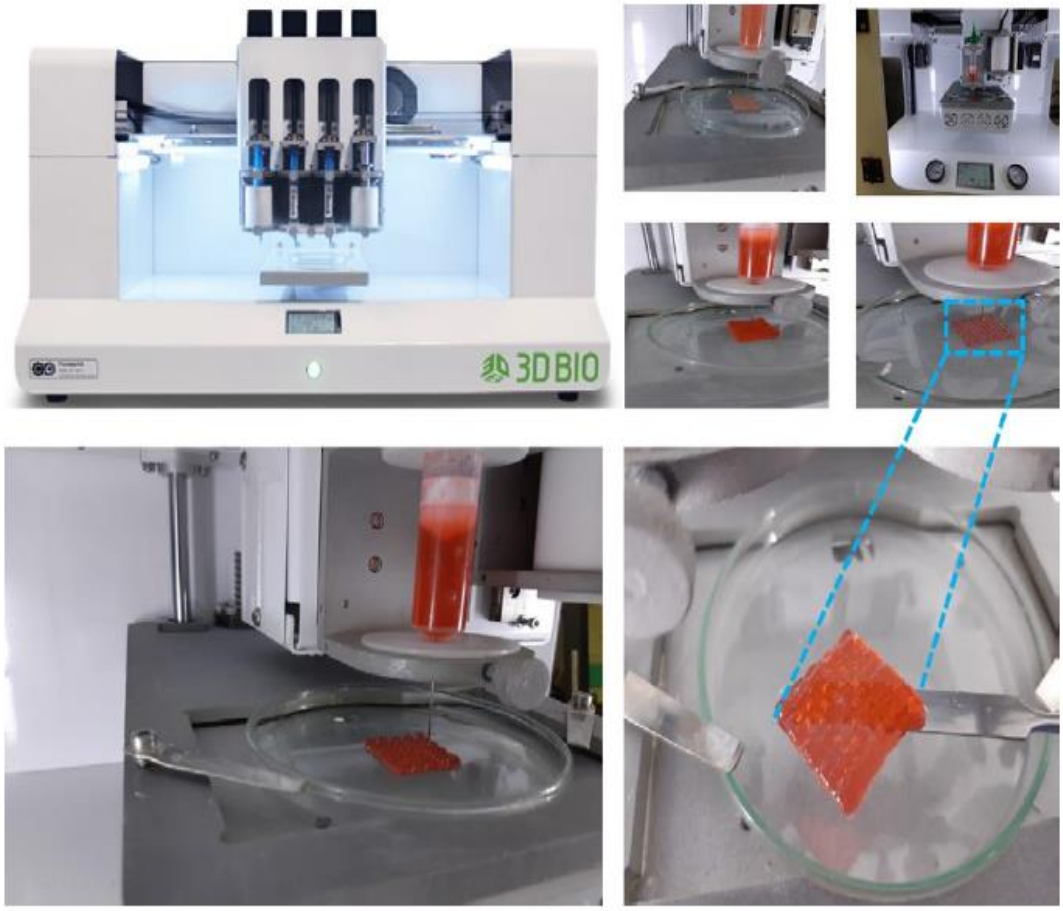

Fig. 2.2. Schematic of the preparation of polymeric filler for CVD application using the bioprinter

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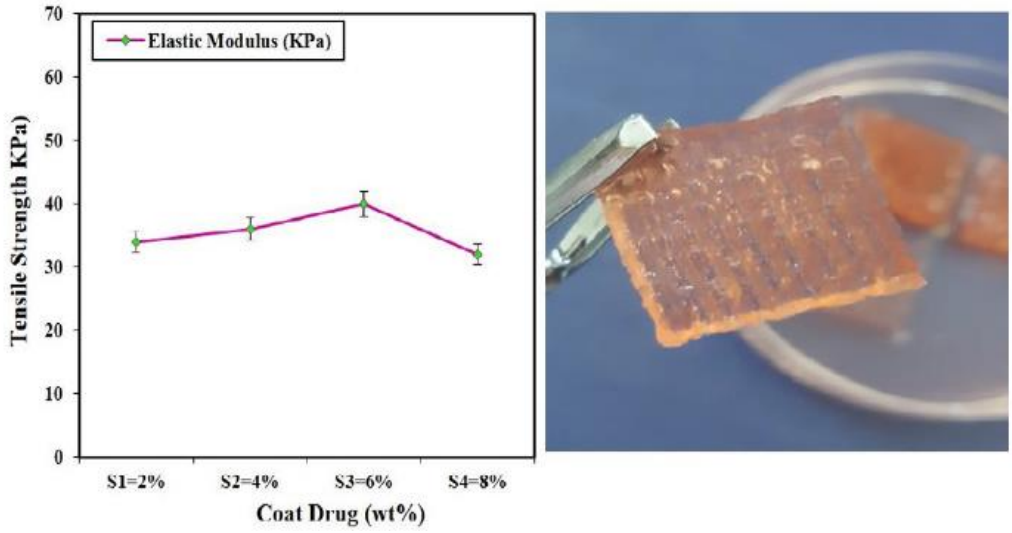

Fig. 2.3. Tensile strength of polymeric patch for CVD application using the bioprinter

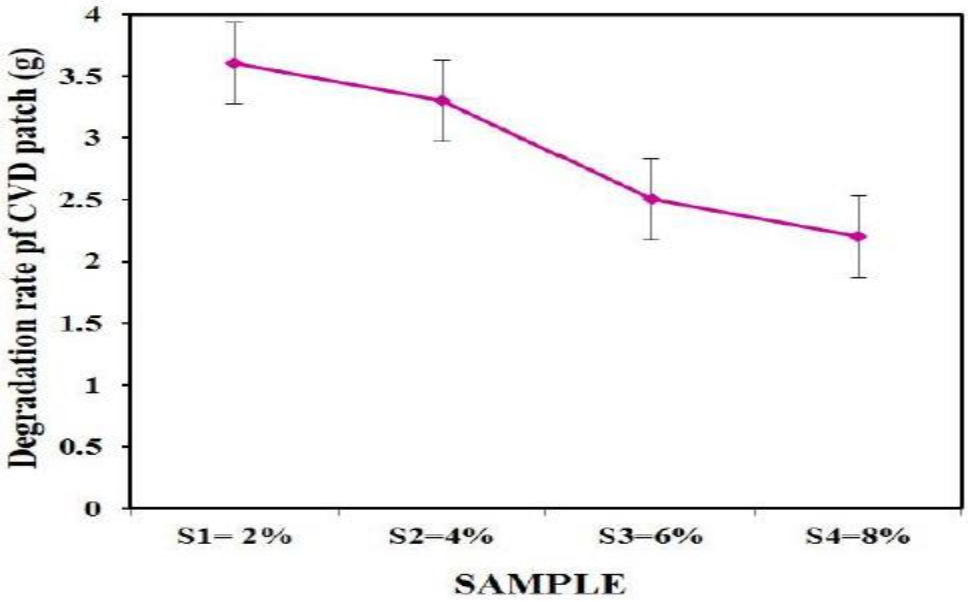

Figure 2.4. Amount of degradation and weight loss of the four samples in the phosphate buffer

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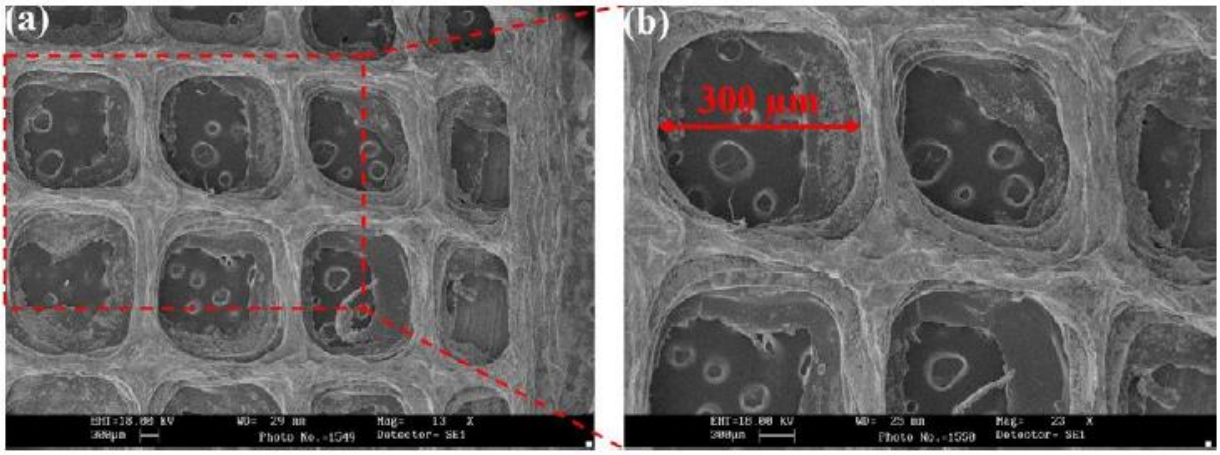

Figure 2.5. SEM images of (a) bioprinted patch with cubic shape, and (b) magnified patch with cubic shape

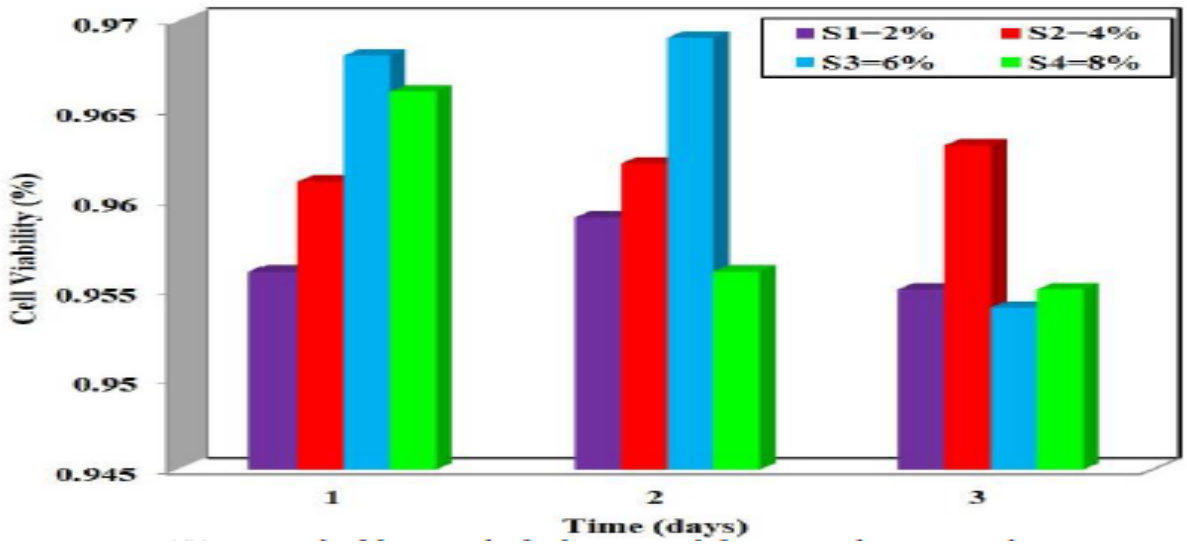

Fig. 2.6. MTT assay result of the strength of polymeric patch for CVD application using bioprinter

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

Table 2.1: Tensile strength, degradation rate, and MTT assay of the bioprinted patch for CVD disease.

Nano-Alginate Polymer Using Bioxfab 3D Bioprinter

Currently available bio-ink formulations are limited due to the requirements of "good" printability (e.g., shear thinning, shape retention). Moreover, high cell viability and long-term function are necessary. Considering the extrusion based 3DBP for instance, Pluronic hydrogels have great printability properties, but cells do not survive and proliferate in the long-term in vitro culture in a Pluronic concentration higher than 5 w:v% (Khattak et al., 2005; Fedorovich et al., 2009). A possible solution was found by nano-structuring these hydrogels to increase their biocompatibility. Pure Pluronic (PF127), combined with diacrylated Pluronic F127 (PF127-DA), creates nano-pores after wash-out of the pure PF127 from the UV-crosslinked structure. Muller et al. combined the new hydrogel formulation with bovine chondrocytes, bioprinted them in a simple structure and cultivated them up to 14 days. Cell viability at the end of the experiment was significantly higher compared to their viability in the bulk Pluronic gel structure, reported to be 86.3%

compared to 61.6% (Müller et al., 2015). Complementary to the creation of nano-pores inside a hydrogel, embedding nanobiomaterials inside the bio-ink allowed for nano-composite bioinks formulation directed to specific achievements in cellular constructs for TERM here reported.

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Nano-Composite Bio-Inks for Biomolecule Release Chemical cues are important for stem cell differentiation,and specific stimuli are particularly necessary in order to induce differentiation in bone or cartilage tissues. Growth factors are commonly provided in tissue engineering because they are responsible for spatial and temporal cell functions. Unfortunately, without protecting these molecules, they experience degradation and quick elimination even though they are administrated in high dose (Chen et al., 2010). This is even more critical when applied within a bio-ink, which process may further decrease stability of fragile proteins. A novel approach introduced by Zhu et al. allows for nano-carriers of transforming growth factor beta 1 (TGF-b1) directly inside the bioprinted cartilage construct, from which TGF-b1 can be sustained released, obtaining a significant improved MSCs chondrogenesis differentiation. In this study stereolithography based 3DBP was used to create the cartilage construct.

A movable head with a UV-source was used to crosslink the square shaped cartilage construct. The bioink was a gelatin methacrylate (GelMA)-based hydrogel combined with TGF-b1 loaded nano-spheres (average size of 120 nm) which where fabricate through a co-axial electrospraying method (Zhu et al., 2015). The nano-carrier's surrounding shell of poly(lactic-co-glycolicacid) (PLGA) slowly degrades and sustains the release of its content (Danhier et al., 2012). The qPCR analysis revealed that the expression levels of collagen II and aggrecan of MSCs laden into the hydrogel increased when the bioink was enriched with TGF-b1 nano-carriers (Zhu et al., 2018). This is a valid strategy for embedding biomolecules into bioprinted constructs. Indeed, with this approach is possible to recapitulate the dynamic presence of biomolecules into the ECM. However, tuning the biomolecule release could require a long optimization time to achieve the desired release profile. This top-down fabrication approach, though, leaves back a not neglectable amount of unused material, which is not optimal if the material is precious or its availability is limited. Envisioning clinical applications, the donor biological material is often a limiting factor. Moreover, the fabrication time could be reduced if the UV-light exposure could be volumetric instead of punctual as with a UV-light laser source.

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Cell Instructive Nano-Composite Bio-Inks for Matrix Properties Enhancement

NANO-COMPOSITE BIO-INKS FOR BIONIC TISSUES AND SOFT ROBOTS

Exploring the synergistic combination of 3DBP and nanotechnology, fabrication of complex biologic structures with active properties is the futuristic application of such technologies. Sensing, moving capabilities and shape modification have been included in biologic constructs. Functional electronic components and biological tissue have been integrated through bioprinting of nano-enriched bio-ink. For example, nanoelectronics materials and cell-laden hydrogels have been deposited together in a model of a human hear. With this knowhow, it is possible to manufacture cyborg tissues which are three-dimensional combination of electronics and engineered tissue. In the example of the bionic hear, the bioprinted construct could receive and transmit radiofrequency sounds. This proof of concept showed the integration of electronic circuits built up with nano-elements in a 3D biological construct. It overtakes the planar flexible electronic devices and sensors previously used, even though now the conductive elements are discrete and not continuous (Mannoor et al., 2013).

A bionic tissue must mimic the native organ's functionality, replacing a lost body function or even improving it. Designed nano-biomaterials surfaces combined with additive manufacturing have shown potential in engineering bioactive devices. For example, natural red blood cell (RBC) membrane have been wrapped onto PLGA nano-particles (RGB-NPs) to mimic natural RBCs with a final dimension of 133 nm in size. Exploiting the natural behavior of RBC membrane, RBC-NPs have the capacity to bind, through a non-specific bonding, hemolytic toxins. In doing so, they can be integrated as an active component in a detoxification bioprinted device, which was developed by embedding RBC-NPs inside a hydrogel for biomimetic detoxification properties close to the liver function.

Following the application of a magnetic field, the IONPs self-assembled in nano-rods which gave magnetic anisotropy to the hydrogel matrix. Indeed, after the fabrication process the luminescent signal have been detected from the scaffold placed

under ˜0.6 mm layer of chicken breast muscle upon excitation at 800 nm. Also, orientation and rotation of the construct along the direction of an applied external magnetic field have been shown to be possible (Augurio et al., 2019).

The excellent biocompatibility and contactless manipulation of this nano-composite hydrogels open the way toward future applications of the synergy nano-technology-bioprinting where soft-robotics devices will be fabricated for minimally invasive endoscopic tools.

3D Bioinks Based on Alginate for Biomedical Applications

Three-dimensional (3D) bioprinting is an appealing and revolutionary manufacturing approach for the accurate placement of biologics, such as living cells and extracellular matrix (ECM) components, in the form of a 3D hierarchical structure to fabricate synthetic multicellular tissues. Many synthetic and natural polymers are applied as cell printing bioinks. One of them, alginate (Alg), is an inexpensive biomaterial that is among the most examined hydrogel materials intended for vascular, cartilage, and bone tissue printing. It has also been studied pertaining to the liver, kidney, and skin, due to its excellent cell response and flexible gelation preparation through divalent ions including calcium. Nevertheless, Alg hydrogels possess certain negative aspects, including weak mechanical characteristics, poor printability, poor structural stability, and poor cell attachment, which may restrict its usage along with the 3D printing approach to prepare artificial tissue. In this review paper, we prepare the accessible materials to be able to encourage and boost new Alg-based bioink formulations with superior characteristics for upcoming purposes in drug delivery systems.

Moreover, the major outcomes are discussed, and the outstanding concerns regarding this area and the scope for upcoming examination are outlined. The body system has restricted functionality regarding regeneration. Recent treatment choices to substitute impaired tissue and organs depend on acquiring tissue through an identical person, or transplantation from cadavers that have been developed very quickly.

However, there are restrictions to these treatments that consist of donor position, its side e_ect, and donor shortage [1,2].

These conditions additionally support the demand regarding biological replacements and the areas of tissue engineering (TE), and thus regenerative medicine is an e_ective route in the direction of regeneration development. Work in the area has developed to generate what we consider to be an innovative area—regenerative technological know-how, described as the a_uence of innovative materials engineering, stem cell technology, and clinical translation concerning the reproduction of sophisticated tissues and body organ devices [1,2]. Numerous approaches are employed in this context; among them, the additive manufacturing (AM) has captivated a lot of consideration. AM or 3D printing is designed to incorporate living cells in 3D biomaterials. This innovative system enables the automated and reproducible generation of 3D well-designed living tissues through depositing layer-by-layer biocompatible materials (typically including biochemicals) with a high-accuracy placing of cells [1–3].

This approach enables the manufacturing of 3D, scalable and accurate geometries, which are generally not provided through other methods including 2D and 3D cell cultures [2–4]. Initially, Charles Hull created 3D printing [5], which he described as “stereolithography”, in the beginning of the 1980s, and fromthen on, this technology has developed into numerous kind methods [5]. All 3D printing techniques provide positive aspects and negatives [5,6]. The kind of 3D printer selected regarding an application typically relies on the components to be employed and precisely how the layers in the completed product are attached. The three most frequently used 3D printer technologies for medical purposes are selective laser sintering (SLS), thermal inkjet (TIJ) printing, and fused deposition modeling (FDM) [6,7]. Biomaterials employed in AM approaches comprising cells, base structure material, and some other necessary components are known as “bioinks”. The bioink particles are multicellular aggregates typically in the form of cylinders made up of cell types dependable with the tissue or organ system to be prepared [8]. A perfect bioink material must have a number of characteristics, including

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ALGINATE AS PRINTABLE MATERIAL

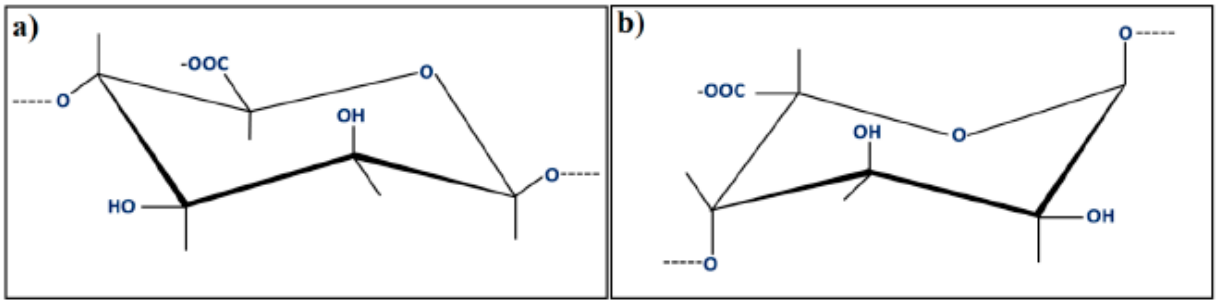

Figure 4.1. Units of the alginate block types: (**a**) β-(1-4)-D-mannuronic acid; (**b**) α-(1-4)-L-guluronic acid [2].

The molecular weight, shown as an average of all the molecules existing in the specimens, of Alg, varies around 33,000–400,000 g/mol. The 1% w/v aqueous Na-Alg solution possesses a dynamic viscosity 20–400 mPa_s at 20 °C. Alternatively, Alg solubility is restricted through the solvent pH (a reduction in pH might result in polymer precipitation), ionic strength, and the number of gelling ions [40,41]. However, the cytocompatibility of Alg is substantially examined in vitro along with in vivo. There still exists an issue concerning the influence of the Alg composition. However, most of this misunderstanding probably pertains to numerous degrees of purity in the Alg examined in several studies. The immunogenic reaction at the injection or implantation sites could be caused by impurities keeping in the Alg. Considering that Alg is acquired through natural resources, several contaminants, including heavy metals and endotoxins, might exist. Significantly, Alg purified through a multi-phase extraction treatment to an extremely high purity did not stimulate any substantial foreign body response as soon as implanted directly into animals [42]. Alg would not induce considerable inflammatory reaction when applied in an in-vivo condition, consisting of the use of bioinks for 3D-printers.

Applications in Biomedical Field

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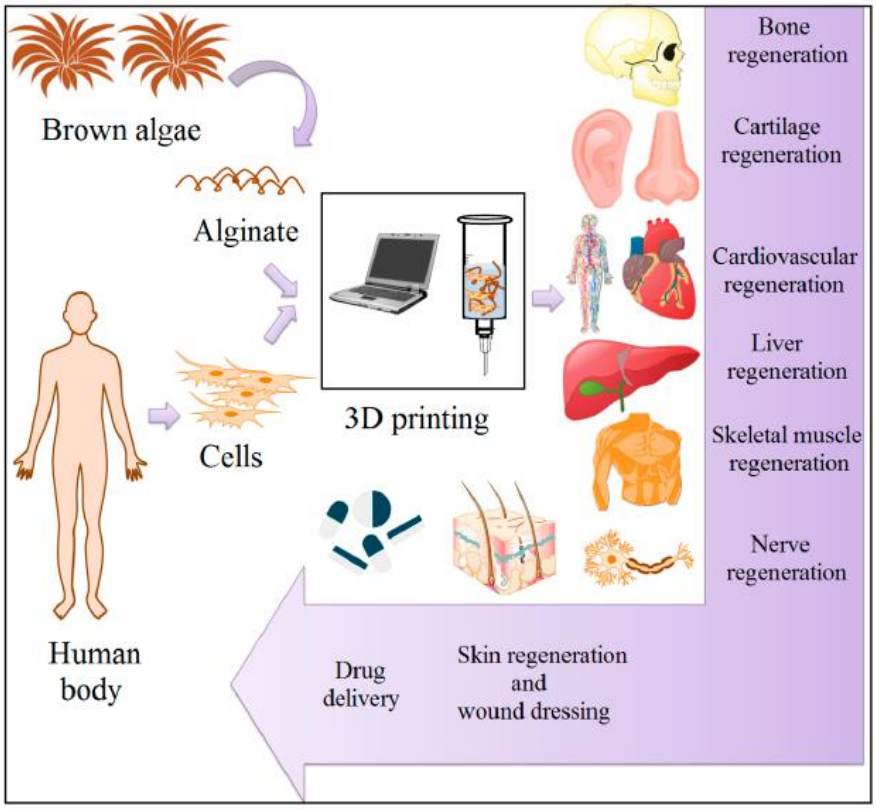

Figure 4.2. Various application of 3D printed alginate constructs in tissue engineering.

Bone Regeneration

Bone defects with small size might be fixed through the self-healing performance of bone tissue (BT), however massive bone defects might merely be fixed via BT transplantation. The current examination methods for restoring impaired bone tissue are bone tissue engineering (BTE) methods, because of the issues encountered in acquiring materials regarding an autogenous bone graft [93,94]. In this investigation, 3D bioprinting approaches were utilized as an appealing approach in the current years [95,96]. Compared to conventional BTE, 3D bioprinting has superb possibilities to create tissues with numerous biomaterials, cells, and bioactive materials in a patient, which are certain disorder forms [97]. Nevertheless, BT bioprinting requires inks with appropriate viscosity, mechanical performance, and apatite formation capacity to enhance

bioactivity and create chemical bonds with adjacent BT following implantation [98,99]. One of the desirable approaches for producing Alg-based inks is encapsulated with bioceramics into a polymeric matrix. An in vivo experiment was conducted by Wang et al. [98] regarding printed sodium Alg/Gel/hASCs (AG construct) and sodium Alg/Gel/nHAp/hASCs (AGH construct) and attained constructs implanted in mice for eight weeks. To evaluate bone formation ability, micro-CT scans were carried out. Outcomes pointed out that the no-cells-encapsulated AG construct and no-cells-encapsulated AGH construct had been reasonably loose. Furthermore, the hydrogel-based constructs pointed out the soft and brittle structure that made the constructs hard to maintain the following implantation. The AG and AGH construct encapsulated with hASCs cells kept the initial shape, with minor alterations in appearance; the structure was reasonably tough, and cell growth was noticeable via the pores (Figure c) [98]. Furthermore, osteogenic di_erentiation of AG and AGH construct encapsulated with hASCs cells exhibited that the presence of nHAp causes enhancement of osteogenic di_erentiation of the AGH constructs in vitro and in vivo experiments, which making it suitable for BTE applications.

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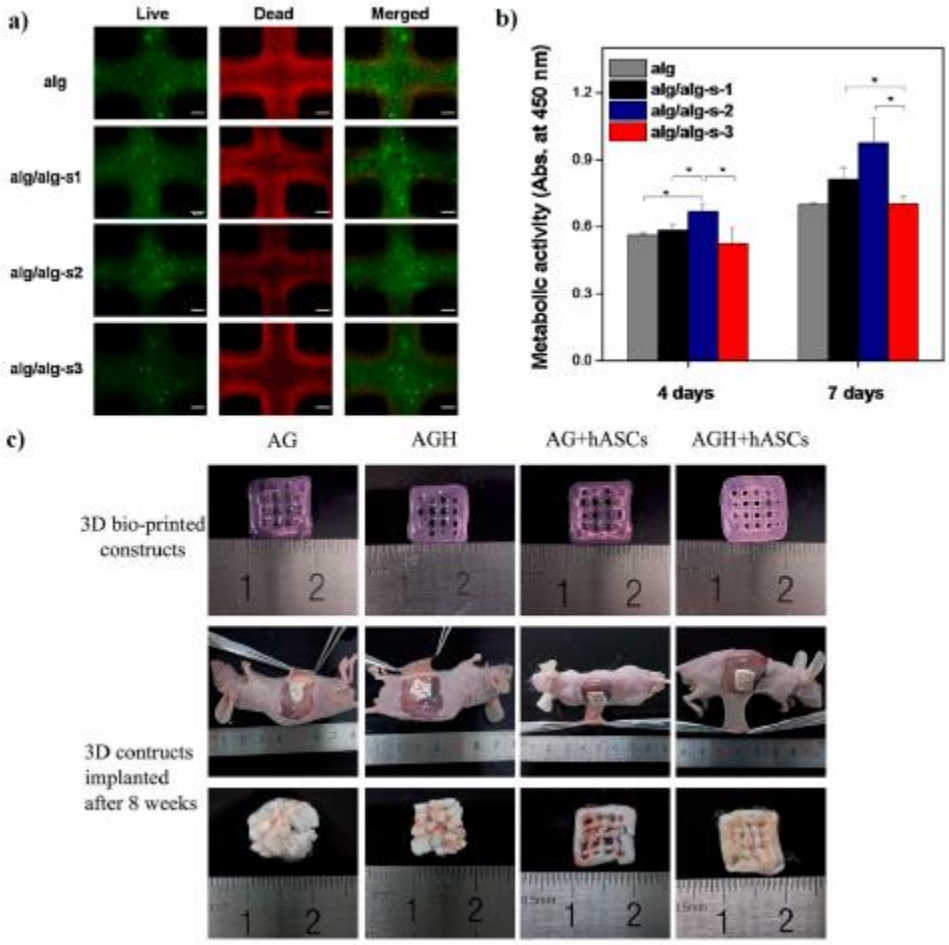

Figure 4.3. Osteoblasts viability and metabolic activity in 3D printed Al/Al-sulfate bio-inks: (**a**) fluorescence images of live/dead; (**b**) 3D-printed cells metabolic activities in hydrogels at day 7 of incubation [102]; (**c**) morphological analysis before and after implantations.

Cartilage Regeneration

Articular cartilage disorders have restricted e_ciency for self-reproduction and recovery. Cartilage injuries frequently lead to discomfort and reduced performance for the a_ected person and usually hasten the progress of osteoarthritis in the joint. 3D bioprinting might provide treatment solution options which may possibly conquer the restrictions of current management options, including the creation of fibrocartilage, donor

site morbidity, hypertrophy of implant. The mixture of cells, natural-based materials, and biochemical components could present the opportunity of true cartilage reproduction [106]. In this perspective, Alg is extensively used in cartilage 3D bioprinting. Markstedt et al. [107] evaluated a shear thinning bioink of NFC encapsulated Alg with the rapid cross-linking capacity regarding the 3D bioprinting of TE. Their 3D product with a small cross-linked grid working as a rigid gel is shown in Figure 4.3A–C [107]. In Markstedt et al. [107] investigated the shapes similar to cartilage tissues, including an ear and a meniscus had been e_ectively printed, Figure 4.3D–F. Actually, with printing periods of as much as 20 min for these larger sized constructs, the printed products lose their form throughout the printing procedure because of the ink's viscosity. It appears that their ink seemed to be among the appealing materials regarding cartilage tissue printing in complicated shapes in the appropriate manufacture period [107]. Muller et al. [108] fabricated Alg sulfate-based bioink loaded with nanocellulose. They revealed that when the bioink was basically printed, the biological response of the cells was extremely influenced by the nozzle dimensions and shape. Cell proliferation and growth were preserved along with the lowest extrusion pressure and shear stress. Nevertheless, extruding the Alg sulfate/nanocellulose bioink and chondrocytes considerably a_ected cell viability, especially if employing nozzles and valves with a small diameter. For this reason, the choice of needle shape and bioink requires tuning the factors for great printing quality and great cell viability, in addition to cell growth, structure and matrix deposition [108].

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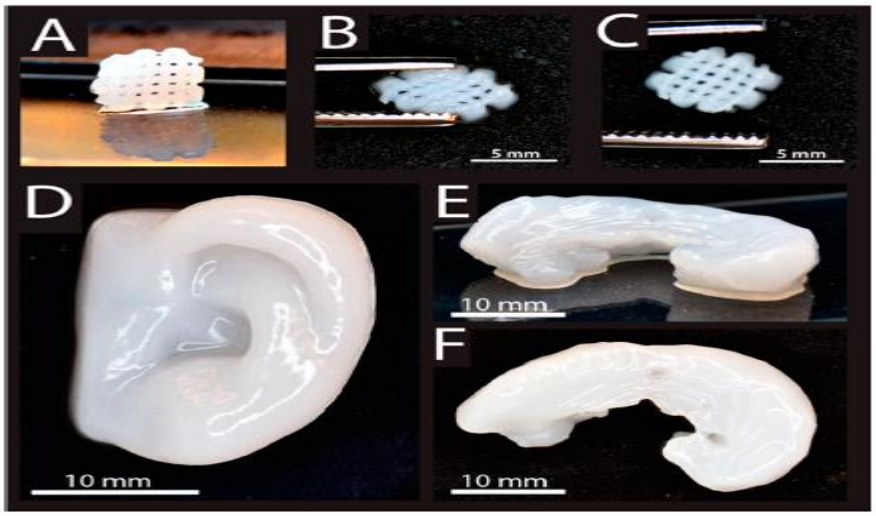

Figure 4.4. (**A**) 3D printed grids (7.2 × 7.2 mm2). (**B**) The deformity of grids with squeezing, and (**C**) restoring after squeezing. (**D**) 3D printed human ear; (**E**) (side view) and (**F**) (top view)

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.

Synergistic combination of bioprinting and nano-technology have been demonstrated valid approach for a biofabrication process more efficient with a clear added value in TERM applications. Two main biofabrication approaches have been adopted: (a) bioprinting of cell-laden nano-composite bio-inks for direct fabrication of nano-functionalized constructs, and (b) cell-free approach where a nano-composite biomaterial is used to fabricate scaffolds. In both cases the presence of nano-features

inside the biological constructs support and increase the cell differentiation toward mature tissue, but the two approaches have different benefits and applications. Nanocomposite bio-inks can modify the pure hydrogel's rheological properties. Meanwhile, the hydrogel crosslinking capability can retain nano-biomaterials inside the gel's matrix after bioprinting with active or passive bindings. Nano-composite bio-inks have been used to trigger specific cell responses. Nevertheless, a strict classification of targeted applications is not completely clear. Overall, the nano-composite's functionalities which were identified in this review work are related to control the biomolecule release close to the cell surrounding, to instruct cells and enhance the matrix's properties, to trigger cellular responses and to remotely control cell behavior. Nanofeaturing inside bioprinted constructs can also be achieved without directly embedding nano-biomaterials inside the bio-ink, but through the design of bio-ink compositions which can self-assemble in nano-structures (e.g., nanofibers or nano-pores). Protein absorption, cell adhesion and differentiation have proven to be enhanced when cells were laden in self-assembled nano-featured bioinks. Moreover, nano-biomaterials can introduce passive components inside biological constructs for remotely control and contactless stimulation of the biological construct. Ultrasounds or magnetic field are useful tools to locally stimulate cells to differentiate or control the substrate movement, recreating the natural tissue's dynamics. Bionic tissue and soft robotics are the future applications of synergistic combination of additive manufacturing and nano-technology, as they open the prospective for organ-inspired active soft tissues fabrication.

REFERENCES

1. Matai, I.; Kaur, G.; Seyedsalehi, A.; McClinton, A.; Laurencin, C.T. Progress in 3D bioprinting technology for
2. tissue/organ regenerative engineering. Biomaterials **2020**, 226, 119536. [CrossRef] [PubMed]
3. Axpe, E.; Oyen, M.L. Applications of Alg-based bioinks in 3D bioprinting. Int. J. Mol. Sci. **2016**, 17, 1976.[CrossRef] [PubMed]

4. Piras, C.C.; Smith, D.K. Multicomponent polysaccharide alginate-based bioinks. J. Mater. Chem B **2020**.[CrossRef] [PubMed]
5. Kim, M.H.; Nam, S.Y. Assessment of coaxial printability for extrusion-based bioprinting of alginate-based tubular constructs. Bioprinting **2020**, 4, e00092. [CrossRef]
6. Schubert, C.; Van Langeveld, M.C.; Donoso, L.A. Innovations in 3D printing: A 3D overview from optics to organs. Br. J. Ophthalmol. **2014**, 98, 159–161. [CrossRef]
7. Banks, J. Adding value in additive manufacturing: Researchers in the United Kingdom and Europe look to 3D printing for customization. IEEE Pulse **2013**, 4, 22–26. [CrossRef] Materials **2020**, 13, 3980 29 of 37
8. Ventola, C.L. Medical applications for 3D printing: Current and projected uses. Pharm. Ther. **2014**, 39, 704.
9. Marga, F.; Jakab, K.; Khatiwala, C.; Shepherd, B.; Dorfman, S.; Hubbard, B.; Colbert, S.; Forgacs, G Toward engineering functional organ modules by additive manufacturing. Biofabrication **2012**, 4, 022001 [CrossRef]
10. Chung, J.H.; Naficy, S.; Yue, Z.; Kapsa, R.; Quigley, A.; Moulton, S.E.;Wallace, G.G. Bio-ink properties and printability for extrusion printing living cells. Biomater. Sci. **2013**, 1, 763–773. [CrossRef]
11. Cantley, W.L.; Du, C.; Lomoio, S.; DePalma, T.; Peirent, E.; Kleinknecht, D.; Hunter, M.; Tang-Schomer, M.D.Tesco, G.; Kaplan, D.L. Functional and sustainable 3D human neural network models from pluripotent ste cells. Acs Biomater. Sci. Eng. **2018**, 4, 4278–4288. [CrossRef]
12. Song, S.J.; Choi, J.; Park, Y.D.; Hong, S.; Lee, J.J.; Ahn, C.B.; Choi, H.; Sun, K. Sodium alginate hydrogel-base bioprinting using a novel multinozzle bioprinting system. Artif. Organs. **2011**, 35, 1132–1136. [CrossRef]
13. Wasko, J.; Fraczyk, J.; Becht, A.; Kaminski, Z.J.; Flinˇcec Grgac, S.; Tarbuk, A.; Kaminska, M.; Dudek, M. Gliscinska, E.; Draczynski, Z.; et al. Conjugates of chitosan and calcium alginate with oligoproline anD oligohydroxyproline derivatives for potential use in regenerative medicine. Materials **2020**, 13, 3079.

14. Kaczmarek, B. Improving sodium alginate films properties by phenolic acid addition. Materials **2020**, 13
15. Rojewska, A.; Karewicz, A.; Karnas, K.; Wolski, K.; Zaja¸c, M.; Kamin´ ski, K.; Szczubiałka, K.; Zapotoczny, S.;Nowakowska, M. Pioglitazone-loaded nanostructured hybrid material for skin ulcer treatment. Materials **2020**, 13, 2050.
16. Elsayed, E.M.S.; Elnouby, M.; Gouda, M.H.; Elessawy, N.A.; Santos, D.M.F. E_ect of the morphology of tungsten oxide embedded in sodium alginate/polyvinylpyrrolidone composite beads on the photocatalytic degradation of methylene blue dye solution. Materials **2020**, 13, 1905. [CrossRef]
17. Parmentier, L.; Ri_ault, M.; Hoey, D.A. Utilizing osteocyte derived factors to enhance cell viability an osteogenic matrix deposition within IPN hydrogels. Materials **2020**, 13, 1690. [CrossRef] [PubMed]
18. Vasile, B.S.; Birca, A.C.; Musat, M.C.; Holban, A.M.Wound dressings coated with silver nanoparticles and essential oils for the management of wound infections. Materials **2020**, 13, 1682. [CrossRef]
19. Norambuena-Contreras, J.; Arteaga-Perez, L.E.; Guadarrama-Lezama, A.Y.; Briones, R.; Vivanco, J.F. Gonzalez-Torre, I. Microencapsulated bio-based rejuvenators for the self-healing of bituminous materials.Materials **2020**, 13, 1446. [CrossRef]
20. Larraza, I.; Ugarte, L.; Fayanas, A.; Gabilondo, N.; Arbelaiz, A.; Corcuera, M.A.; Eceiza, A. Influence of process parameters in graphene oxide obtention on the properties of mechanically strong alginate nanocomposites.Materials **2020**, 13, 1081. [CrossRef]
21. Mokhena, T.C.; Mochane, M.J.; Mtibe, A.; John, M.J.; Sadiku, E.R.; Sefadi, J.S. Electrospun alginate nanofibers toward various applications: A review. Materials **2020**, 13, 934. [CrossRef]
22. Araiza-Verduzco, F.; Rodríguez-Velázquez, E.; Cruz, H.; Rivero, I.A.; Acosta-Martínez, D.R.; Pina-Luis, G.; Alatorre-Meda, M. Photocrosslinked alginate-methacrylate hydrogels with modulable mechanical properties:E_ect of the

molecular conformation and electron density of the methacrylate reactive group. Materials **2020**,13, 534. [CrossRef] [PubMed]

23. Carrow, J.K.; Kerativitayanan, P.; Jaiswal, M.K.; Lokhande, G.; Gaharwar, A.K. Polymers for bioprinting.In Essentials of 3D Biofabrication and Translation; Academic Press: Winston-Salem, NC, USA, 2015; pp. 229–248.
24. Das, D.; Zhang, S.; Noh, I. Synthesis and characterizations of alginate-_-tricalcium phosphate microparticle hybrid film with flexibility and high mechanical property as a biomaterial. Biomed. Mater. **2018**, 13, 025008.[CrossRef]
25. Hernández-González, A.C.; Téllez-Jurado, L.; Rodríguez-Lorenzo, L.M. Alginate hydrogels for bone tissue engineering, from injectables to bioprinting: A review. Carbohydr. Polym. **2020**, 229, 115514. [CrossRef]