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The Role of Cardiovascular 3D Bioprinting in Repairing Damaged Cardiac Tissues: A Critical Review of Engineering Scaffolds

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Abstract

Cardiovascular diseases and damage have become very common and thus medical science focuses on technology and biomedical engineering to improve the quality of concerning treatment. In this context, "cardiovascular bioprinting engineering" endeavours to regenerate and repair damaged heart and blood vessel tissues. Medical science currently focuses on the differentiation between pluripotent and multipotent cells on the basis of "biometrical scaffold design" which promotes the development of functional cardiac tissues. The advanced "3D bioprinting technology" allows heterogeneous biometrics, cells, and signal factors to deposit in precise and organised geometrical figures exactly similar to their surrounding counterparts. This technology is used for fabricating "cardiac tissues" in vitro including inkjet, extrusion, and stereolithography with naturally-derived or synthetic bioinks. This article has evaluated the methods and techniques related to "3D bioprinting" technology including the current practices and procedure of post-fabrication in order to develop effective tissues and concerning engineering in vitro.

Keywords

3D Bioprinting Engineering, Bioink, CVD, ECM, Electrospinning, Inkjet Printing, Laser-Based Stereolithography, Natural Bioinks, Scaffold, Synthetic Bioinks

INTRODUCTION

Symptoms related to ***"cardiovascular diseases"*** (CVD) such as issues related to heart valves, myocardium, and vasculature have become very common in developed countries that lead to mortality and morbidity [1]. It has been noticed that CVD generally affects single or multiple structures of heart cells where replacement becomes necessary at the end-stage to develop the prognoses that have been affected. In this context, the process of grafting tissues from the body of patriots, animals, donors or synthetically manufactured has become a common medical practice. Autografts are widely used in treating ischemia where "coronary artery bypass grafts" perform with the help of harvesting segments of the saphenous veins or other vessels of the patient himself/herself [2]. Accordingly, allografting is used where allografting is used to take a heart from a donor and xenografting is applied to replace porcine or bovine valves of the heart. Furthermore, vascular grafts and synthetic valves are also used for the treatment of CVD.

Choices related to biomaterials involve decellularised matrices, and synthetic or natural hydrogels to create an "interconnected polymer network" for migrating, receiving, and proliferating essential nutrients required for the survival of "cardiac tissues". It has been noticed that the allogenic and autologous cells have adequate potential to immune cardiac damages with the help of "cardiac tissue engineering". The integration of endolithic cells, cardiomyocytes, and cardiac fibroblasts derived from the cardiac stem cells is required in cardiac structure generation.

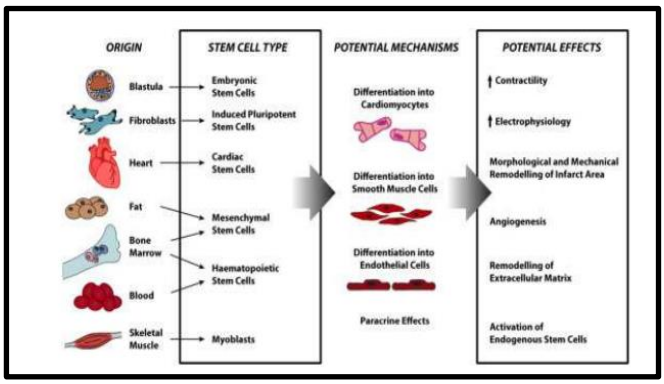

Figure 1: Cardiac cell structure [1]

The "3D bioprinting engineering" involves a manufacturing technology which consists of a "layer by layer" process used for developing "cardiac patches" of the next generation. Efforts have been made on the development of chip-based "3D bioprint" cardiac tissues by utilising bioinks or scaffolds that have the ability for restoring the functionalities of damaged myocardium. ***Collagen, alginate, hyaluronic, gelation, and decellularised matrix*** are some common examples of biomaterials that are used in "3D bioprinting". Despite having a high success rate of "3D printed scaffolds" it includes the possibility of fast degeneration which results in the reduction of mechanical or physical stability [3]. These scaffold patches maintain unique formation architecture according to the shape of native "myocardial tissues". The bioprinting "cardiac patches" consists of fibroblasts, ECs, and hiPS-CMs ("pluripotent

stem cell-derived cardiomyocytes") [1]. Accordingly, these patches promote the coupling between ***"cardiac fibroblasts" (CFs) and cardiomyocytes (CMs)***. This article is going to focus on different types of bioinks and methodologies related to "3D bioprinting" technology in order to repair damaged heart cells and tissues.

REVIEW OF LITERATURE

Prefabrication of "3D bioprinting patches"

A particular "3D bioprinting model" for a patient is based on the computer-aided design or clinical imaging in reference to damaged or diseased "cardiac tissues". According to [4], the medical imaging process for developing relevant "3D models" include the techniques of ***"volumetric 3D echocardiography", "computer tomography" based electrocardiography (CT), and CMR ("Cardiac magnetic response").*** However, "3D echocardiography" is considered an appealing source of data due to its high availability, low ionizing radiation and low cost [1]. Generally, "3D transesophageal echocardiography" data sources are prioritised for generating perfect cardiac structure models including valve leaflets and ventricular chambers. It has been observed that "ultrasound imaging" has specific limitations and artefacts that can lead to anatomic loss of data due to the ultrasound shadow. It makes the CMR and CT the most capable techniques regarding "3D bioprinting" engineering [5]. CT technology has a significant spatial resolution that enables it to generate images if patients wear metal implants or pacemakers whereas CMR is not capable of this scanning. Contradictorily, it is possible to get a "high-resolution image" without ionised radiation through CMR. In addition, the CMR technique owes the capacity to discern the composition of "cardiac tissue" without the influence of "iodinated contrast media". Henceforth, images generated from CMR are widely utilised for developing "3D bioprinting models" for vasculature and congenital cardiac chambers. It is also used for developing reconstructive models for "intracardiac tumours".

The "Image segmentation" method is applied to prepare a patient-specific "3D digital model" by interpreting "volumetric imaging" datasets received from CMR, CT, and "3D echocardiography". This segmentation method attributes to increase fascination related to "anatomical modelling" and required pressing in terms of personalised cardiac structural interventions [1]. Recently, CMR has been included along with the CT technique to replicate diseases related to systematic vasculature and congenital heart through the "image segmentation" process. At the initial stage, CT/MRI-generated dataset is exported in the format of ***DICOM*** ("Digital Imaging and Communication in Medicine"). After that, the required "anatomic geometry" is recognised and segmented depending on the threshold pixels intensity in "greyscale 2-dimensional projections" which includes axial projection, sagittal projection and coronal projection consecutively. Furthermore, computer aid software is used to "stack" each 2D image of "cardiac tissue" in particular groups according to the similar pixel intensity range for assigning them in a single material print. Later, these segmentation masks are transmitted into the final Patient-specific "3D cardiac model" by utilising rendering techniques and solved through ***STL*** ("Standard Tessellation Language") within computer programs [6]. Here, these models can be adjusted or altered according to the requirements of the patients to make them more user-specific and then finally exported for "3D bioprinting".

Types of bioink in 3D bioprinting"

Bioink is used for "3D bioprinting" in order to recover damaged "cardiac issues". As opined by [3], bioink can be broadly categorised into two segments such as:

"Scaffold-based bioinks": This type of bioink consists of cells which are hydrogels encapsulated and contain biomaterials including fibrin, collagen, alginate, gelatin, chitosan and ***PGD*** ("polyethylene glycol diacrylate"). These aforementioned biomaterials are used along with cytocompatibility, cross-linking ability and viscosity in the "extrusion-based bioprinting technology". As suggested by [3], scaffold materials can be broadly categorised into two segments such as natural materials and synthetic materials. Natural biomaterials can be generated from human or animal tissues such as fibrin, collagen, chitosan and gelatin. On the other hand, biomaterial polymers come under synthetic materials including "polyethylene glycol" and "polyethylene glycol diacrylate" [3].

It has been observed that gelatin, alginate and gelatin methacrylate are used as the most common natural bioink materials. There are certain benefits of using "hydrogel-based natural materials" compared to synthetic biomaterials including better cell viability, biocompatibility, and lower "immunogenic properties". In addition, most natural biomaterials used to have better cell cohesion compared to synthetic materials due to having signalling molecules. The process of "cell encapsulation" by using hydrogels aids in enhancing adhesion in 3D environments that develops homogeneity related to cell communication, cell distribution, and cell migration.

Generally, bioinks do not adversely impact "cardiomyocyte viability" thus resulting in significant differentiation. Moreover, decellularised ***ECM*** ("Extracellular matrix") is also used as a scaffold component in cardiovascular engineering because it shows better results in terms of structural maturity and signalling factors that make it enable to create a closer resemblance with "native ECM" compared to natural and "synthetic biomaterials" [7]. According to the result of several tests, "cell-based ECM" is beneficial with respect to scalability; however, its capabilities in mimicking "native ECM" in vivo and in vitro. In most cases, growth factors are added during the "cell culture phase" in the process of "bioink solution" production.

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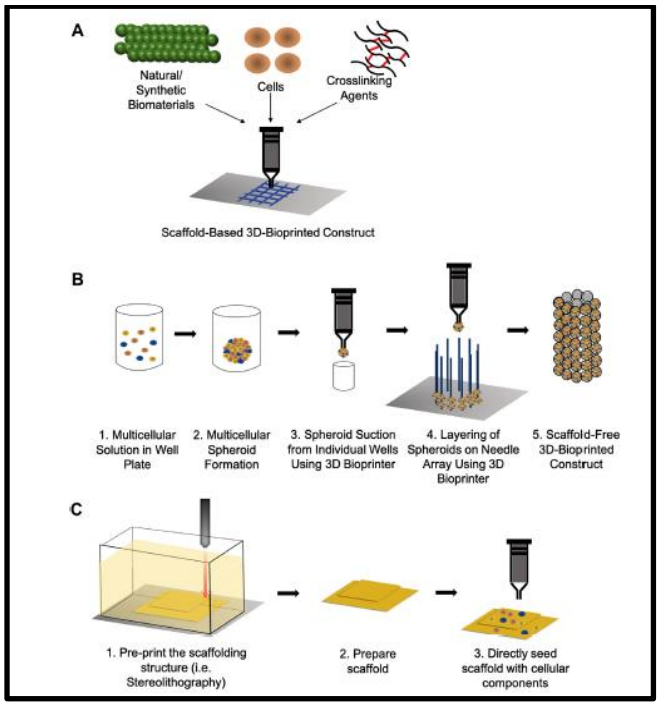

Figure 2: Types of "bioprinting method" [3]

"Scaffold-free bioinks": It generally comprises multicellular tissue spheroids which are used to construct bio cardiac tubular and "cardiac patches". However, this method is rarely used in "3D bioprinting" technology as it endures limitations regarding the mechanical support of cells provided by biomaterials [8]. Thus, this article is going to mainly focus on the "scaffold-based bioinks" in the next segment to evaluate materials used for producing "3D bioprinting".

MATERIALS AND METHODS

Materials

It has been previously discussed those scaffold biomaterials are widely used to produce bioink as the highly porous nature and crosslinked hydrogel allow "cardiac cells' to populate and migrate nutrients to fulfil metabolic needs and help in repairing damages. Therefore, this section is going to illustrate "scaffold based biomaterials" that mostly used as bioinks in "3D bioprinting".

Gelatin

It is a hydrosoluble portion which is produced by partial hydrolysis of collagen. Collagen is extracted from bones, skin and connective tissues of animals, especially fish and pigs. In past, gelatin was chemically synthesised by photo cross-linking, disulphide crosslinking, and enzymatic crosslinking. Gelatin is recently used on a big margin in "cardiovascular printing engineering" due to its high biocompatibility and biodegradability, commercial availability at reasonable costs and low immunogenicity.

It has been recognised from the test results that gelatin has the potential for fabricating "cardiac tissues". Moreover, the application of fibrin into gelation hydrogel (CM encapsulated) develops the functionality of "3D print patches" that can easily mimic the cell behaviour and mechanical properties of "native myocardium" cells [9]. MRI results suggest that gelatin has a remarkable degradation in adverse remodelling of "cardiac performance". Gelatin has better performance as a bioink used in "bioprinting technology" compared to the previously preferred biomaterial ***"PEGDA-Eosin Y system"*** in terms of CMs viability percentage. Accordingly, gelatin retains ***78%*** live cells on average when kit plated in "24 wells"; the proportion is only ***31%*** in the case of "PEGDA-Eosin Y" on ***2D TCPS*** ("tissue culture polystyrene") wells [1].

Alginate

"Alginate" is normally obtained from seaweed which is a copolymer based on a polysaccharide block. The backbone of its linear molecule involves ***"a-L-guluronate" (G) and (1, 4) "β-D-mannuronate" (M)*** [1]. Alginate can be strongly crosslinked with the higher proportion of G substance along with Ca^{++} as divalent cations. However, alginate does not have the capacity of facilitating cell adhesion by itself as it is biologically inert. Henceforth, peptides of immobilised "arginine glycine aspartate" (RGD) are used with alginate scaffolds to facilitate the generation of "cardiac bioprinting" [10]. As a result, alginate is used as bioink along with "adhesion moieties" such as matrigel or ***HBP ("heparin-binding peptide")*** to generate "cardiac tissue".

Alginate creates an ideal environment so that progenitor cells of the heart can be easily differentiated, developed, and printed into CMs. It also has the ability to tune the crosslinking degree of polymer comfortably which allows it to match the mechanical properties of "surrounding counterparts". In order to construct fetal progenitor cells of CM, cell-leaden alginate is widely used in the "extrusion-based printing method". Later, it was also used in the inkjet process to generate a hierarchical design of "pseudo tissue" along with the required porosity balance and cell response. It has been noted that $CaCl_2$ layers are used in "cardiac 3D bioprinting" to develop crosslinking by using alginate as a hydrogel precursor along with "feline adult CMs" [1]. These $CaCl_2$ layers and "adhesion moieties" aids alginates to adhere to "cardiac cells', and mimic the cell behaviours of native ECM.

Fibrin

Fibrin is investigated and exhibited as a natural biomaterial that is widely used in "cardiac tissue engineering" as a bioink. It is derived from the plasma of the patient's own blood and thus the risk regarding "immunogenic reaction" can be neglected. This autologous scaffold biomaterial can be utilised as glue, gel or microbeads with different peptides and it leads fibrin to facilitate the growth factors as well as the mimicking ability to the "native tissue" microenvironment. As a natural bioink material, fibrin enhances cell migration, attachment, ECM synthesis, and proliferation with the help of the "transforming growth factor" (β) and "platelet-derived growth factor" [1].

Fibrin is used in multiple cardiovascular segments

including cardiac mussels, heart valves, blood vessels and aortic conduits for its specific advantages. A silicone tube containing fibrin gel can be utilised in the femoral artery or vein to replace and repair cardiac damage. Accordingly, valve conduits can be fabricated by using fibrin gel and myofibroblast suspension. It has been noticed that multiple silicon layers are utilised to construct cusp structure and vascular wall. After 4 weeks of synthesising fibrin hydrogels, the microscopic image reveals a porous scaffold with high ECM and well-structured collagen bundles without any nutritional issues [1]. A supplement of aprotinin is added to fibrin gel to control the degradation in the "tissue bioprinting" process. According to [4], aprotinin works as a "protease inhibitor" that can slow down or nearly stop the degradation related to f8ibrin in order to ensure effective "cardiac tissue" development. Different experiments have been conducted by suspending the aortic myofibroblast of humans in fibrin gel for 4 weeks [1]. The results reflect that the 3-dimensional fibrin hydrogel along with aprotinin inhibits constant collagen production and homogenous growth of cells without major inflammatory reactions or degradation. It clearly conveys that the degradation of the "fibrin scaffold" can be controlled by the adjustment of aprotinin concentration.

Fibrin is used as a major biocomponent for bioink which is widely used in "cardiac bioprinting" engineering. It is generally derived from fibrinogen through enzymatic polymerisation and it helps to heal the natural wound, develop functional tissue and control degradation [11]. In addition, it also offers specific constraints including insufficient mechanical stiffness, shrinkage, and gradual disintegration during the development of tissue structures. However, these limitations can be reduced by maximising fibrinogen concentration, cross-linking, and combining Ca^{++} ion, pH and aprotinin with "fibrin polymer" [1].

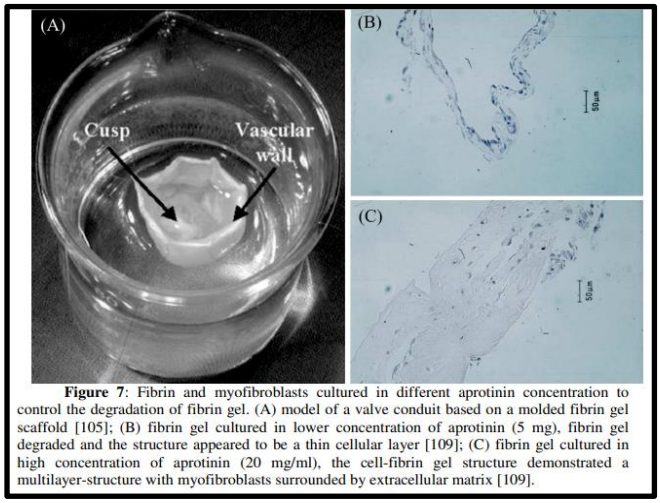

Figure 7: Fibrin and myofibroblasts cultured in different aprotinin concentration to control the degradation of fibrin gel. (A) model of a valve conduit based on a molded fibrin gel scaffold [105]; (B) fibrin gel cultured in lower concentration of aprotinin (5 mg), fibrin gel degraded and the structure appeared to be a thin cellular layer [109]; (C) fibrin gel cultured in high concentration of aprotinin (20 mg/ml), the cell-fibrin gel structure demonstrated a multilayer-structure with myofibroblasts surrounded by extracellular matrix [109].

Figure 3: Fibrin synthesis with aprotinin concentration [1]

Synthetic bioinks

Synthetic biomaterials are also used besides natural bioink that comprises similar properties of native CMs in "cardiac tissue bioprinting". Here, the properties of bioinks completely depend on the chemical substances and reactions. For instance, PCL (polycaprolactone) is used as a synthetic biomaterial for cardiac scaffolding under its melting point of 60°C. According to the research report, ***PCL along with CNTs*** ("carbon nanotubes") used in tissue development technology where PCL acts as biodegradable and allows the proliferation of myoblasts tissues in vitro [1]. It indicates that PCL can be utilised as a biocompatible and biodegradable synthetic bioink that facilitates significant growth of "cardiac cells". ***PLA*** ("polylactic acid") is another example of synthetic biomaterial capable of cell viability, vascular grafts, and slow degradation. Synthetic bioinks are used in vascular grafts as it inhibits a low degradation along with the prolonged duration of structural support and cell viability that makes the "tissue development" process withstand a long process [12]. Thus, "synthetic bioinks" are used in "3D bioprinting" to support a biocompatible durable frame structure and the formation of vascular grafts for bypass surgeries and cardiac implantation.

Methods of "3D bioprinting technology"

There are several methods related to "3D bioprinting" in terms of tissue development such as "extrusion-based printing", stereolithography, scaffold-free method and filament extraction. However, the key challenges in bioprinting include developing perfect biocompatible materials which are ideal for cell viability. In "bioprinting tissue development engineering", the formation of scaffolds, maintaining scaffold stiffness, fibre alignment direction and chemical composition play a remarkable role.

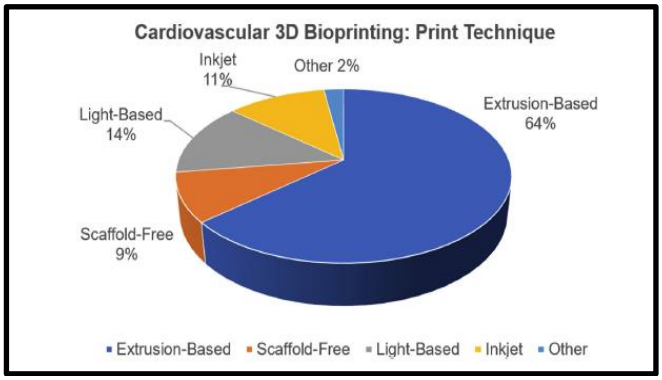

Figure 4: Methods of "3D bioprinting" [3]

Conventional methods include the practice of constructing scaffold materials that can provide adequate temporary support and viability to the "cardiac cells". Then, stem cells are collected to seed on the scaffold material to permeate the material through diffusion. This scaffold material degrades naturally over time because seeded cells secrete native ECMs which provide support to the initial bioprinting structure. Traditional methods involve certain limitations including the time taken by cells to diffuse in scaffold material and achieve expected cell viability and acceptable thickness. In the field of tissue engineering, the viability of "cardiac cells" reduces substantially in scaffold materials of thickness between 100-200 μm due to the lack of nutrient diffusion [3].

Extrusion-based methods

This method is used extensively in "3D bioprinting" due to its effectiveness, advantages and convenience. Nozzles are used in this approach to handling a wide variety of biocompatible soft materials including hydrogels, polymers and colloids. These materials don't require intense heat or radiation to print designed scaffold architecture. This particular benefit allows this method to preserve construction shapes during the bioprinting procedure without replacing cells which can affect cell viability.

"Extrusion-based methods" can be categorised on the basis of single and multi-cartridge systems. As opined by [5], the "single-cartridge extrusion-based" printing system includes a single mix bioink component that consists of both ECM and cells. The main advantage of "single cartridge bioprint" is the requirement of producing a single bioink in the overall "tissue development bioprinting" process for creating a scaffold. Here, the scaffold and cell are combined into a single bioink and thus the biomaterial needs to have proper chemical properties that lead it to the required viability. It limits the variety of the final composition of the scaffold in single cartridge bioink. On the contrary, the "multi-cartridge extrusion-based" printing approaches offer additive flexibility and advantages regarding bioinks and biomaterials which can be utilised in the printing process. In order to construct a "complex cardiac tissue system", the multi-cartridge approach is preferred due to the wide versatility of biomaterial in ECM choices. In this system, each cartridge may contain different bioinks as well as scaffold materials comprising varied cellular compositions. A dedicated scaffold solution or gel in a "multi-cartridge system" can provide additional benefits of heating the "scaffold solution" at a high temperature to make extrusion smoother. It has been recognised that "scaffold cartridges" enables too viscous polymers to print tissue development model at low temperatures [13]. This attribute makes this method more effective by printing and filling "porous scaffold structures" or different types of cells with bioinks. Henceforth, "extrusion-based" printing methods show promising outcomes, where "scaffold alignment" plays a critical role in "cardiac tissue engineering" by developing a synchronised contraction with "surrounding cardiac tissues".

Non-extrusion-based method

There are different approaches related to "non-extrusion–based 3D bioprinting methods" which are used in developing "cardiac cells" and repairing the damage. The method includes electrospinning, inkjet printing, and a light-based approach. According to [3], bioink deposition is delivered in the form of droplets through cartridges in the "inkjet printing" method. Here, droplets are delivered individually instead of a continuous stream of the selected biomaterials. As the "droplet approach" needs a specific viscosity for depositing droplets by maintaining consistency, it creates constraints which limit the versatility of the "inkjet printing" approach compared to the "extrusion-based method".

Electrospinning and "laser-based stereolithography" are other available options regarding "3D bioprinting" which comes under the "non-extrusion-based method". These approaches provide significant flexibility in printing and developing complex "scaffold structures" including high-speed cardiac valves, but harsher in terms of "cell viability". A UV source is used in the "laser-based" method to cure and shape biomaterials into desired geometrics according to "native tissue counterparts". In addition, the electrospinning approach s[pins biomaterials at a high speed to convert them into fine weaves that can be seeded with cells later [14]. Stereolithography and electrospinning methods were not used directly in printing with bioinks in the past as they can impact scaffold structure due to harsh environments during the printing process. However, advanced "fabrication techniques" such as ***"stereolithography"*** and ***"electrospinning bioprinting"*** enable better cell viability. Still, these aforementioned methods are not as prevalent as "inkjet printing" and "intrusion-based methods" in terms of bioprinting, cell development and cell viability.

Scaffold-free bioprinting technique

It is developed and started widely used in the last decade due to limitations related to scaffold production. One of the key drawbacks of scaffold bioprinting includes the process of solidifying and crosslinking which are essential for scaffold functionality but impact the "cell viability": due to the application of UV rays and heat. In this "scaffold-free" approach cell-based bioinks are used in place of ECM materials. This method is beneficial as cell-based bioinks aid in secreting raw "native ECM" that creates cell-to-cell direct communication. It improves the synchronisation of cellular contraction and electrical signalling in "cardiac tissue engineering".

The "scaffold-free printing technique" can be critically categorised into two parts as ***"magnetic suspension method" (MSM)*** and the ***"spheroid droplet method" (SDM)*** [3]. It has been identified that the "spheroid droplet method" mostly use in developing "cardiac cells". These "cardiac cells" are then seeded with the endothelial cells' mixture with fibroblasts in "96-well plates". This mixture is properly cultured till the cells are transmitted into spheroids under specific culture media. Moreover, these formulated spheroids are printed either in the ***Kenzan method*** (droplets method) or the ***LaBarge method*** (Layers method) [3]. On contrary, a magnetic nanoparticles-based solution is used in the MSM approach where cells are expected to take particles. Thereafter, a magnetic field is applied externally to hold various structures up to those cells secrete native ECM for supporting their particular shapes.

RESULTS AND DISCUSSION

Post fabrication

The "post fabrication" approach is needed to accustom the biomaterials to their required function followed by the "3D bioprinting design". It ensures the optimal performance of scaffolds or new tissues by improving the properties related

to "cardiac tissue" such as "electrical signalling", contraction, and reception of blood. Continuous contraction and stretching of material are used to ensure mechanical simulation of cardiac patches. It assures the capability of biomaterial in relaxing and contracting for a long time by retaining actual morphology. This "Bioprinting" technology resists permanent change of structure by maintaining an average heart rest of 70-80 bpm for an adult [1].

The process of developing "cardiac tissues" involves certain limitations such as cell attachment, cell viability, oxygen diffusion and deeding cells within scaffold materials. In this context, perfusion techniques are used to deliver adequate nutrients to bioprinting scaffolds and "cardiac cells" including ***CFs, CMs, and ECs***. As suggested by [1], the method of "perfusion cell seedings" aids in placing "cardiac cells" uniformly all over the scaffold biomaterials which improves the proliferation and survival of new "cardiac cells". Accordingly, electrical conditioning is utilised along with the process of mechanical simulation and perfusion to initiate contraction in the cardiac muscles. The simulation related to electrical pulses can be applied to "bioprinted tissues" to develop conductive properties and contraction rate of pacemaker cells or "cardiac cells". As per the studies of [15], electrical simulation plays a significant role in "cardiac tissue engineering" because the contraction of cardiomyocyte cells occurs simultaneously and it is necessary to organise ventricular and atrial structures by maintaining the beating rate of CMs. In the post-fabrication process, perfusion, electrical conditioning and mechanical simulation are considered necessary procedures in terms of "3D bioprinted cardiac tissue" development.

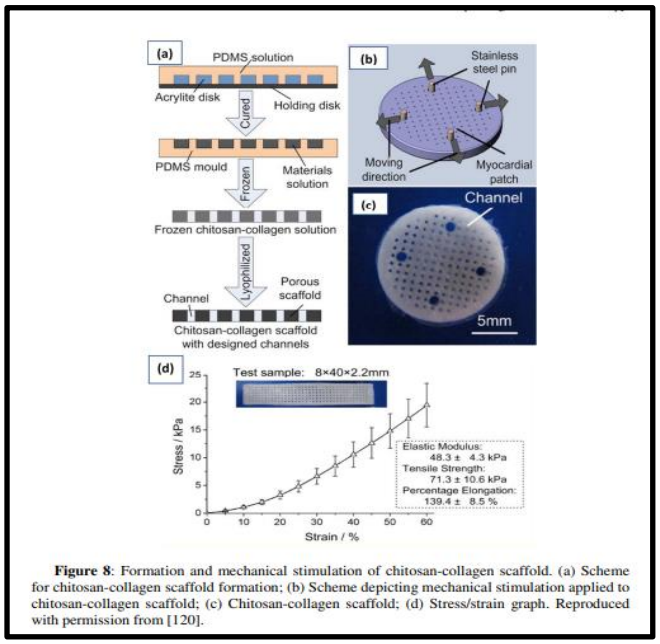

Figure 8: Formation and mechanical stimulation of chitosan-collagen scaffold. (a) Scheme for chitosan-collagen scaffold formation; (b) Scheme depicting mechanical stimulation applied to chitosan-collagen scaffold; (c) Chitosan-collagen scaffold; (d) Stress/strain graph. Reproduced with permission from [120].

Figure 5: Post-fabrication [1]

CONCLUSION AND RECOMMENDATION

The overall article has shed light on "3D bioprinting" engineering in order to replace and repair "cardiac tissues" for curing cardiovascular diseases. The prefabrication method for bioprinting technology has been discussed in this article. It has been spotted that natural and synthetic biomaterials and hydrogels are used as bioink to prepare scaffolds. The use of natural biomaterials such as gelatine, alginate, collagen, and fibrin has been evaluated in this study as bioinks to generate scaffolds in bioprinting. On the other hand, the use of ***PCL and PLA*** has been discussed as synthetic bioink materials.

The methods of "3D bioprinting" have been discussed in this article including the "extrusion-based method" and the "non-extrusion-based method". It has been recognised that the Inkjet approach is more effective compared to other "non-extrusion methods". Furthermore, the ***"scaffold-free method"*** has been discussed and the advantages of "***SDM and MSM"*** have been spotted. There is no requirement for scaffold biomaterial in "scaffold-free methods" where cells from patients' bodies are used for "bioprinting". Thus, the development of "SDM and MSM" methods is highly recommended in the upcoming times to develop the "3D printing engineering" and cardiac tissue development process without depending on the scaffold biomaterials.

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