

Review*

Chitosan Scaffolds for Cardiac Tissue Engineering

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Abstract: Cardiovascular diseases are the leading cause of death worldwide. Cardiovascular diseases complication can give rise to myocardial infarction which produces cell death by blockage of blood flow, leading to loss of heart function. Current treatments directed at heart repair have several disadvantages such as the lack of donors for heart transplantation or the use of non-bioactive inert materials for replacement of the damage tissue. New treatment strategies involve stimulation of heart tissue regeneration with the use of bioactive materials like chitosan, in combination with cells and biochemical factors. Chitosan scaffolds have the necessary proprieties of biocompatibility, porosity, and biodegradation, that imitates the heart extracellular matrix. Chitosan scaffolds physical proprieties, such as electrical conductivity and mechanical proprieties, can be improved by different preparation techniques and by the functionalization with other materials.

Keywords: chitosan; cardiac tissue engineering; scaffolds

1. Heart regeneration

The heart has three main tissue layers know as endocardium (inner layer), myocardium (muscle layer), and epicardium (outer layer). As a primary organ, the heart is fundamentally a two-sided pump responsible for maintaining blood flow throughout the body. Blood flow is kept unidirectional through a series of valves or cusps. These valves are formed from tissue folds that open and close by dilation and contraction of the heart. The smallest contractile unit of the heart are cells known as cardiomyocytes (CM), primarily residing in the myocardium. CM make up about 1/3 of the cells in the atria and about half of the cells in ventricle [1]. Unfortunately, because of the lack of heart regenerative capabilities, CM regeneration has been one of the greatest challenges cardiac tissue engineers have sought to overcome in the past two decades of the field [2].

1.1. Mammalian heart cells lack from regenerative capabilities

Aging in the heart is accompanied by changes in vascular structure and function, especially in large arteries. While it is known that the mitotic proliferative state of human CM ceases before birth, some CM plasticity remains active over a lifetime. With the time, CM regenerative activity decreases from 1% per year (at the age of 20 years old) to 0.4% (at the age of 75 years old) [3]. This implies that less than 50% of CM would be renewed

throughout a lifetime and about 55% would be persisting CM from birth [4]. The deterioration of vascular function in elderly patients is the result of phenotypic alterations of different types of cells, such as smooth muscle cells (SMC), pericytes [5] and endothelial cells (EC) that become more heterogeneous in size, shape, and axial orientation. These changes produce less laminar blood flow, increasing lipid deposition and developing atherosclerotic plaques (cardiac ischemia) [6]. Cardiac ischemia is characterized by less oxygen flow into the heart muscle [7]. When there is ischemic injury, a pathological remodeling of the heart leads to CM loss and scar formation, caused by cardiac fibroblast (CF) activation. This scar formation eventually generates fibrosis and ventricle dysfunction, leading to heart failure [8]. Mammalian hearts, as opposed to amphibians, lack from regenerative capabilities that can revert the fibrotic scar and replenish CM population and cardiac function. The mechanisms of differences are still not well understood [9]. In zebrafishes, transcription factor *Prrx1b* promotes Neuregulin 1 that acts as a mitogen and activates CM proliferation [10]. Adult mammalian hearts exhibit minimal regenerating capacity due to a lack of a reserve population of cardiac progenitor stem cells, and, due to the almost-null proliferative ability of the postnatal differentiated CM [11]. There is a set of chronic diseases that can injure the non-regenerable heart tissues, called cardiovascular diseases.

1.2. Cardiovascular diseases

Cardiovascular diseases include acute myocardial infarction, arterial hypertension, cerebrovascular diseases, coronary diseases, congenital diseases, and rheumatic heart diseases [12]. Cardiovascular diseases tend to evolve gradually throughout life and are typically asymptomatic for a long time. It is estimated that by the year 2030, 23.6 million people will die of cardiovascular diseases per year [13]. There are multiple risk factors for developing cardiovascular disease, including genetics, smoking, physical inactivity, poor eating habits, dyslipidemias [14], type 2 diabetes, obesity, and insulin resistance. These are also associated with premature features of vascular and cardiac senescence [15]. It is important to mention that the most important risk factors are age and gender. Older people and women have more cardiovascular comorbidities and have a higher incidence of adverse cardiovascular outcomes, including mortality after an acute myocardial infarction [16].

In terms of treatment, current approaches are characterized by improving disease symptoms and slowing adverse cardiac remodeling, but treatments fail to reverse the irreversible loss of cardiac tissue. For this reason, innovative therapies such as tissue engineering, have been proposed for its potential of stimulating the regeneration of the affected myocardium [17].

2. Cardiac tissue engineering

The process of combining extracellular matrices, specific cell types, and bioactive molecules together to create specific human tissues is called tissue engineering (Figure 1). The goal of tissue engineering is to replace damaged or malfunctioning tissues in human patients [1,18–22]. Delay in cardiovascular disease treatment can often create a situation where the patient needs, for example, a valve replacement. Furthermore, heart tissue damage produced by cardiovascular diseases can lead to further heart degeneration and failure, requiring at the extreme, full heart replacement. Sadly, donor hearts are not just in significant demand, but also in very short supply. In addition, there is a potential for organ rejection because of the need of tissue compatibility. Hence, being able to construct specific tissue sizes, and replacement valves of the heart, would lessen the time before treatment and thus relieve the demand on full heart replacements [22–24].

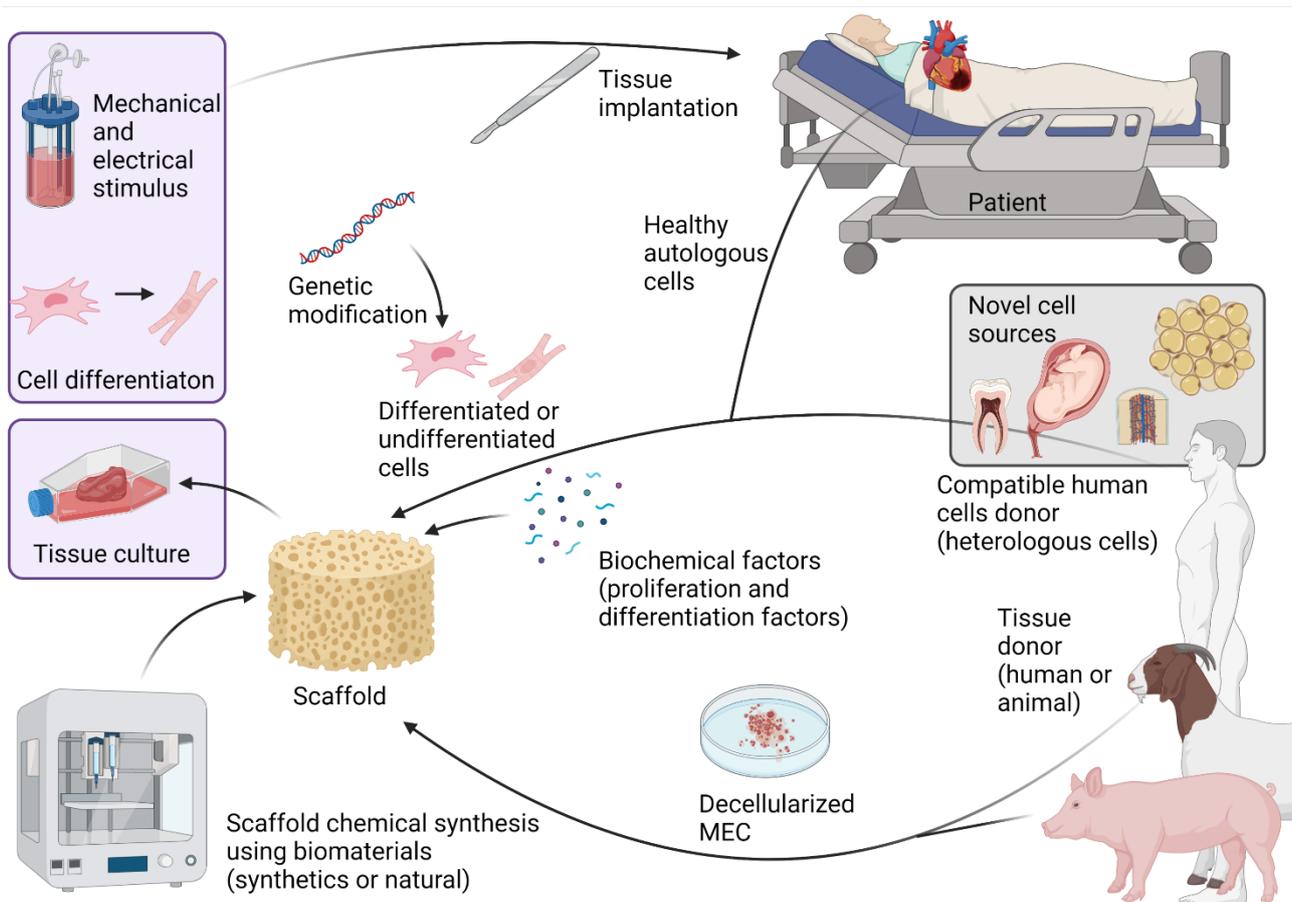


Figure 2. Scheme cartoon of cardiac tissue engineering development. Tissue engineering is the result of combining three basic elements: scaffolds that imitate extracellular matrix, different sources cells, and biochemical factors. Created with BioRender.com

Tissue engineers seek to create tissues and specific organs as models to elucidate normal and diseased tissue states. More specifically, cardiac tissue engineering has many advantages that make the heart an ideal focus for tissue engineers. Cardiac tissue engineering can yield significant advancements in drug development by offering a platform for testing drug effects before costly clinical trials. Recent research has shown engineered cardiac tissue and organoids react in biologically relevant ways that recapitulate clinically-presented side effects [1,18–22]. This opens the door to high-throughput screening techniques that can shorten the time of drug development and allow assessment of off-target effects that drugs may have on the heart.

The idea of repairing an injured heart by growing cardiac cells in the lab has long been a goal of cardiac tissue engineers, however, progress to a fully functioning model has been slow. This is in part due to the wide variety of challenges due to the complex structural, metabolic, and electrical demands of an organ that never stops moving. Cell type and configuration, scaffolding, and bioactive molecule composition all contribute to enhancing the cardiac tissue engineering process.

3. Cells

Cells that constitute the heart are CM (25-30% of heart cells) and the other 70% are blood and lymphatic EC, CF, vascular smooth muscle cells (VSMC), cardiac progenitor cells (CPC), pericytes, and immune cells. CM are the contractile unit, responsible for pushing blood and delivering oxygen and nutrients. EC and CF are key to tissue function and homeostasis. Vascular EC are metabolically active, control vasomotor tone, and regulate

angiogenesis. CF constantly maintain the ECM through a degradation-deposition equilibrium. VSMC and pericytes regulate blood flow in the cardiac vasculature. CPC are multipotent cells with an expression of surface markers like c-Kit, Sca-1, MESP1+ or Isl1+. CPC comprise the stage of nascent cardiac mesoderm and they can differentiate *in vitro* and *in vivo* into the three major different layers of cells in the cardiovascular system: SMC, EC, and CM. Each cell type composition is crucial in cardiac biology. This complexity undoubtedly raises the challenge of designing artificial heart muscle. In the last decade, great efforts have been made to obtain human cardiac lineage for various injury cardiac applications [3]. Below, in Table 1, are summarize the cell sources used in cardiac tissue engineering.

Table 1. Cell sources in cardiac tissue engineering

Source	Cells	Definition	Advantages	Disadvantages	Reference
	Fetal CM	Fetal heart.	Potential for cardiac integration and regeneration.	Immunogenicity. Malignant potential. Ethical questions. Limited availability.	[25]
Embryonic	Human umbilical cord blood derived cells	Pluripotent stem cells, mesenchymal stem cells (MSC), hematopoietic stem cells (HSC) and non-hematopoietic stem cells (NHSC) that can differentiate into several distinct cell types.	Reduction of infarct after intramyocardial injection.	Immunogenicity. Need to standardize isolation and culture procedures. Senescence and mutational acquisition during <i>in vitro</i> expansion.	[26,27]
	Embryonic MSC	Pluripotent stem cell derived from inner cell mass of blastocyst in embryo.	Potential to differentiate into cells from all three embryonic germ layers.	Only a small number of differentiated CM can be generated. Associated with malignant transformation. Legal issues.	[28]
	Adipose stem cells	Cells derived from adipose tissue: heterogeneous mixture of MSC, HSC, and endothelial progenitor cells (EPC).	Multipotent potential. Easily source with minimal effort. Easy harvesting. Low cost. No ethical issues.	Potentially tumorigenic. Limited understanding of mechanism associated to cardiac repair.	[29]
Adult stem cells	Bone marrow stem cells	Stem cells derived from bone marrow.	Well-known cell precursors. Easy collection.	Extracted in low numbers. Take on fused donor/recipient characteristics. Potential contamination during <i>in vitro</i> expansion.	[29,30]
	EPC	Originated from different tissues. Have been classified into hematopoietic and nonhematopoietic progenitor cells.	Increase its numbers in response to ischemia/cytokine stimuli. Can migrate to injury site and differentiate into new myocytes. Participate in the repair and maintenance of vascular homeostasis.	Low numbers in peripheral blood and bone marrow makes <i>ex vivo</i> expansion difficult.	[31]
	Resident cardiac stem cells (RCSC)	Represent a responsive stem cell reservoir within the adult associated myocardial homeostasis.	Capable of differentiating into multiple cell types such as CM or VSMC.	Limited repair.	[32]

	Human induced pluripotent stem cells (iPSC)	Autologous somatic cells that can be converted into pluripotent cells.	Possibilities of large-scale production. Ability to differentiate into CM, SMC, and vascular EC.	Poor purity. Heterogeneity. Laborious/inefficient techniques of isolation. Can generate teratomas.	[29,33]
Adult somatic cells	CF	Source of induced pluripotent cells. CF can be reprogrammed directly into CM, EC and SMC.	Available in large numbers. Phenotypically plastic. Promote the proliferation of endogenous CM by upregulating the production of ECM proteins that activate the CM cell-cycle, or by altering structural components to reduce ECM stiffness.	CF are the primary drivers of fibrosis. Unclear how the <i>in vivo</i> environment with changed ECM compositions influences CF plasticity and integration of transplanted CM.	[34]
	Skeletal myoblasts	Derived from muscle biopsy.	Ability to engraft, create myotubules, and improve cardiac function after transfer into infarcted myocardium.	Heterogeneous. Have been associated with arrhythmias due to separation of CM by islands of skeletal muscle cells. Interfering with the propagation of electrical potentials.	[35]

4. Biochemical factors

De novo CM, in the postnatal period, can be differentiated from a subpopulation of undifferentiated cardiac progenitors, or they can be transdifferentiated from CF. Both, induction of CM proliferation and CF reprogramming have granted significant benefits in pre-clinical experimental studies of heart regeneration. Several challenges must be overcome before this could translate into novel therapies for human heart regeneration.

CF represent an ideal cell source for heart regeneration due to its relative abundance in this tissue. The landmark discovery that CF could be reprogrammed into a pluripotent state (iPSC) by the overexpression of different transcription factors (SOX2, KLF4, C-MYC, and OCT3/4) yielded new paradigms in cell-based therapies [36]. Ieda et al. identified a set of core cardiomyogenic transcription factors (GATA4, MEF2C, and TBX5) that induced the expression of cardiac Troponin T in mouse CF [37]. These transcription factors are part of a core set of evolutionarily conserved genes (*Mesp1*, *Gata4*, *Hand2*, *Mef2c*, *Nkx2-5*, and *Tbx5*) involved in heart development [38]. *Mesp1*, that is highly conserved in metazoans, is considered the cardiac master gene that drives mesoendoderm differentiation, via DKK1-inhibition of the Wnt/ β -catenin signaling pathway [38,39]. Downstream of the transient expression of *Mesp1*, comes a transcriptional network regulated by transcription factors GATA4, HAND2, MEF2C, MYOCD, NKX2-5, FOXH1, ISL1 and TBX5. This transcriptional network differentiates cell subpopulations to cardiac mesoderm lineages, consisting in the first and second heart field [38,40].

Subsequent studies, using different combinations of transcription factors and gene delivery methods (for the expression of transcription factors) have demonstrated successful reprogramming of neonatal and adult fibroblast into cardiomyocyte like cells in experiments both *in vitro* and *in vivo* [8,41]. GATA, MESP1, TBX5 (GMT) overexpression has been shown to induce the expression of sarcomere structures in fibroblasts, moreover, the addition of HAND2 markedly improves reprogramming efficiency to CPC and enhances function of injured hearts following myocardial infarction [41]. CPC maintenance and differentiation is regulated via NOTCH and WNT signaling [38]. NOTCH inhibition substantially increases MEF2C binding to its target genes, producing cardiac differentiation (in cell clusters) marked by raise in calcium flux and beating colonies [42]. BMP and WNT

signaling inhibition differentiate CPC to a myocardial lineage marked by expression of cardiac isoform troponin T and the expression of the transcription factor NKX2.5 [39,43]. The combination of the histone deacetylase inhibitor sodium butyrate, the WNT inhibitor ICG-001, and the cardiac growth regulator retinoic acid, have been shown to enhanced cardiomyocyte-like cells generation in rat CF [44]. Other strategies for cell differentiation implicates the cell mRNA degradation using miRNAs. The combination of miR-1, miR-133, miR-208, and miR-499 induced expression of the α MHC-CFP reporter in 1.5 to 7.7% of neonatal CF *in vitro*, and *in vivo* administration into ischemic mice myocardium induce conversion from CF to CM [45]. Delivery of miR-106b~25 into mouse heart produce CM proliferation by targeting a network of negative cell cycle regulators, including E2f5, Cdkn1c, Ccne1 and Wee1. Proliferation produces almost complete regeneration of the adult myocardium after ischemic injury [46]. Three different miRNAs (miR-548c-3p, miR-509-3p and miR-23b-3p) were identified to inhibit posttranscriptional activity of the anti-mitotic gene Meis1 inducing CM proliferation [47].

5. Scaffolds

Scaffolds are tridimensional porous structures, prepared with biocompatible and bioactive materials that stimulate the differentiation and proliferation of cells, once cells are attached inside the porous structure of the scaffold (Figure 2). Porous allow cell migration and facilities nutrient supplementation [1]. From a technical standpoint, scaffolds should have a permeability of 50–90% to promote the diffusion of nutrients, oxygen, and other fluids [48]. Scaffolds mimic the extracellular matrix (EMC) proprieties giving cells mechanical support for their proliferation and tissue formation. Mechanical proprieties of scaffolds need to be similar to the mechanical proprieties of the native tissue that is to be regenerated. Scaffolds must be biodegradable to gradually disappear while cells generate their own EMC that will replace the scaffold. Materials chosen for the scaffold construction should be non-toxic nor immunogenic. This also applies to the residual molecules generated by the scaffold during biodegradation. In addition, these molecules should be eliminated easily from the body of the patient, or they can be integrated to the different cellular metabolic routes [1].

5.1. Decellularized extracellular matrices

Decellularized extracellular matrices are a natural source for scaffolds. Decellularization is the total elimination of cells, from a tissue, by physical or chemical methods. After the removal of cells, the obtained scaffold is comprised of natural basic structural ECM proteins, and glycosaminoglycans [49]. The lack of cellular components eliminates the risk of adverse side effects such as inflammatory response or an immunological rejection. If the obtained 3D structure is not warranted, matrices might pass thought drying and pulverization for further reconstitution into molds or shapes of interest [50–52].

Data from preclinical and clinical evaluation of acellular biologic EMC scaffolds in cardiac pump dysfunction and ischemic heart failure showed reduced fibrotic tissue, improved perfusion of infarcted myocardium, and reverse structural remodeling [53]. A decellularized pericardial matrix colonized with human viable Wharton's jelly-derived mesenchymal stromal cells, was implanted in patients with non-revascularizable myocardial scars, and has shown reduction in scar mass after three months [54]. A previous study using human decellularized pulmonary heart valves engineered with autologous EPC in pediatric patients with pulmonary valve pathology reported that these valves have the potential to remodel and grow along with the somatic growth of the patient [55].

A potential disadvantage of the use of decellularized extracellular matrices is the existence of remaining cells from the native tissue, in the scaffold. This cellular remaining can be dangerous if it triggers a patient immune response [49]. In addition, an alternative source for scaffolding materials is to synthesize them.

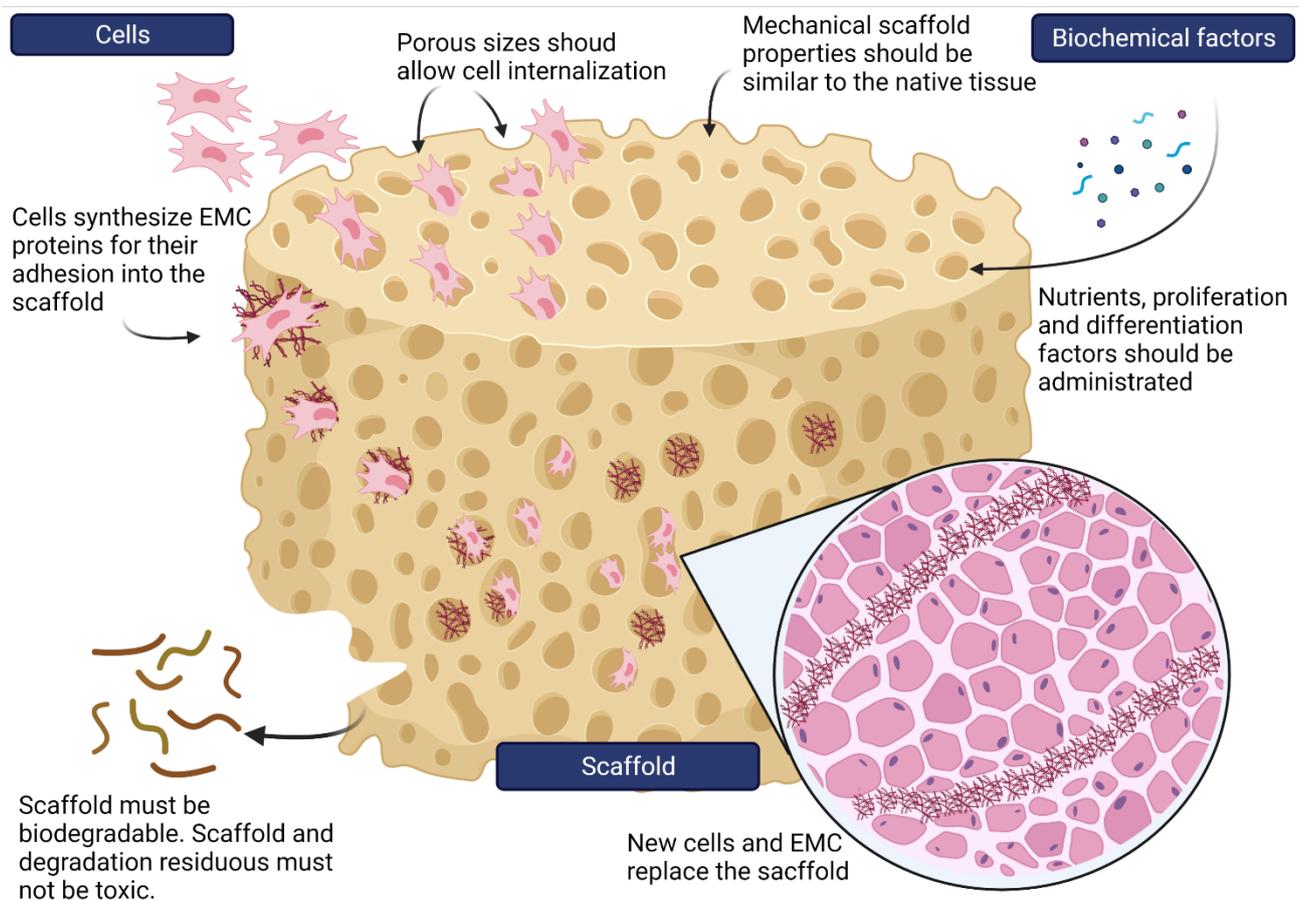


Figure 2. Scheme cartoon of principal properties of scaffolds. Created with BioRender.com

5.2. Synthetic biomaterials for scaffolds construction

Synthetic biodegradable scaffolds hold the innate advantage of being easily crafted into any shape and engineered for desired mechanical properties [52]. Synthetic biomimetic materials are an attractive option for tissue engineering, as we can monitor their development, thus controlling topological, mechanical, and structural properties with the added benefit of functionalizing or adding other materials to enhance attachment [56,57]. Although not all synthetic biomaterials are directly biodegradable, non-biodegradable biomaterials can be engineered to biodegrade. Biodegradable materials have a great advantage as they have the capability of being able to be replaced when cells mature and produce their own EMC.

Using synthetic materials in cardiac tissue engineering has grown over recent years, with emphasis on developing patches for cardiac repair with the use of biodegradable polymers like poly(ϵ -caprolactone) (PCL), poly(glycerol sebacate) (PGS), polyethylene glycol (PEG), and poly(L-lactide acid) (PLLA), poly(L-lactic-co- ϵ -caprolactone) (PLCL), and poly(lactic-co-glycolic acid) (PLGA) [48]. In the early part of the 21st century research from Matsubayashi et al developed a porous PCL cardiac patch which were seeded with VSMC for the intent to repair aneurysm and designed to prevent ventricular dilation post myocardial infarction [58]. Moreover, other porous structures using PGS were developed by Morgan et al, to control orientation of cardiac cells [59]. Improving on cardiac orientation, Hu et al. also developed patches using PGS copolymerized with aniline trimer to obtain electroactive material significantly enhancing cell interactions [60]. An interesting part of heart mending is from the aspect of valve repair, wherein materials like PEG-based hydrogels are used as scaffolds. By crosslinking PEG with peptides, biomimetic properties

were obtained resulting hydrogels influenced the elongation. De novo EMC deposition and hydrogel degradation behavior of encapsulated valvular cells, potentiating the use of these material for the development of future heart valves [61]. In another interesting approach, the implantation of tissue-engineered vascular grafts (PLLA and PLCL) seeded with autologous bone marrow mononuclear cells were used as extracardiac total cavopulmonary conduits in pediatric univentricular physiology. No evidence of aneurysmal formation, graft rupture, graft infection, or calcification were reported, and seven (28%) patients had asymptomatic graft stenosis [62].

5.3. Natural biomaterials for scaffolds construction

In the search for natural biomaterials, researchers have investigated the individual components of the EMC as platforms. A considerable number of biomaterials for scaffold construction are under research, such as collagen, fibrinogen, silk, elastin, alginates, and chitosan [50,63–66].

5.3.1. Collagen scaffolds

Collagen is commonly used in myocardial tissue engineering, given that it is one of the major components of the myocardium ECM [67]. In the heart, collage type I, is the major constituent of the EMC representing 75-85% [68], with the added advantage of having low immunogenicity. Collagen type I comprises two alpha 1 chains and one alpha 2 chain, creating long fibers whose properties depend on the density and spatial alignment [56]. Albeit collagens can divide into fibrillar and non-fibrillar. Those non-fibrillar can help form networks or associate with either fibrillar collagens or membranes [69,70]. Recent research has focused on utilizing collagen-based biomaterials on pathologies like myocardial infarction, as these materials can deliver particulates, such as growth factors or peptides to induce differentiation and patterning [71]. Researchers based initial approaches to deliver these materials on intra myocardial injection, as this permits the direct and precise delivery to the affected area. Yet, with this approach, surgery becomes a must and there is a second issue of potential leaking of the material to other areas [72]. An alternative to this delivery system is the formation of so called “cardiac patches”. Even though they are not exclusive to collagen, cardiac patches hold impressive properties. One such feature is their potential to cultivate cells *ex vivo* to promote a proper invasion of the patch, further these patches can go into models and have high levels of engraftment [63]. Lastly, a 3D collagen type I matrix seeded with autologous bone marrow mononuclear cells (for the regeneration of ischemic myocardium) showed to increase the thickness of the infarct scar with viable tissues and helped to normalize cardiac wall stress in injured regions, limiting ventricular remodeling and improving diastolic function [73].

5.3.2. Fibrinogen scaffolds

Previous works have used both collagen and fibrin/fibrinogen patches to enhance maturation of cardiac cells by simulating cardiac muscle development [63,64]. One clear advantage of these materials was their help in promoting electrical conductivity, particularly on differentiating cells, along with other stimulants, certain maturation properties are achieved like the induction of Purkinje-like cells [63,74,75]. Fibrin is a naturally occurring biomaterial; a biopolymer formed during coagulation [68,76]. It has interested many researchers as alone or in combination, fibrin/fibrinogen helps with certain properties, including biocompatibility and biodegradability, when used as a scaffold. Fibrin comprises a 3D structure randomly organized with high interconnectivity, yet the threads forming the network are soft, allowing for deformation without a break. Fibrinogen decomposition (fibrinopeptides) which depends on the amount of thrombin used and the re-polymerization, regulates the mechanical properties of fibrin gels [76]. In clinical applications, fibrin is obtained from plasma for autologous applications such as to treat osteoarthritis [66]. It is important to mention that fibrin glue is another important application used typically in

surgical procedures to replace sutures [77]. Other useful applications include assisting in the repair of urinary tract, eye, liver, lung, spleen, and to fill bone cavities and heart valves [68,77–79].

5.3.3. Silk scaffolds

Using silk as a biomaterial for tissue engineering has been on the rise over several years, as it not only holds several similarities to other materials, such as fibrin/fibronectin (architecture, mechanical properties, and degradation rates) because silk, in comparison with fibronectin, does not contribute to pathological hypertrophy [80]. In animal models, silk-based scaffolds have shown therapeutic effects, as well as the capacity to maintain differentiation of cell to a cardiac lineage [81]. It is important to note that orientation is key, as defined in cardiac tissue studies, by demonstrating that the maintenance and development of sarcomeres, particularly there was an upregulation in the synthesis of titin protein [82].

6. Chitosan scaffolds

Chitosan is a polysaccharide obtained through the deacetylation of chitin. Normally found in insects and crustaceans like shrimps, crabs, and lobsters. It is a natural polymer, with a linear structure consisting of β (1-4) glycosidic bonds linked to d-glucosamine residues with a variable number of randomly located N-acetyl-d-glucosamine (NAG) groups. It is soluble in dilute or weak acids, but insoluble in aqueous solutions above pH 6.5 [69]. Recently, chitosan has been widely used in tissue engineering because of its capacity to protonate amino groups in acidic medium, providing high biocompatibility, non-toxicity, anti-thrombogenic, biodegradability properties, and possessing a hydrophilic surface [83]. These properties make chitosan scaffolds suitable for cell attachment and proliferation.

As we mentioned in scaffold section, all scaffolds used in tissue engineering must have the following essential properties [84]:

- Ability to promote adhesion, migration, differentiation, and proliferation cellular of tissues test *in vitro* and *in vivo*;
- Ability to interaction with tissues, including cells and extracellular matrixes;
- Be non-toxic, biodegradable and easily eliminable from the body;
- To have desirable mechanical, physicochemical, and structural properties, similar to tissue that be will repair;
- To have a bioactivity a surface;
- To have an appropriate porosity, through pore interconnection, pore size, and pore size distribution.

Chitosan scaffolds can exhibit all of these properties due to their chemical structure, as it resembles the cell's outer composite. However, the chitosan scaffolds alone have a limitation because weak mechanical properties and high rate of degradation [84]. To improve the properties of chitosan scaffolds, researchers have carried many investigations to elaborate chitosan composite scaffolds (chitosan combined with other materials including metallic nanoparticles, silk, collagen, hyaluronan, PEG, and alginate) and to improve mechanical, electrical, and biological properties. Other proposals used PLGA as a reinforcing material to chitosan [85], multiwalled carbon nanotubes [86], polyurethane [87] or PLLA to increase the mechanical, thermal and bioactivity properties of chitosan scaffolds [88].

6.1. Chitosan composite scaffolds

Over time, many formulations of scaffolds have been developed by either mixing two or more different biomaterials to mimic ECM native myocardium and its mechanical and biological properties. Recent studies have further used chitosan composite scaffolds for

myocardial tissue engineering, finding positive results. Studies show that chitosan composite scaffolds can increase cell survival and proliferation in EC [89]. The combination of chitosan scaffolds with stem cells has shown positive results in the delivery of stem cells to infarcted myocardium, and increase cell retention, while preserving cardiac function [90].

Among the most current investigations in cell delivery (primarily in small animal models e.g., rodent and rabbit), polyurethane/chitosan/CNT nano-fibrous scaffolds have shown appropriate biocompatibility to support cell attachment and proliferation, demonstrated the potential of these scaffolds in cardiac patch applications [91]. Composite poly lactic acid (PLA)/chitosan nanofibrous scaffolds support CM viability and elicit cell elongation. Immunofluorescence staining has further revealed high expression of cardiac marker, obtaining a promise scaffold for regenerating myocardia *in vitro* CM cultures [92]. Polycaprolactone/gelatin/chitosan hydrogel scaffolds with excellent biodegradable and tensile strength properties can be used for developing patches with the potential for surgical reconstruction of congenital heart defects [93], Chitosan/ polyvinyl alcohol/ multi-walled carbon nanotubes scaffolds have also showed positive results with similar properties in cardiac tissue differentiation process [94]. According to research from Liu et. al., a cardiac patch loaded with nanoparticles of TiO₂ into PEGylated chitosan hydrogel scaffold enhances the physicochemical, mechanical, and biological properties [95]. Equally, using a similar method for chitosan gelation, this has been injected in rats as hydrogel combined with MSC directly in left ventricular infarcted, showing MSC differentiation to CM and the increase of neo vasculature formation. In addition, chitosan scaffold cultured with brown adipose-derived stem cells have shown improvement of cardiac function and hemodynamics in an animal model, preventing adverse in matrix remodeling, increased angiogenesis and heart function preservation [96]. Biodegradable chitosan-based patches have also been developed with the potential of surgical reconstruction of heart defects. One such example can be seen in the work by Pok et al., wherein they developed a hydrogel based on a PCL self-assembled sandwiched in gelatin-chitosan. Their biomaterial mixing procedure allowed for optimal suturing properties, due to PCL tensile strength; on the other hand, gelatin-chitosan scaffold allows cell attachment, in addition to mechanical properties that promote CM migration and function [97].

At present, there is no evidence on the clinical use of chitosan-based tissue engineering therapies. Nevertheless, recent research has been devoted to study the potential use of chitosan-based biomaterials as an injectable therapy delivering progenitor cells [93]. Because of its hemostatic properties, chitosan has been used in the form of chitosan-based pads to improve hemostasis following transradial arterial access in a couple of clinical studies [98,99], and a few more ongoing clinical trials (NCT04380883, NCT04857385, NCT03522077, NCT02837744).

7. Preparation techniques of chitosan scaffolds

Different techniques are used to create chitosan composite scaffolds with the purpose of improving the mechanical and biocompatibility properties. To prepare chitosan scaffolds that mimic ECM native myocardium, various techniques have been developed these include but are not limited to electrospinning, phase-separation, and 3D printing (Figure 3) [100]. Understandably, each technique has its advantages and disadvantages, which we will discuss in a general in this section.

7.1. Electrospinning

Electrospinning is a technique used for micro-and nanoscale fiber production from an electrically forced solution/melt of polymers. It is a versatile electrohydrodynamic process with simple and relatively low preparation cost, generating fibers smaller than a micrometer. This method helps obtain a tunable porosity, unique pore shapes, and being able to control the scaffold composition. Various chitosan-based composite scaffolds have

been design using electrospinning, for example poly vinyl alcohol (PVA)/keratin/chitosan nonfibrous scaffolds from which we can obtain a uniform fibrous structures and suitable porosity [101]. One advantage is that the fibers are made to increase the surface-to-volume ratio, which improves the cell attachment, proliferation, and differentiation [102,103].

To implement electrospinning the four basic elements required are: a dosing pump with a syringe (containing the polymer solution inside), a needle (Taylor cone), a collector drum (can be a plate, metal screen, or rotating mandrel), and a high-voltage power supply (up to 30 kV). Briefly, electrospinning involves the generation of a charged polymer jet that is ejected through a high-voltage electric field; randomly rotating polymeric fibers rest on a grounded complex to create a scaffold, the solvent is evaporated, and the fibers of the polymer solidify [103,104]. It is important to consider every single parameter, like chitosan molecular weight, voltage, the distance between capillary and collector, polymer concentration, solution conductivity, and solvent volatility, which impact on characteristics and properties of the fibers [105]. Some additional advantages are uniformity, aligned fiber, the porosity of over 80%, strong interconnectivity of porosity, and superior mechanical properties. Disadvantages are the need for high-voltage appliances, solvents can be toxic, packaging, shipping, and handling [106].

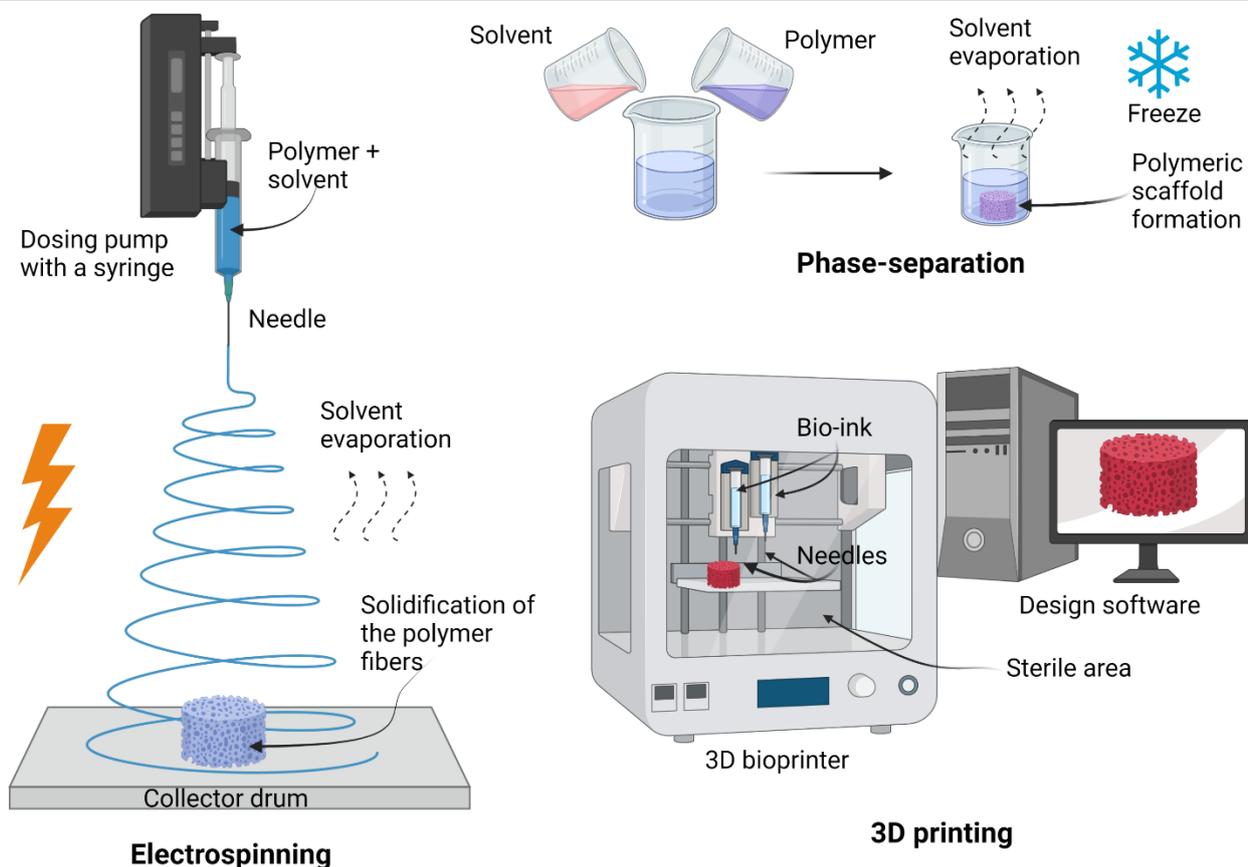


Figure 2. Scheme cartoon of principal preparation techniques of scaffolds. Electrospinning consists in the formation of fibers using a voltage source. It involves the generation of a charged polymer jet that is ejected through a high-voltage electric field. The phase separation method consists in a solution preparation of polymers and solvents, followed by the sublimation of the solvent after freezing. 3D printing generates a scaffold by computer design software, printing the scaffold layer by layer. Created with BioRender.com

7.2. Phase-separation

Also called thermally induced phase separation, is a simple method that comprises a solution preparation with biopolymers and solvents, followed by freezing. Once the mix

is prepared, the solvent is removed without degrading the polymer by sublimation via freeze-drying to obtain scaffolds with high porosity and interconnectivity [107]. Freezing temperature, concentration, nature of solvent and solute can be tunable to modify some pore characteristics [108]. As an advantage, the preservation of the scaffold structure stands out because of the freeze-drying process since it does not involve high temperatures that could damage the scaffold. Despite the simplicity of the technique, the modification of the pore size is limited, generating irregular porosity, moreover it is also necessary to have a freeze dryer, and it is important to note that the process can be quite lengthy in time. One of the major limitations of this technique is that the mechanical properties obtained are not suitable for replacing human tissue. Furthermore, this technique offers poor architecture, limited control of pore size range and remaining solvents are potentially toxic [106].

7.3. 3D printing

Another novel method for fabricating chitosan scaffolds is 3D printing. This technique is probably the most attractive in terms of micro-architecture, its use involves bio-printers, needles, bio-inks, and design software. Extrusion-based 3D printing has been a widely used technique to create polymer scaffolds given it allows usage of a wide range of materials, including chitosan due to excellent controllability over structural characteristics, such as porosity, pore size, and pore interconnectivity. For example, chitosan/alginate/hydroxyapatite scaffolds [109], chitosan/silk fibroin/cellulose nanoparticle scaffolds [110], and PLA/chitosan scaffolds [111] have been manufactured with this method.

Three-dimensional printing can use different technologies, mainly based on extrusion, light, and inkjet 3D printing [112]. Basically, a 3D model is generated using computer-aided design (CAD) software to be converted to an STL format. This is the 3D format of the scaffold, which is sliced into 2D layers to be successively printed and bound layer by layer. Hence, the resolution level depends on the diameter of needles used [112,113]. Some current bio-printers models mimic a sterile environment like a biosafety cabinet, using HEPA filters and UV lamps for sterilization. The process can be observed because of their clear windows. The drawbacks are the initial investment of the instrument, toxic solvents, and mechanical instability [104].

7.4. Solvent casting and solvent casting/particulate leaching

Solvent casting method is based on polymer and organic solvents mixing, followed by casting into 3D mold, or immersing the mold into the polymeric solution to get a scaffold once the solvent has been removed by simple evaporation, vacuum drying, or lyophilization. After the solvent removal, the scaffold is washed with water leaving the porous structure. The internal size of pores is related and controlled by salt granules, therefore maintaining desired porosity and uniform salt dispersion is a challenge. This method is simple, mechanically stable, and does not need sophisticated instruments. However, the disadvantages are related to the time for solvent evaporation and sometimes, the complete salt leach out. Also, complete removal of the solvent is important due to residuals can denature proteins [106,108,114].

7.5. Soft lithography

This is a set of microfabrication techniques that uses molds, elastomeric stamps, and conformable photomask, to microstructure fabrication around 1-1000 μm . This allows the control of molecules distribution, which subsequently improves cell distribution. Among its advantages, clean rooms and photolithographic equipment are unnecessary. The technique can be a cheap and rapid method for fabricating microfluidic channels and scaffolds because of the material used (PDMS-polydimethylsiloxane). This is capable for in vitro applications since is flexible, allowing the spread of CO_2 and O_2 , and can seal several surfaces. Furthermore, it can generate high fidelity of surfaces. The standard protocol for

generating elastomeric molds consists of pouring PDMS in viscous solution, onto a photoresist patterned surface, to be cured afterward, silicon wafer is used. Finally, PDMS mold is detached from the silicon wafer and the PDMS can be used as a new mold to generate hydrogels scaffolds, such as those based on chitosan [115].

8. Improving the physical proprieties of chitosan

Current limitations of cellular scaffolds include reduce electrical conductivity and suboptimal mechanical properties. The properties of chitosan alone offer great advantages over other polymers, so the study and analysis of its properties offer a better understanding of how these structures can be applied and improved in biological applications. The design of a suitable scaffold is the first step in the fabrication of a tissue engineering product, which should be able to support cell growth under *in vitro* conditions. In the heart, there is an important requirement to provide cellular attachment but also permit mechanical contractility and electrical conductivity [50].

8.1. Mechanical proprieties

While there are many aspects to consider in biomaterial design, mechanical properties may be of particular importance in this dynamically remodeling tissue to achieve the desired mechanical behavior [116,117]. The applications of chitosan scaffolds can be enhanced by adding fibers of the same material (chitosan fibers) and studying the effects of fiber/scaffold mass ratio, fiber mechanical properties, and fiber length. When fibers are incorporated, the strength and stiffness of the scaffold can be improved. Thus, heart valves reinforced with chitosan fibers achieved tensile strength values of 220 ± 17 kPa, comparable to human pulmonary valve values. The effects of 2 mm fibers were found to be up to three times greater than those of 10 mm fibers at identical mass ratios. The results show that chitosan fiber reinforcement can achieve a porous chitosan scaffold strength approaching that of tissue, and that fiber length and mechanical properties are important parameters in defining mechanical improvement, and as other studies have shown, varying chitosan concentrations increase strength by up to 9MPa [118].

But not only chitosan can improve itself, it is possible to find suitable materials that increase the properties of scaffolds, this is important for intracellular support and myocardial tissue function. The focus has been on natural polymers that include collagen, gelatin, chitosan, fibrin, alginate, etc. Among these, collagen/chitosan composite for their physical characteristics, biocompatibility, and contractile performance. These showed excellent mechanical properties, with an elastic modulus (81.0 ± 8.1 kPa) compared to natural myocardial tissue (20~100 kPa) [69]. Another particularity of the improvement of chitosan scaffolds is the addition of PLA by conventional electrospinning. The addition of PLA fibers to chitosan significantly improved both elongation and tensile strength, increasing Young's modulus from 57.38 ± 13.21 MPa to 78.67 ± 14.15 MPa. This large increase is attributable to the thermodynamic immiscibility and inherent incompatibility between thermoplastic polymers and chitosan, thus composite scaffolds consisting of PLA have great potential for cardiac tissue enhancement and regeneration [92]. Even non-polymeric aggregates have been effective for the enhancement of chitosan scaffolds, such as graphene, its tensile strength of 130 GPa compared to 0.4 GPa of structural steel or 70 GPa of Kevlar, make it a perfect candidate for these purposes [119,120].

8.2. Electrical proprieties

In addition to the improvement of the mechanical properties, it is possible to improve the electrical properties by including a carbon fiber coating on the chitosan scaffolds. Cardiac muscle is an electroactive tissue that can transfer electrical signals across the heart. Cardiac tissue engineering requires materials that can faithfully recapitulate and support the native *in vivo* microenvironment while providing a seamless bioelectronic interface. An important property for the selection of the scaffold is the electrical behavior.

Conductive materials for preparing scaffolds are preferred in cardiac regeneration, and although chitosan is a nonconductive material, its electrical potential can improve with the combination of some conductive materials such as carbon nanotubes or carbon nanofibers [121].

Carbon nanostructures are commonly used in combination with other materials for preparing biological matrices, to improve multiple functions, such as electrical conductivity. Interestingly, carbon nanofibers have more sites on the outer wall than carbon nanotubes, which can facilitate the electron transfer of electroactive analytes. Carbon nanofibers, can further reinforce polymer scaffolds and produce excellent mechanical properties. Chitosan/carbon scaffolds, prepared by Martins et al, had elastic modulus of 28.1 ± 3.3 KPa, similar to rat myocardium and an electrical property of conductivity of 0.25 ± 0.09 S/m [120,121]. Conductivity properties can also be promoted by gold nanoparticle-chitosan hydrogels, or graphene oxide-chitosan scaffolds by freezing and lyophilization, which have been observed that enhanced cardiomyogenic differentiation and protein expression involved in muscle conduction [122,123].

An example of a conductive chitosan composite was reported by Shabnam Mombini et. al., and was based on chitosan-PVA-carbon nanotube, prepared by electrospinning. This scaffold showed optimal properties for cardiac differentiation, showing an elastic modulus of 130 ± 3.605 MPa, 70-80% porosity, and >80% cell viability. Also, nanofibers content of 1% carbon nanotubes, providing an electrical conductivity of 3.4×10^{-6} S/Cm. It could enhance MSC differentiation to CM grown in a media supplied with 5-azacytidine, TGF-B, and ascorbic acid. They also observed a significant increase in cardiac markers as Nkx2.5, troponin I, and β -MHC [124]. Similarly, Chiu Loraine et. al, added that not only electrical stimulation is important but also fiber orientation to achieve beating and optimal cellular phenotype maintenance in a scaffold based on collagen-chitosan hydrogel by soft lithography and photo cross-linking, cultured with CM in a bioreactor which had an array aligning microgrooves with electrical field lines, stimulated with biphasic square pulses (1 Hz and 2.5 V/cm) [125]. The diameter of the fiber in electrospinning plays an important role in conductivity; according to Abedi et al., reduction of average fiber diameter from 225 to 110 nm in a scaffold based on chitosan and PVA with multi-wall carbon nanotubes, increased electrical conductivity, from 8×10^{-5} S/m to 9×10^{-3} S/m. Therefore, it is recommended to deploy the conductivity, electrical stimulation, and chemical differentiation factors to obtain effective cardiac tissue engineering [94].

5. Conclusions

Undoubtedly, there are multiple models and proposals of chitosan scaffolds, and composite chitosan scaffolds, in combination with cells and biochemical factors, to provide an adequate microenvironment to mimic the characteristics necessary for the maintenance of the cardiac phenotype: the structural, mechanical, and conductivity properties of the native tissue. Nevertheless, we have a long road ahead to expand the knowledge on cell-electrical interactions and promoting electroactive tissue repair, to finally, be able to generate artificial hearts, which would reach the goal of medicine and tissue engineering field.

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Abbreviations

CF	Cardiac fibroblast
CM	Cardiomyocytes
CPC	Cardiac progenitor cells
EC	Endothelial cells
EPC	Endothelial progenitor cells
EMC	Extracellular matrix
HSC	Hematopoietic stem cells
iPSC	Induced pluripotent stem cells
MSC	Mesenchymal stem cells
NHSC	Non-hematopoietic stem cells
PCL	Poly(ϵ -caprolactone)
PEG	Polyethylene glycol
PGS	Poly(glycerol sebacate)
PLA	Poly lactic acid
PLCL	Poly l-lactic-co- ϵ -caprolactone
PLGA	Poly(lactic-co-glycolic acid)
PLLA	Poly(l-lactide acid)
PVA	Polyvinyl alcohol
RCSC	Resident cardiac stem cells
SMC	Smooth muscle cells
VSMC	Vascular smooth muscle cells

References

- Rossi, G.; Broguiere, N.; Miyamoto, M.; Boni, A.; Guiet, R.; Girgin, M.; Kelly, R.G.; Kwon, C.; Lutolf, M.P. Capturing Cardiogenesis in Gastruloids. *Cell Stem Cell* **2021**, *28*(2), 230-240.e6. doi: 10.1016/j.stem.2020.10.013. Epub 2020 Nov 10. PMID: 33176168; PMID: PMC7867643.
- Huang, N.F.; Serpooshan, V.; Morris, V.B.; Sayed, N.; Pardon, G.; Abilez, O.J.; Nakayama, K.H.; Pruitt, B.L.; Wu, S.M.; Yoon, Y.S.; Zhang, J.; Wu, J.C. Big bottlenecks in cardiovascular tissue engineering. *Commun Biol* **2018**, *1*, 199. doi: 10.1038/s42003-018-0202-8. PMID: 30480100; PMID: PMC6249300.
- Bergmann, O.; Zdunek, S.; Felker, A.; Salehpour, M.; Alkass, K.; Bernard, S.; Sjostrom, S.L.; Szewczykowska, M.; Jackowska, T.; Dos Remedios, C.; Malm, T.; Andr a, M.; Jashari, R.; Nyengaard, J.R.; Possnert, G.; Jovinge, S.; Druid, H.; Fris n, J. Dynamics of Cell Generation and Turnover in the Human Heart. *Cell* **2015**, *161*(7), 1566-75. doi: 10.1016/j.cell.2015.05.026. Epub 2015 Jun 11. PMID: 26073943.
- Bergmann, O.; Bhardwaj, R.D.; Bernard, S.; Zdunek, S.; Barnab e-Heider, F.; Walsh, S.; Zupicich, J.; Alkass, K.; Buchholz, B.A.; Druid, H.; Jovinge, S.; Fris n, J. Evidence for cardiomyocyte renewal in humans. *Science* **2009**, *324*(5923), 98-102. doi: 10.1126/science.1164680. PMID: 19342590; PMID: PMC2991140.
- Sawabe, M. Vascular aging: from molecular mechanism to clinical significance. *Geriatr Gerontol Int* **2010**, *10* Suppl 1, S213-20. doi: 10.1111/j.1447-0594.2010.00603.x. PMID: 20590836.
- Chiu, J.J.; Chien, S. Effects of disturbed flow on vascular endothelium: pathophysiological basis and clinical perspectives. *Physiol Rev* **2011**, *91*(1), 327-87. doi: 10.1152/physrev.00047.2009. PMID: 21248169; PMID: PMC3844671.
- Severino, P.; D'Amato, A.; Pucci, M.; Infusino, F.; Adamo, F.; Birtolo, L.I.; Netti, L.; Montefusco, G.; Chimenti, C.; Lavalle, C.; Maestrini, V.; Mancone, M.; Chilian, W.M.; Fedele, F. Ischemic Heart Disease Pathophysiology Paradigms Overview: From Plaque Activation to Microvascular Dysfunction. *Int J Mol Sci* **2020**, *21*(21), 8118. doi: 10.3390/ijms21218118. PMID: 33143256; PMID: PMC7663258.
- Riching, A.S.; Song, K. Cardiac Regeneration: New Insights Into the Frontier of Ischemic Heart Failure Therapy. *Front Bioeng Biotechnol* **2021**, *8*, 637538. doi: 10.3389/fbioe.2020.637538. PMID: 33585427; PMID: PMC7873479.
- de Bakker, D.E.M.; Bouwman, M.; Dronkers, E.; Sim es, F.C.; Riley, P.R.; Goumans, M.J.; Smits, A.M.; Bakkers, J. Prrx1b restricts fibrosis and promotes Nrg1-dependent cardiomyocyte proliferation during zebrafish heart regeneration. *Development* **2021**, *148*(19), dev198937. doi: 10.1242/dev.198937. Epub 2021 Oct 4. PMID: 34486669; PMID: PMC8513610.
- Gemberling, M.; Karra, R.; Dickson, A.L.; Poss, K.D. Nrg1 is an injury-induced cardiomyocyte mitogen for the endogenous heart regeneration program in zebrafish. *Elife* **2015**, *4*, e05871. doi: 10.7554/eLife.05871. PMID: 25830562; PMID: PMC4379493.
- Locatelli, P.; Gim enez, C.S.; Vega, M.U.; Crottogini, A.; Belaich, M.N. Targeting the Cardiomyocyte Cell Cycle for Heart Regeneration. *Curr Drug Targets* **2019**, *20*(2), 241-254. doi: 10.2174/1389450119666180801122551. PMID: 30068271.

12. Sánchez-Arias, A.G.; Bobadilla-Serrano, M.E.; Dimas-Altamirano, B.; Gómez-Ortega, M.; González-González, G. Enfermedad cardiovascular: primera causa de morbilidad en un hospital de tercer nivel. *Rev Mex Cardiol* **2016**, *27*(Suppl: 3), 98-102.
13. Francula-Zaninovic, S.; Nola, I.A. Management of Measurable Variable Cardiovascular Disease' Risk Factors. *Curr Cardiol Rev* **2018**, *14*(3), 153-163. doi: 10.2174/1573403X14666180222102312. PMID: 29473518; PMCID: PMC6131408.
14. European Association for Cardiovascular Prevention & Rehabilitation; Reiner, Z.; Catapano, A.L.; De Backer, G.; Graham, I.; Taskinen, M.R.; Wiklund, O.; Agewall, S.; Alegria, E.; Chapman, M.J.; Durrington, P.; Erdine, S.; Halcox, J.; Hobbs, R.; Kjekshus, J.; Filardi, P.P.; Riccardi, G.; Storey, R.F.; Wood, D.; ESC Committee for Practice Guidelines (CPG) 2008-2010 and 2010-2012 Committees. ESC/EAS Guidelines for the management of dyslipidaemias: the Task Force for the management of dyslipidaemias of the European Society of Cardiology (ESC) and the European Atherosclerosis Society (EAS). *Eur Heart J* **2011**, *32*(14), 1769-818. doi: 10.1093/eurheartj/ehr158. Epub 2011 Jun 28. PMID: 21712404.
15. Costantino, S.; Paneni, F.; Cosentino, F. Ageing, metabolism and cardiovascular disease. *J Physiol* **2016**, *594*(8):2061-73. doi: 10.1113/JP270538. Epub 2015 Oct 22. PMID: 26391109; PMCID: PMC4933114.
16. Shufelt, C.L.; Pacheco, C.; Tweet, M.S.; Miller, V.M. Sex-Specific Physiology and Cardiovascular Disease. *Adv Exp Med Biol* **2018**, *1065*, 433-454. doi: 10.1007/978-3-319-77932-4_27. PMID: 30051400; PMCID: PMC6768431.
17. Müller, P.; Lemcke, H.; David, R. Stem Cell Therapy in Heart Diseases - Cell Types, Mechanisms and Improvement Strategies. *Cell Physiol Biochem* **2018**, *48*(6), 2607-2655. doi: 10.1159/000492704. Epub 2018 Aug 17. PMID: 30121644.
18. Giacomelli, E.; Bellin, M.; Sala, L.; van Meer, B.J.; Tertoolen, L.G.; Orlova, V.V.; Mummery, C.L. Three-dimensional cardiac microtissues composed of cardiomyocytes and endothelial cells co-differentiated from human pluripotent stem cells. *Development* **2017**, *144*(6), 1008-1017. doi: 10.1242/dev.143438. PMID: 28279973; PMCID: PMC5358113.
19. Tavakol, D.N.; Fleischer, S.; Vunjak-Novakovic, G. Harnessing organs-on-a-chip to model tissue regeneration. *Cell Stem Cell* **2021**, *28*(6), 993-1015. doi: 10.1016/j.stem.2021.05.008. PMID: 34087161; PMCID: PMC8186820.
20. Augustine, R.; Dan, P.; Hasan, A.; Khalaf, I.M.; Prasad, P.; Ghosal, K.; Gentile, C.; McClements, L.; Maureira, P. Stem cell-based approaches in cardiac tissue engineering: controlling the microenvironment for autologous cells. *Biomed Pharmacother* **2021**, *138*, 111425. doi: 10.1016/j.biopha.2021.111425. Epub 2021 Mar 23. PMID: 33756154.
21. Campostrini, G.; Windt, L.M.; van Meer, B.J.; Bellin, M.; Mummery, C.L. Cardiac Tissues From Stem Cells: New Routes to Maturation and Cardiac Regeneration. *Circ Res* **2021**, *128*(6), 775-801. doi: 10.1161/CIRCRESAHA.121.318183. Epub 2021 Mar 18. Erratum in: *Circ Res.* 2021 Sep 17;129(7):e144. PMID: 33734815; PMCID: PMC8410091.
22. Miyamoto, M.; Nam, L.; Kannan, S.; Kwon, C. Heart organoids and tissue models for modeling development and disease. *Semin Cell Dev Biol* **2021**, *118*, 119-128. doi: 10.1016/j.semcdb.2021.03.011. Epub 2021 Mar 26. PMID: 33775518; PMCID: PMC8513373.
23. Davidson, S.M.; Padró, T.; Bollini, S.; Vilahur, G.; Duncker, D.J.; Evans, P.C.; Guzik, T.; Hofer, I.E.; Waltenberger, J.; Wojta, J.; Weber, C. Progress in cardiac research: from rebooting cardiac regeneration to a complete cell atlas of the heart. *Cardiovasc Res* **2021**, *117*(10), 2161-2174. doi: 10.1093/cvr/cvab200. PMID: 34114614; PMCID: PMC8344830.
24. Voges, H.K.; Mills, R.J.; Elliott, D.A.; Parton, R.G.; Porrello, E.R.; Hudson, J.E. Development of a human cardiac organoid injury model reveals innate regenerative potential. *Development* **2017**, *144*(6), 1118-1127. doi: 10.1242/dev.143966. Epub 2017 Feb 7. PMID: 28174241.
25. Montero, P.; Flandes-Iparraguirre, M.; Musquiz, S.; Pérez Araluce, M.; Plano, D.; Sanmartín, C.; Orive, G.; Gavira, J.J.; Prosper, F.; Mazo, M.M. Cells, Materials, and Fabrication Processes for Cardiac Tissue Engineering. *Front Bioeng Biotechnol* **2020**, *8*, 955. doi: 10.3389/fbioe.2020.00955. PMID: 32850768; PMCID: PMC7431658.
26. Alrefai, M.T.; Murali, D.; Paul, A.; Ridwan, K.M.; Connell, J.M.; Shum-Tim, D. Cardiac tissue engineering and regeneration using cell-based therapy. *Stem Cells Cloning* **2015**, *8*, 81-101. doi: 10.2147/SCCAA.S54204. PMID: 25999743; PMCID: PMC4437607.
27. Roura, S.; Pujal, J.M.; Gálvez-Montón, C.; Bayes-Genis, A. Impact of umbilical cord blood-derived mesenchymal stem cells on cardiovascular research. *Biomed Res Int* **2015**, *2015*, 975302. doi: 10.1155/2015/975302. Epub 2015 Mar 10. PMID: 25861654; PMCID: PMC4377460.
28. Sun, Q.; Zhang, Z.; Sun, Z. The potential and challenges of using stem cells for cardiovascular repair and regeneration. *Genes Dis* **2014**, *1*(1), 113-119. doi: 10.1016/j.gendis.2014.07.003. PMID: 25642448; PMCID: PMC4307803.
29. Bruun, K.; Schermer, E.; Sivendra, A.; Valaik, E.; Wise, R.B.; Said, R.; Bracht, J.R. Therapeutic applications of adipose-derived stem cells in cardiovascular disease. *Am J Stem Cells* **2018**, *7*(4), 94-103. PMID: 30510844; PMCID: PMC6261868.
30. Arjmand, B.; Abedi, M.; Arabi, M.; Alavi-Moghadam, S.; Rezaei-Tavirani, M.; Hadavandkhani, M.; Tayanloo-Beik, A.; Kordi, R.; Roudsari, P.P.; Larijani, B. Regenerative Medicine for the Treatment of Ischemic Heart Disease; Status and Future Perspectives. *Front Cell Dev Biol* **2021**, *9*, 704903. doi: 10.3389/fcell.2021.704903. PMID: 34568321; PMCID: PMC8461329.
31. Chopra, H.; Hung, M.K.; Kwong, D.L.; Zhang, C.F.; Pow, E.H.N. Insights into Endothelial Progenitor Cells: Origin, Classification, Potentials, and Prospects. *Stem Cells Int* **2018**, *2018*, 9847015. doi: 10.1155/2018/9847015. PMID: 30581475; PMCID: PMC6276490.
32. Liu, N.; Qi, X.; Han, Z.; Liang, L.; Kong, D.; Han, Z.; Zhao, S.; He, Z.X.; Li, Z. Bone Marrow Is a Reservoir for Cardiac Resident Stem Cells. *Sci Rep* **2016**, *6*, 28739. doi: 10.1038/srep28739. PMID: 27345618; PMCID: PMC4921812.
33. Fan, C.; Zhang, E.; Joshi, J.; Yang, J.; Zhang, J.; Zhu, W. Utilization of Human Induced Pluripotent Stem Cells for Cardiac Repair. *Front Cell Dev Biol* **2020**, *8*, 36. doi: 10.3389/fcell.2020.00036. PMID: 32117968; PMCID: PMC7025514.
34. Chen, W.; Bian, W.; Zhou, Y.; Zhang, J. Cardiac Fibroblasts and Myocardial Regeneration. *Front Bioeng Biotechnol* **2021**, *9*, 599928. doi: 10.3389/fbioe.2021.599928. PMID: 33842440; PMCID: PMC8026894.

35. Durrani, S.; Konoplyannikov, M.; Ashraf, M.; Haider, K.H. Skeletal myoblasts for cardiac repair. *Regen Med* **2010**, *5*(6), 919-32. doi: 10.2217/rme.10.65. PMID: 21082891; PMCID: PMC3074361.
36. Takahashi, K.; Tanabe, K.; Ohnuki, M.; Narita, M.; Ichisaka, T.; Tomoda, K.; Yamanaka, S. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* **2007**, *131*(5), 861-72. doi: 10.1016/j.cell.2007.11.019. PMID: 18035408.
37. Ieda, M.; Fu, J.D.; Delgado-Olguin, P.; Vedantham, V.; Hayashi, Y.; Bruneau, B.G.; Srivastava, D. Direct reprogramming of fibroblasts into functional cardiomyocytes by defined factors. *Cell* **2010**, *142*(3), 375-86. doi: 10.1016/j.cell.2010.07.002. PMID: 20691899; PMCID: PMC2919844.
38. Burridge, P.W.; Keller, G.; Gold, J.D.; Wu, J.C. Production of de novo cardiomyocytes: human pluripotent stem cell differentiation and direct reprogramming. *Cell Stem Cell* **2012**, *10*(1), 16-28. doi: 10.1016/j.stem.2011.12.013. PMID: 22226352; PMCID: PMC3255078.
39. Liu, Y.; Chen, L.; Diaz, A.D.; Benham, A.; Xu, X.; Wijaya, C.S.; Fa'ak, F.; Luo, W.; Soibam, B.; Azares, A.; Yu, W.; Lyu, Q.; Stewart, M.D.; Gunaratne, P.; Cooney, A.; McConnell, B.K.; Schwartz, R.J. Mesp1 Marked Cardiac Progenitor Cells Repair Infarcted Mouse Hearts. *Sci Rep* **2016**, *6*, 31457. doi: 10.1038/srep31457. PMID: 27538477; PMCID: PMC4990963.
40. Chiapparò, G.; Lin, X.; Lescroart, F.; Chabab, S.; Paulissen, C.; Pitisci, L.; Bondue, A.; Blanpain, C. Mesp1 controls the speed, polarity, and directionality of cardiovascular progenitor migration. *J Cell Biol* **2016**, *213*(4), 463-77. doi: 10.1083/jcb.201505082. Epub 2016 May 16. PMID: 27185833; PMCID: PMC4878090.
41. Song, K.; Nam, Y.J.; Luo, X.; Qi, X.; Tan, W.; Huang, G.N.; Acharya, A.; Smith, C.L.; Tallquist, M.D.; Neilson, E.G.; Hill, J.A.; Bassel-Duby, R.; Olson, E.N. Heart repair by reprogramming non-myocytes with cardiac transcription factors. *Nature* **2012**, *485*(7400), 599-604. doi: 10.1038/nature11139. PMID: 22660318; PMCID: PMC3367390.
42. Abad, M.; Hashimoto, H.; Zhou, H.; Morales, M.G.; Chen, B.; Bassel-Duby, R.; Olson, E.N. Notch Inhibition Enhances Cardiac Reprogramming by Increasing MEF2C Transcriptional Activity. *Stem Cell Reports* **2017**, *8*(3), 548-560. doi: 10.1016/j.stemcr.2017.01.025. Epub 2017 Mar 2. PMID: 28262548; PMCID: PMC5355682.
43. den Hartogh, S.C.; Wolstencroft, K.; Mummery, C.L.; Passier, R. A comprehensive gene expression analysis at sequential stages of in vitro cardiac differentiation from isolated MESP1-expressing-mesoderm progenitors. *Sci Rep* **2016**, *6*, 19386. doi: 10.1038/srep19386. PMID: 26783251; PMCID: PMC4726039.
44. Singh, V.P.; Pinnamaneni, J.P.; Pugazenthi, A.; Sanagasetti, D.; Mathison, M.; Wang, K.; Yang, J.; Rosengart, T.K. Enhanced Generation of Induced Cardiomyocytes Using a Small-Molecule Cocktail to Overcome Barriers to Cardiac Cellular Reprogramming. *J Am Heart Assoc* **2020**, *9*(12), e015686. doi: 10.1161/JAHA.119.015686. Epub 2020 Jun 5. PMID: 32500803; PMCID: PMC7429035.
45. Jayawardena, T.M.; Egemnazarov, B.; Finch, E.A.; Zhang, L.; Payne, J.A.; Pandya, K.; Zhang, Z.; Rosenberg, P.; Mirotsov, M.; Dzau, V.J. MicroRNA-mediated in vitro and in vivo direct reprogramming of cardiac fibroblasts to cardiomyocytes. *Circ Res* **2012**, *110*(11), 1465-73. doi: 10.1161/CIRCRESAHA.112.269035. Epub 2012 Apr 26. PMID: 22539765; PMCID: PMC3380624.
46. Raso, A.; Dirx, E.; Sampaio-Pinto, V.; El Azzouzi, H.; Cubero, R.J.; Sorensen, D.W.; Ottaviani, L.; Olieslagers, S.; Huijbers, M.M.; de Weger, R.; Siddiqi, S.; Moimas, S.; Torrini, C.; Zentilin, L.; Braga, L.; Nascimento, D.S.; da Costa Martins, P.A.; van Berlo, J.H.; Zacchigna, S.; Giacca, M.; De Windt, L.J. A microRNA program regulates the balance between cardiomyocyte hyperplasia and hypertrophy and stimulates cardiac regeneration. *Nat Commun* **2021**, *12*(1), 4808. doi: 10.1038/s41467-021-25211-4. PMID: 34376683; PMCID: PMC8355162.
47. Pandey, R.; Yang, Y.; Jackson, L.; Ahmed, R.P. MicroRNAs regulating meis1 expression and inducing cardiomyocyte proliferation. *Cardiovasc Regen Med* **2016**, *3*, e1468. PMID: 28111633; PMCID: PMC5245919.
48. Trombino, S.; Curcio, F.; Cassano, R.; Curcio, M.; Cirillo, G.; Iemma, F. Polymeric Biomaterials for the Treatment of Cardiac Post-Infarction Injuries. *Pharmaceutics* **2021**, *13*(7), 1038. doi: 10.3390/pharmaceutics13071038. PMID: 34371729; PMCID: PMC8309168.
49. Parmaksiz, M.; Dogan, A.; Odabas, S.; Elçin, A.E.; Elçin, Y.M. Clinical applications of decellularized extracellular matrices for tissue engineering and regenerative medicine. *Biomed Mater* **2016**, *11*(2), 022003. doi: 10.1088/1748-6041/11/2/022003. PMID: 26989100.
50. Kc, P.; Hong, Y.; Zhang, G. Cardiac tissue-derived extracellular matrix scaffolds for myocardial repair: advantages and challenges. *Regen Biomater* **2019**, *6*(4), 185-199. doi: 10.1093/rb/rbz017. Epub 2019 Apr 22. PMID: 31404421; PMCID: PMC6683951.
51. Taylor, D.A.; Parikh, R.B.; Sampaio, L.C. Bioengineering Hearts: Simple yet Complex. *Curr Stem Cell Rep* **2017**, *3*(1), 35-44. doi: 10.1007/s40778-017-0075-7. Epub 2017 Feb 10. PMID: 28261549; PMCID: PMC5315720.
52. Tang-Quan, K.R.; Mehta, N.A.; Sampaio, L.C.; Taylor, D.A. Whole Cardiac Tissue Bioscaffolds. *Adv Exp Med Biol* **2018**, *1098*, 85-114. doi: 10.1007/978-3-319-97421-7_5. PMID: 30238367.
53. Svystonyuk, D.A.; Mewhort, H.E.M.; Hassanabad, A.F.; Heydari, B.; Mikami, Y.; Turnbull, J.D.; Teng, G.; Belke, D.D.; Wagner, K.T.; Tarraf, S.A.; DiMartino, E.S.; White, J.A.; Flewitt, J.A.; Cheung, M.; Guzzardi, D.G.; Kang, S.; Fedak, P.W.M. Acellular bioscaffolds redirect cardiac fibroblasts and promote functional tissue repair in rodents and humans with myocardial injury. *Sci Rep* **2020**, *10*(1), 9459. doi: 10.1038/s41598-020-66327-9. PMID: 32528051; PMCID: PMC7289874.
54. Prat-Vidal, C.; Rodríguez-Gómez, L.; Aylagas, M.; Nieto-Nicolau, N.; Gastelurrutia, P.; Agustí, E.; Gálvez-Montón, C.; Jorba, I.; Teis, A.; Monguió-Tortajada, M.; Roura, S.; Vives, J.; Torrents-Zapata, S.; Coca, M.I.; Reales, L.; Cámara-Rosell, M.L.; Cediél, G.; Coll, R.; Farré, R.; Navajas, D.; Vilarrodona, A.; García-López, J.; Muñoz-Guijosa, C.; Querol, S.; Bayes-Genis, A. First-in-human PeriCord cardiac bioimplant: Scalability and GMP manufacturing of an allogeneic engineered tissue graft. *EBioMedicine* **2020**, *54*, 102729. doi: 10.1016/j.ebiom.2020.102729. Epub 2020 Apr 15. PMID: 32304998; PMCID: PMC7163319.

55. Cebotari, S.; Lichtenberg, A.; Tudorache, I.; Hilfiker, A.; Mertsching, H.; Leyh, R.; Breymann, T.; Kallenbach, K.; Maniuc, L.; Batrinac, A.; Repin, O.; Maliga, O.; Ciubotaru, A.; Haverich, A. Clinical application of tissue engineered human heart valves using autologous progenitor cells. *Circulation* **2006**, *114*(1 Suppl), I132-7. doi: 10.1161/CIRCULATIONAHA.105.001065. PMID: 16820562.
56. Jang, Y.; Park, Y.; Kim, J. Engineering Biomaterials to Guide Heart Cells for Matured Cardiac Tissue. *Coatings* **2020**, *10*, 925. <https://doi.org/10.3390/coatings10100925>
57. Huyer, L.D.; Montgomery, M.; Zhao, Y.; Xiao, Y.; Conant, G.; Korolj, A.; Radisic, M. Biomaterial based cardiac tissue engineering and its applications. *Biomed Mater* **2015**, *10*(3), 034004. doi: 10.1088/1748-6041/10/3/034004. PMID: 25989939; PMCID: PMC4464787.
58. Matsubayashi, K.; Fedak, P.W.; Mickle, D.A.; Weisel, R.D.; Ozawa, T.; Li, R.K. Improved left ventricular aneurysm repair with bioengineered vascular smooth muscle grafts. *Circulation* **2003**, *108* Suppl 1, II219-25. doi: 10.1161/01.cir.0000087450.34497.9a. PMID: 12970236.
59. Morgan, K.Y.; Sklaviadis, D.; Tochka, Z.L.; Fischer, K.M.; Hearon, K.; Morgan, T.D.; Langer, R.; Freed, L.E. Multi-Material Tissue Engineering Scaffold with Hierarchical Pore Architecture. *Adv Funct Mater* **2016**, *26*(32), 5873-5883. doi: 10.1002/adfm.201601146. Epub 2016 Jun 13. PMID: 27942257; PMCID: PMC5142531.
60. Hu, T.; Wu, Y.; Zhao, X.; Wang, L.; Bi, L.; Ma, P.X.; Guo, B. Micropatterned, electroactive, and biodegradable poly(glycerol sebacate)-aniline trimer elastomer for cardiac tissue engineering. *Chem Eng J* **2019**, *366*, 208–222. doi:10.1016/j.cej.2019.02.072.
61. Zhang, X.; Xu, B.; Puperi, D.S.; Yonezawa, A.L.; Wu, Y.; Tseng, H.; Cuchiara, M.L.; West, J.L.; Grande-Allen, K.J. Integrating valve-inspired design features into poly(ethylene glycol) hydrogel scaffolds for heart valve tissue engineering. *Acta Biomater* **2015**, *14*, 11–21. doi:10.1016/j.actbio.2014.11.042.
62. Sugiura, T.; Matsumura, G.; Miyamoto, S.; Miyachi, H.; Breuer, C.K.; Shinoka, T. Tissue-engineered Vascular Grafts in Children With Congenital Heart Disease: Intermediate Term Follow-up. *Semin Thorac Cardiovasc Surg* **2018**, *30*(2), 175-179. doi: 10.1053/j.semtcvs.2018.02.002. Epub 2018 Feb 7. PMID: 29427773; PMCID: PMC6380348.
63. Mohamed, M.A.; Islas, J.F.; Schwartz, R.J.; Birla, R.K. Electrical Stimulation of Artificial Heart Muscle: A Look Into the Electrophysiologic and Genetic Implications. *ASAIO J* **2017**, *63*(3), 333-341. doi: 10.1097/MAT.0000000000000486. PMID: 28459744; PMCID: PMC5469367.
64. Salazar, B.H.; Reddy, A.K.; Tao, Z.; Madala, S.; Birla, R.K. 32-Channel system to measure the electrophysiological properties of bioengineered cardiac muscle. *IEEE Trans Biomed Eng* **2015**, *62*, 1614–1622. doi:10.1109/TBME.2015.2399437.
65. Gupta, N.; Cruz, M.A.; Nasser, P.; Rosenberg, J.D.; Iatridis, J.C. Fibrin-Genipin Hydrogel for Cartilage Tissue Engineering in Nasal Reconstruction. *Ann Otol Rhinol Laryngol* **2019**, *128*, 640–646. doi:10.1177/0003489419836667.
66. Setayeshmehr, M.; Esfandiari, E.; Hashemibeni, B.; Tavakoli, A.H.; Rafienia, M.; Samadikuchaksaraei, A.; Moroni, L.; Joghataei, M.T. Chondrogenesis of human adipose-derived mesenchymal stromal cells on the [devitalized costal cartilage matrix/poly(vinyl alcohol)/fibrin] hybrid scaffolds. *Eur Polym J* **2019**, *118*, 528–541. doi:10.1016/j.eurpolymj.2019.04.044.
67. Saeedi Garakani, S.; Khanmohammadi, M.; Atoufi, Z.; Kamrava, S.K.; Setayeshmehr, M.; Alizadeh, R.; Faghihi, F.; Bagher, Z.; Davachi, S.M.; Abbaspourrad, A. Fabrication of chitosan/agarose scaffolds containing extracellular matrix for tissue engineering applications. *Int J Biol Macromol* **2020**, *143*, 533-545. doi: 10.1016/j.ijbiomac.2019.12.040. Epub 2019 Dec 6. PMID: 31816374.
68. Majid, Q.A.; Fricker, A.T.R.; Gregory, D.A.; Davidenko, N.; Hernandez Cruz, O.; Jabbour, R.J.; Owen, T.J.; Basnett, P.; Lukasiewicz, B.; Stevens, M.; Best, S.; Cameron, R.; Sinha, S.; Harding, S.E.; Roy, I. Natural Biomaterials for Cardiac Tissue Engineering: A Highly Biocompatible Solution. *Front Cardiovasc Med* **2020**, *7*, 554597. doi: 10.3389/fcvm.2020.554597. PMID: 33195451; PMCID: PMC7644890.
69. Fang, Y.; Zhang, T.; Song, Y.; Sun, W. Assessment of various crosslinking agents on collagen/chitosan scaffolds for myocardial tissue engineering. *Biomed Mater* **2020**, *15*, doi:10.1088/1748-605X/ab452d.
70. Gotenstein, J.R.; Koo, C.C.; Ho, T.W.; Chisholm, A.D. Genetic suppression of basement membrane defects in *Caenorhabditis elegans* by gain of function in extracellular matrix and cell-matrix attachment genes. *Genetics* **2018**, *208*, 1499–1512. doi:10.1534/genetics.118.300731.
71. Wu, W.; Peng, S.; Song, Z.; Lin, S. Collagen biomaterial for the treatment of myocardial infarction: an update on cardiac tissue engineering and myocardial regeneration. *Drug Deliv Transl Res* **2019**, *9*, 920–934. doi:10.1007/s13346-019-00627-0.
72. Johnson, T.D.; Christman, K.L. Injectable hydrogel therapies and their delivery strategies for treating myocardial infarction. *Expert Opin Drug Deliv* **2013**, *10*, 59–72. doi:10.1517/17425247.2013.739156.
73. Chachques, J.C.; Trainini, J.C.; Lago, N.; Masoli, O.H.; Barisani, J.L.; Cortes-Morichetti, M.; Schussler, O.; Carpentier, A. Myocardial assistance by grafting a new bioartificial upgraded myocardium (MAGNUM clinical trial): one year follow-up. *Cell Transplant* **2007**, *16*(9), 927-34. doi: 10.3727/096368907783338217. PMID: 18293891.
74. Raghunathan, S.; Islas, J.F.; Mistretta, B.; Iyer, D.; Shi, L.; Gunaratne, P.H.; Ko, G.; Schwartz, R.J.; McConnell, B.K. Conversion of human cardiac progenitor cells into cardiac pacemaker-like cells. *J Mol Cell Cardiol* **2020**, *138*, 12-22. doi: 10.1016/j.yjmcc.2019.09.015. Epub 2019 Oct 31. PMID: 31678351; PMCID: PMC7035977.
75. Islas, J.F.; Abbasgholizadeh, R.; Dacso, C.; Potaman, V.N.; Navran, S.; Bond, R.A.; Iyer, D.; Birla, R.; Schwartz, R.J. β -Adrenergic stimuli and rotating suspension culture enhance conversion of human adipogenic mesenchymal stem cells into highly conductive cardiac progenitors. *J Tissue Eng Regen Med* **2020**, *14*, 306–318. doi:10.1002/term.2994.
76. de Melo, B.A.G.; Jodat, Y.A.; Cruz, E.M.; Benincasa, J.C.; Shin, S.R.; Porcionatto, M.A. Strategies to use fibrinogen as bioink for 3D bioprinting fibrin-based soft and hard tissues. *Acta Biomater* **2020**, *117*, 60–76. doi:10.1016/j.actbio.2020.09.024.

77. Evans, L.A.; Ferguson, K.H.; Foley, J.P.; Rozanski, T.A.; Morey, A.F. Fibrin sealant for the management of genitourinary injuries, fistulas and surgical complications. *J Urol* **2003**, *169*(4), 1360-2. doi: 10.1097/01.ju.0000052663.84060.ea. PMID: 12629361.
78. Abbasgholizadeh, R.; Islas, J.F.; Navran, S.; Potaman, V.N.; Schwartz, R.J.; Birla, R.K. A Highly Conductive 3D Cardiac Patch Fabricated Using Cardiac Myocytes Reprogrammed from Human Adipogenic Mesenchymal Stem Cells. *Cardiovasc Eng Technol* **2020**, *11*(2), 205-218. doi: 10.1007/s13239-019-00451-0. Epub 2020 Jan 8. PMID: 31916039.
79. Litvinov, R.I.; Pieters, M.; de Lange-Loots, Z.; Weisel, J.W. Fibrinogen and Fibrin. In *Macromolecular Protein Complexes III: Structure and Function*; Harris, J.R., Marles-Wright, J., Eds.; Springer, 2021; pp. 471–501.
80. Stoppel, W.L.; Hu, D.; Domian, I.J.; Kaplan, D.L.; Black, L.D. Anisotropic silk biomaterials containing cardiac extracellular matrix for cardiac tissue engineering. *Biomed Mater* **2015**, *10*, 034105. doi:10.1088/1748-6041/10/3/034105.
81. Song, Y.; Wang, H.; Yue, F.; Lv, Q.; Cai, B.; Dong, N.; Wang, Z.; Wang, L. Silk-Based Biomaterials for Cardiac Tissue Engineering. *Adv Healthc Mater* **2020**, *9*, 2000735. doi:10.1002/adhm.202000735.
82. Di Felice, V.; Serradifalco, C.; Rizzuto, L.; De Luca, A.; Rappa, F.; Barone, R.; Di Marco, P.; Cassata, G.; Puleio, R.; Verin, L.; Motta, A.; Migliaresi, C.; Guercio, A.; Zummo, G. Silk fibroin scaffolds enhance cell commitment of adult rat cardiac progenitor cells. *J Tissue Eng Regen Med* **2015**, *9*(11), E51-64. doi: 10.1002/term.1739. Epub 2013 Apr 17. PMID: 23592297.
83. Tamimi, M.; Rajabi, S.; Pezeshki-Modaress, M. Cardiac ECM/chitosan/alginate ternary scaffolds for cardiac tissue engineering application. *Int J Biol Macromol* **2020**, *164*, 389-402. doi: 10.1016/j.ijbiomac.2020.07.134. Epub 2020 Jul 20. PMID: 32702419.
84. Rezaei, F.S.; Sharifianjazi, F.; Esmailkhanian, A.; Salehi, E. Chitosan films and scaffolds for regenerative medicine applications: A review. *Carbohydr Polym* **2021**, *273*, 118631. doi: 10.1016/j.carbpol.2021.118631. Epub 2021 Sep 2. PMID: 34561021.
85. Martel-Estrada, S.A.; Martínez-Pérez, C.A.; Chacón-Nava, J.G.; García-Casillas, P.E.; Olivas-Armendáriz, I. Synthesis and thermo-physical properties of chitosan/poly(dl-lactide-co-glycolide) composites prepared by thermally induced phase separation. *Carbohydrate Polymers* **2010**, *81* Issue 4, 775-783. <https://doi.org/10.1016/j.carbpol.2010.03.032>.
86. Olivas-Armendariz, I.; Martel-Estrada, S.; Mendoza-Duarte, M.; Jiménez-Vega, F.; García-Casillas, P.E.; Martínez-Pérez, C.A. Biodegradable Chitosan/Multiwalled Carbon Nanotube Composite for Bone Tissue Engineering. *Journal of Biomaterials and Nanobiotechnology* **2013**, *4*(2), 204-211. doi: 10.4236/jbnb.2013.42025.
87. Olivas-Armendariz, I.; García-Casillas, P.; Martel Estrada, A.; Martínez-Villafañe, A.; De la Rosa, L.A.A.; Martínez-Pérez, C.A. In Vitro Evaluation of Polyurethane-Chitosan Scaffolds for Tissue Engineering. *Journal of Biomaterials and Nanobiotechnology* **2012**, *3*, 440-445. <http://dx.doi.org/10.4236/jbnb.2012.34044>
88. De la Paz Orozco, A.; Vega, F.; Martel-Estrada, S.; Aguilar, A.; Mendoza-Duarte, M.; Chavarría-Gaytán, M.; Rodríguez-González, C.; Olivas-Armendáriz, I. Development of Chitosan/Poly(L-Lactide)/Multiwalled Carbon Nanotubes Scaffolds for Bone Tissue Engineering. *Open Journal of Regenerative Medicine* **2016**, *5*, 14-23. doi: 10.4236/ojrm.2016.51002
89. Lv, J.; Liu, W.; Shi, G.; Zhu, F.; He, X.; Zhu, Z.; Chen, H. Human cardiac extracellular matrix-chitosan-gelatin composite scaffold and its endothelialization. *Exp Ther Med* **2020**, *19*(2), 1225-1234. doi: 10.3892/etm.2019.8349. Epub 2019 Dec 19. PMID: 32010293; PMCID: PMC6966153.
90. Lu, W.N.; Lü, S.H.; Wang, H.B.; Li, D.X.; Duan, C.M.; Liu, Z.Q.; Hao, T.; He, W.J.; Xu, B.; Fu, Q.; Song, Y.C.; Xie, X.H.; Wang, C.Y. Functional improvement of infarcted heart by co-injection of embryonic stem cells with temperature-responsive chitosan hydrogel. *Tissue Eng Part A* **2009**, *15*(6), 1437-47. doi: 10.1089/ten.tea.2008.0143. PMID: 19061432.
91. Ahmadi, P.; Nazeri, N.; Derakhshan, M.A.; Ghanbari, H. Preparation and characterization of polyurethane/chitosan/CNT nanofibrous scaffold for cardiac tissue engineering. *Int J Biol Macromol* **2021**, *180*, 590-598. doi: 10.1016/j.ijbiomac.2021.03.001. Epub 2021 Mar 9. PMID: 33711373.
92. Liu, Y.; Wang, S.; Zhang, R. Composite poly(lactic acid)/chitosan nanofibrous scaffolds for cardiac tissue engineering. *Int J Biol Macromol* **2017**, *103*, 1130-1137. doi: 10.1016/j.ijbiomac.2017.05.101. Epub 2017 May 18. PMID: 28528953.
93. Pok, S.; Myers, J.D.; Madihally, S.V.; Jacot, J.G. A multilayered scaffold of a chitosan and gelatin hydrogel supported by a PCL core for cardiac tissue engineering. *Acta Biomater* **2013**, *9*(3), 5630-42. doi: 10.1016/j.actbio.2012.10.032. Epub 2012 Nov 2. PMID: 23128158; PMCID: PMC3562398.
94. Abedi, A.; Bakhshandeh, B.; Babaie, A.; Mohammadnejad, J.; Vahdat, S.; Mombeiny, R.; Moosavi, S.R.; Amini, J.; Tayebi, L. Concurrent application of conductive biopolymeric chitosan/ polyvinyl alcohol/ MWCNTs nanofibers, intracellular signaling manipulating molecules and electrical stimulation for more effective cardiac tissue engineering. *Mater Chem Phys* **2021**, *258*. <https://doi.org/10.1016/j.matchemphys.2020.123842>
95. Liu, N.; Chen, J.; Zhuang, J.; Zhu, P. Fabrication of engineered nanoparticles on biological macromolecular (PEGylated chitosan) composite for bio-active hydrogel system in cardiac repair applications. *Int J Biol Macromol* **2018**, *117*, 553–558.
96. Xu, B.; Li, Y.; Deng, B.; Liu, X.; Wang, L.; Zhu, Q.L. Chitosan hydrogel improves mesenchymal stem cell transplant survival and cardiac function following myocardial infarction in rats. *Exp Ther Med* **2017**, *13*(2), 588-594. doi: 10.3892/etm.2017.4026. Epub 2017 Jan 5. PMID: 28352335; PMCID: PMC5348688.
97. Wang, H.; Shi, J.; Wang, Y.; Yin, Y.; Wang, L.; Liu, J.; Liu, Z.; Duan, C.; Zhu, P.; Wang C. Promotion of cardiac differentiation of brown adipose derived stem cells by chitosan hydrogel for repair after myocardial infarction. *Biomaterials* **2014**, *35*(13), 3986-98. doi: 10.1016/j.biomaterials.2014.01.021. Epub 2014 Feb 6. PMID: 24508080.
98. Roberts, J.S.; Niu, J.; Pastor-Cervantes, J.A. Comparison of Hemostasis Times with a Chitosan-Based Hemostatic Pad (Clo-Sur-Plus Radial™) vs Mechanical Compression (TR Band®) Following Transradial Access: A pilot Study. *Cardiovasc Revasc Med* **2019**, *20*(10), 871-874. doi: 10.1016/j.carrev.2018.11.026. Epub 2018 Dec 6. PMID: 30553816.

99. Pathan, A.Z.; Aijaz, S.; Sheikh, S.; Sattar, S. Randomized trial comparing radial hemostasis techniques; catechol conjugated chitosan pad (InnoSEAL) versus pneumatic compression band. *Catheter Cardiovasc Interv* **2021**, *98*(2), E181-E187. doi: 10.1002/ccd.29578. Epub 2021 Mar 2. PMID: 33655650.
100. Lu, T.; Li, Y.; Chen, T. Techniques for fabrication and construction of three-dimensional scaffolds for tissue engineering. *Int J Nanomedicine* **2013**, *8*, 337-50. doi: 10.2147/IJN.S38635. Epub 2013 Jan 18. PMID: 23345979; PMCID: PMC3551462.
101. Islam, M.T.; Laing, R.M.; Wilson, C.A.; McConnell, M.; Ali, M.A. Fabrication and characterization of 3-dimensional electrospun poly(vinyl alcohol)/keratin/chitosan nanofibrous scaffold. *Carbohydr Polym* **2022**, *275*, 118682. doi: 10.1016/j.carbpol.2021.118682. Epub 2021 Sep 21. PMID: 34742412.
102. Anisie, A.; Oancea, F.; Marin, L. Electrospinning of chitosan-based nanofibers: From design to prospective applications. *Rev Chem Eng* **2021**. doi:10.1515/revce-2021-0003
103. Xue, J.; Wu, T.; Dai, Y.; Xia, Y. Electrospinning and Electrospun Nanofibers: Methods, Materials, and Applications. *Chem Rev* **2019**, *119*(8), 5298-5415. doi: 10.1021/acs.chemrev.8b00593. Epub 2019 Mar 27. PMID: 30916938; PMCID: PMC6589095.
104. Salazar-Brann, S.A.; Patiño-Herrera, R.; Navarrete-Damián, J.; Louvier-Hernández, J.F. Electrospinning of chitosan from different acid solutions. *AIMS Bioeng* **2021**, *8*, 112–129.
105. Izadifar, Z.; Chen, X.; Kulyk, W. Strategic design and fabrication of engineered scaffolds for articular cartilage repair. *J Funct Biomater* **2012**, *3*(4), 799-838. doi: 10.3390/jfb3040799. PMID: 24955748; PMCID: PMC4030923.
106. Rahmati, M.; Mills, D.K.; Urbanska, A.M.; Saeb, M.R.; Venugopal, J.R.; Ramakrishna, S.; Mozafari, M. Electrospinning for tissue engineering applications. *Prog Mater Sci* **2021**, *117*, 100721.
107. Perez-Puyana, V.; Rubio-Valle, J.F.; Jiménez-Rosado, M.; Guerrero, A.; Romero, A. Alternative processing methods of hybrid porous scaffolds based on gelatin and chitosan. *J Mech Behav Biomed Mater* **2020**, *102*, 103472. doi: 10.1016/j.jmbbm.2019.103472. Epub 2019 Oct 5. PMID: 31605930.
108. Sitab, A.A.; Joya, J.S.; Barman, J.P.; Biswas, S.; Rashid, T.U. Fabrication of Chitosan-Based Biomaterials: Techniques and Designs. in *Engineering Materials for Stem Cell Regeneration* (ed. Sheikh, F. A.) 455–518 (Springer Singapore, 2021). doi:10.1007/978-981-16-4420-7_18
109. Sadeghianmaryan, A.; Naghieh, S.; Yazdanpanah, Z.; Alizadeh Sardroud, H.; Sharma, N.K.; Wilson, L.D.; Chen, X. Fabrication of chitosan/alginate/hydroxyapatite hybrid scaffolds using 3D printing and impregnating techniques for potential cartilage regeneration. *Int J Biol Macromol* **2022**, *204*, 62-75. doi: 10.1016/j.ijbiomac.2022.01.201. Epub 2022 Feb 3. PMID: 35124017.
110. Patel, D.K.; Dutta, S.D.; Hexiu, J.; Ganguly, K.; Lim, K.T. 3D-printable chitosan/silk fibroin/cellulose nanoparticle scaffolds for bone regeneration via M2 macrophage polarization. *Carbohydr Polym* **2022**, *281*, 119077. doi: 10.1016/j.carbpol.2021.119077. Epub 2022 Jan 3. PMID: 35074128.
111. Singh, S.; Singh, G.; Prakash, C.; Ramakrishna, S.; Lamberti, L.; Pruncu, C.I. 3D printed biodegradable composites: An insight into mechanical properties of PLA/chitosan scaffold. *Polymer Testing* **2020**, *89*. <https://doi.org/10.1016/j.polymeresting.2020.106722>
112. Rajabi, M.; McConnell, M.; Cabral, J.; Ali, M.A. Chitosan hydrogels in 3D printing for biomedical applications. *Carbohydr Polym* **2021**, *260*, 117768. doi: 10.1016/j.carbpol.2021.117768. Epub 2021 Feb 10. PMID: 33712126.
113. Camman, M.; Joanne, P.; Agbulut, O.; Hélar, C. 3D models of dilated cardiomyopathy: Shaping the chemical, physical and topographical properties of biomaterials to mimic the cardiac extracellular matrix. *Bioact Mater* **2021**, *7*, 275-291. doi: 10.1016/j.bioactmat.2021.05.040. PMID: 34466733; PMCID: PMC8379361.
114. Roshandel, M.; Dorkoosh, F. Cardiac tissue engineering, biomaterial scaffolds, and their fabrication techniques. *Polym Adv Technol* **2021**, *32*, 2290–2305.
115. Hacking, S.A.; Du, Y.; Khademhosseini, A. Patterning of Polymeric Materials for Biological Applications. in *Polymer Science: A Comprehensive Reference* 9, 439–456 (Elsevier, 2012).
116. Patel, B.; Manne, R.; Patel, D.B.; Gorityala, S.; Palaniappan, A.; Kurakula, M. Chitosan as Functional Biomaterial for Designing Delivery Systems in Cardiac Therapies. *Gels* **2021**, *7*(4), 253. doi: 10.3390/gels7040253. PMID: 34940314; PMCID: PMC8702013.
117. Nguyen-Truong, M.; Li, Y.V.; Wang, Z. Mechanical Considerations of Electrospun Scaffolds for Myocardial Tissue and Regenerative Engineering. *Bioengineering (Basel)* **2020**, *7*(4), 122. doi: 10.3390/bioengineering7040122. PMID: 33022929; PMCID: PMC7711753.
118. Abedi, A.; Hasanzadeh, M.; Tayebi, L. Conductive nanofibrous Chitosan/PEDOT:PSS tissue engineering scaffolds. *Mater Chem Phys* **2019**, *237*, 121882. doi: 10.1016/j.matchemphys.2019.121882.
119. Saravanan, S.; Sareen, N.; Abu-El-Rub, E.; Ashour, H.; Sequiera, G.L.; Ammar, H.I.; Gopinath, V.; Shamaa, A.A.; Sayed, S.S.E.; Moudgil, M.; Vadivelu, J.; Dhingra, S. Graphene Oxide-Gold Nanosheets Containing Chitosan Scaffold Improves Ventricular Contractility and Function After Implantation into Infarcted Heart. *Sci Rep* **2018**, *8*(1), 15069. doi: 10.1038/s41598-018-33144-0. PMID: 30305684; PMCID: PMC6180127.
120. Savchenko, A.; Yin, R.T.; Kireev, D.; Efimov, I.R.; Molokanova, E. Graphene-Based Scaffolds: Fundamentals and Applications for Cardiovascular Tissue Engineering. *Front Bioeng Biotechnol* **2021**, *9*, 797340. doi: 10.3389/fbioe.2021.797340. PMID: 34950649; PMCID: PMC8688816.
121. Martins, A.M.; Eng, G.; Caridade, S.G.; Mano, J.F.; Reis, R.L.; Vunjak-Novakovic, G. Electrically conductive chitosan/carbon scaffolds for cardiac tissue engineering. *Biomacromolecules* **2014**, *15*(2), 635-43. doi: 10.1021/bm401679q. Epub 2014 Jan 28. PMID: 24417502; PMCID: PMC3983145.

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122. Baei, P.; Jalili-Firoozinezhad, S.; Rajabi-Zeleti, S.; Tafazzoli-Shadpour, M.; Baharvand, H.; Aghdami, N. Electrically conductive gold nanoparticle-chitosan thermosensitive hydrogels for cardiac tissue engineering. *Mater Sci Eng C Mater Biol Appl* **2016**, *63*, 131-41. doi: 10.1016/j.msec.2016.02.056. Epub 2016 Feb 22. PMID: 27040204.
 123. Jiang, L.; Chen, D.; Wang, Z.; Zhang, Z.; Xia, Y.; Xue, H.; Liu, Y. Preparation of an Electrically Conductive Graphene Oxide/Chitosan Scaffold for Cardiac Tissue Engineering. *Appl Biochem Biotechnol* **2019**, *188*(4), 952-964. doi: 10.1007/s12010-019-02967-6. Epub 2019 Feb 11. PMID: 30740624.
 124. Mombini, S.; Mohammadnejad, J.; Bakhshandeh, B.; Narmani, A.; Nourmohammadi, J.; Vahdat, S.; Zirak, S. Chitosan-PVA-CNT nanofibers as electrically conductive scaffolds for cardiovascular tissue engineering. *Int J Biol Macromol* **2019**, *140*, 278-287. doi: 10.1016/j.ijbiomac.2019.08.046. Epub 2019 Aug 7. PMID: 31400428.
 125. Chiu, L.L.; Janic, K.; Radisic, M. Engineering of oriented myocardium on three-dimensional micropatterned collagen-chitosan hydrogel. *Int J Artif Organs* **2012**, *35*(4), 237-50. doi: 10.5301/ijao.5000084. Epub 2012 Apr 13. PMID: 22505198.