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Electrically Conductive Nanomaterials for Cardiac Tissue Engineering

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Abstract

Patient deaths resulting from cardiovascular diseases are increasing across the globe, posing the greatest risk to patients in developed countries. Myocardial infarction, as a result of inadequate blood flow to the myocardium, results in irreversible loss of cardiomyocytes which can lead to heart failure. A sequela of myocardial infarction is scar formation that can alter the normal myocardial architecture and result in arrhythmias. Over the past decade, a myriad of tissue engineering approaches has been developed to fabricate engineered scaffolds for repairing cardiac tissue. This paper highlights the recent application of electrically conductive nanomaterials (carbon and gold-based nanomaterials, electroactive polymers) to the development of scaffolds for cardiac tissue engineering. Moreover, this work summarizes the effects of these nanomaterials on cardiac cell behavior such as proliferation and migration, as well as cardiomyogenic differentiation in stem cells.

Keywords

Electrically conductive scaffolds; Cardiac tissue engineering; Carbon-based nanomaterials; Gold nanoparticles; Electroactive polymers; Conductive nanomaterials; Cardiovascular diseases

1. Introduction

In the United States cardiovascular diseases (CVDs) are responsible for one death every 40 seconds (1). Loss of blood circulation to regions of the heart muscle due to coronary artery occlusion can damage the myocardium, causing electrophysiological and morphological disorders of the heart (2, 3). Ischemia may result in cardiac cell death through necrosis, apoptosis, or autophagy and the subsequent formation of scar tissue reduces the cardiac contractile capacity (4). Since adult cardiomyocytes have a limited regenerative capacity, the damage can be permanent and lead to heart failure and death (5). Complex surgical treatments have been developed over the past two decades for cardiac transplantation; however, donor shortage is a major challenge that limits this approach. In addition, transplant patients must receive immunosuppressive drug therapy after surgery to decrease the risk of transplant rejection (5). The disadvantages of heart transplantations highlight the need for alternative therapies for the prevention and remediation of cardiac failure. In the

past decade, regeneration of the heart, using approaches ranging from cell therapy to tissue engineering, has been extensively investigated as an alternative method of managing CVDs. Cardiac cell-based therapy is a concept in which different cell sources such as mesenchymal stem cells (MSCs), induced pluripotent stem cells (iPSCs), and embryonic stem cells (ESCs) (6–9) or their derivatives are used alone, or in combination, with scaffolds to treat the disease (10, 11).

In the extracellular matrix (ECM) of the heart, collagen and elastin form fibers which weave to compose a dense, elastic molecular network. The micro- and nanoscale topography of the matrix causes mechanical coupling of cardiomyocytes, providing the unique electrical and mechanical characteristics of the heart (12). The biochemical, electrical, and mechanical functions of the myocardial ECM are dependent on its nanofeatures (13). Cardiac tissue engineering can be defined as the field that aims to generate or repair the myocardium by combining knowledge and techniques from materials science, micro/nano-engineering, cellular biology, and biochemistry (14). The reconstruction of effective cardiac tissue requires proper selection of cell sources, establishment of the myocardial ECM, electromechanical stimulation of cells, fabrication of robust contractile bundles, and inclusion of vascular channels.

Recently, there has been considerable effort to develop functional scaffolds that are designed for cardiac repair, including cardiac patches, injectables, and nanofibrous or nano-patterned scaffolds (15, 16). To improve scaffold functionality, various nanomaterials in the form of nanofibers (11, 17–19), mesoporous and composite materials (20), nanoparticles (21), and modified nano-patterned surfaces have been adopted. These technologies help recreate biomimetic microenvironments for cells to reach their full biological potential in the engineering of a functional myocardium (Fig. 1).

Fabrication of scaffolds is influenced by the integration of chemical, biological and physical properties (19, 22). An ideal scaffold for cardiac tissue engineering must be electrically conductive, mechanically stable, biocompatible, topographically suitable, and possess similar elasticity to the native myocardium (23, 24). The material’s ability to propagate electrical impulses and translate them into synchronized contractions is necessary to maintain circulation by pumping blood through the organ (25). Both the engineered cardiac constructs and injected cells must integrate into the electrical syncytium of the myocardium to maintain spontaneous contractile activity (26). Electroactive biomaterials can transmit electromechanical, electrochemical, and electrical stimulation to cells (27). In cardiac tissue engineering, development and utilization of electroactive materials (conductive polymers, piezoelectric materials, carbon nanotubes, carbon nanofibers, as well as graphene and gold nanostructures) has been a flourishing area of research in recent years. This review summarizes the advancement of electroactive nanomaterials for cardiac regeneration, and highlights the possibility of using these systems to regenerate cardiac tissue (Fig. 2).

2. Biological response of cardiomyocytes to nanomaterials

It is important to understand the role of key genes and signaling pathways in cardiac tissue development and function. These genes and pathways play an important role in nanomaterial

interaction with cardiac cells. Cardiomyocytes are formed as a result of cardiac progenitor cell differentiation in the body in which several cardiac transcription factors, such as Tbx5, Nkx-2.5, and GATA-4 help to activate the transcription of structural genes for cardiomyocytes, such as myosin heavy chain, desmin, cardiac troponins, and myosin light chain (28). The upregulation of these genes often occurs after 7 days of differentiation on two-dimensional (2D) culture systems. In particular, Nkx-2.5 is expressed in cardiomyocytes with positive cTnT after 10 days of differentiation (29). Major signaling pathways involved in cardiac differentiation are BMP, FGF, Wnt, and TGFβ/Activin/Nodal pathways. Other molecular pathways include Notch and p38 MAPK signaling pathways (30). Commonly used differentiation protocols result in a mixture of atrial, ventricular, and nodal cells (31). However, it is possible to enrich a specific population of cardiomyocytes compared to others. For example, it was shown that BMP antagonist Grem2 is able to preferentially differentiate cardiomyocytes to atrial cell type (32). Nanomaterials can affect stem cell differentiation toward cardiomyocytes. Moreover, they have shown great promise to maintain the function of primary cardiomyocytes *in vitro* and enhance their function and survival *in vivo*.

Mechanical and electrical integrity of the heart is crucial for cardiomyocyte function. The connexin (Cx) genes encode Cx proteins to link cardiomyocytes in the heart. In particular, Cx43 is synthesized in the plasma membrane of cardiomyocytes making intercellular channels between the cytoplasmic components of neighboring cardiomyocytes (33). Cx43 plays an important role in direct transferring signaling molecules and ions from the cell membrane. These signaling molecules and ions regulate cell survival and intracellular calcium transition through releasing glutamate and ATP and electrical pulse propagation (34). Moreover, Cx43 localization on the cell membrane has cardioprotective characteristic and avoids ischemia (35). Electrically conductive and mechanically strong nanomaterials have shown great promise to connect individual cardiomyocytes resembling the role of Cx43 in tissue development and function.

Cardiac tissues have been engineered using different sources of cardiomyocytes (36). Foetal and neonatal cardiomyocytes from animal models, such as rats and mice have largely been used in cardiac tissue engineering as they are easy to obtain and have high regenerative ability (37). These early stage cardiomyocytes have higher survival rate and regeneration capability compared to adult cardiomyocytes (38). However, there are some issues regarding the use of primary cardiomyocytes, such as immunogenicity, malignancy, and ethical concern (39). Nanomaterials can be helpful to remodel the microenvironment of primary cardiomyocytes *in vitro* and enhance their survival and function *in vivo*. Differentiated cardiomyocytes from stem cells, such as MSCs, iPSCs, and ESCs have also shown great promise in cardiac tissue engineering (40). In particular, cardiomyocyte-derived iPSCs can be obtained from human fibroblasts to make personalized tissue constructs. However, there is still required to enhance the efficiency of differentiation protocols to make highly pure and functional cardiomyocytes. Here, nanomaterials can be useful in regulating stem cell differentiation to cardiomyocytes. Moreover, they can provide reliable and biomimetic scaffolds for engineered cardiac tissues.

3. Carbon-based nanomaterials

3.1 Carbon nanotubes

Carbon nanotubes (CNTs) have been utilized extensively in biomedical and biological applications such as imaging, regenerative medicine, and pharmaceutical applications like drug delivery (41–43). CNTs are interesting candidates as substrates or additives in biomaterials for tissue regeneration due to their mechanical and electrical properties (44, 45). These cylindrical nano-structured carbon molecules have a high aspect ratio. There are three classes of carbon nanotubes based on the number of graphite cylinders in the structure: single-walled carbon nanotubes (SWCNTs, 1–2 nm diameter), double-walled carbon nanotubes (DWCNTs), and multi-walled carbon nanotubes (MWCNTs, 10–100 nm diameter). The electrical properties of CNTs are influenced by the orientation and wrapping of the hexagonal bond structure. CNTs are known for their mechanical strength and can be integrated into materials to increase the tensile strength and Young's modulus of composites (46). There are many methods available to produce CNTs including physical methods, like the electric-arc technique (47) and laser ablation (48), and chemical methods, like chemical vapor deposition (49).

CNTs have been used for a wide variety of applications in cellular biology ranging from *in vivo* cell tracking, labeling, and transfection to improving the conductivity of scaffolds (21, 50, 51). A major hurdle to mass adoption of CNTs for biomedical applications was cytotoxicity (52–54); however, advanced surface modifications have significantly improved the biocompatibility of these nanotubes (55, 56). Due to their biocompatibility and physical properties, CNTs are promising reinforcement materials and good conductive agents for cardiac (57–59) and neural (60, 61) tissue engineering (62). Biocompatibility of a purified suspension of CNTs interacting with mouse cardiomyocytes (H9c2) has shown that cell viability was unaffected by the presence of CNTs for the first three days (short-term biocompatibility). However, the long-term toxicity became apparent as apoptosis occurred after three days of cell culture in the presence of the nanotubes (63).

In other studies, pure CNTs were deposited on glass surfaces to investigate cardiomyocyte behavior. Martinelli *et al.* cultured neonatal rat cardiomyocytes on glass modified with MWCNTs (162 nm diameter). They discovered that the cardiomyocytes formed tight contacts and showed enhanced proliferation. After two to three days in culture, shorter action potentials of cardiomyocytes in the presence of MWCNTs were reported (64). In 2013, Martinelli and colleagues further demonstrated that deposition of 20–30 nm diameter MWCNTs on a glass substrate can promote cardiomyocyte growth and differentiation by altering gene expression and electrophysiological properties. MWCNTs (Fig. 3.A.a) improved the electrophysiological characteristics of the cardiomyocytes, enhanced intracellular calcium signaling (Fig. 3A.b), and accelerated the maturation of functional syncytia. The expression of the Cx43 gene (Fig. 3A.c) was also increased; suggesting that CNTs may play a role in improving electrical conductivity by reinforcing electrical coupling between cardiomyocytes (65).

Liao *et al.* have demonstrated the production of MWCNT-incorporated polyvinyl alcohol (PVA)/chitosan nanofibers by electrospinning. MWCNTs (30–70 nm diameter and 100–400

nm length) were incorporated in a blend of PVA and chitosan fibers (160 nm diameter). Incorporation of MWCNTs improved the protein adsorption ability of the nanofibers (Fig. 3B.a) and significantly promoted cell proliferation and adhesion (Fig. 3B.b and 3B.c) (66). Wickham and colleagues have conjugated MWCNTs (7–15 nm diameter) to the surface of hydrophobic polycaprolactone (PCL) sheets and nanofiber meshes via thiophene. This group was able to increase the fiber's mechanical strength without changing the mesh morphology. The addition of thiophene-conjugated CNTs to PCL polymers also resulted in increased proliferation of cardiac progenitor cells (CPCs) (67). Incorporation of CNTs in other materials, such as gelatin nanofibers and poly(glycerol sebacate) (PGS), notably enhanced the alignment, mechanical toughness, and electrical conductivity of fibers. The enhanced material resulted in strong and synchronized beating of cardiomyocytes. By incorporating CNTs, the excitation threshold was 3.5 times lower and expression of Cx43 in cardiomyocytes was higher. In addition, CNTs add to the scaffold's ability to mimic the anisotropic structure of the left ventricle (68).

Incorporation of CNTs in nanofibrous scaffolds has also been applied to cardiomyogenic differentiation of stem cells. In one study, researchers incorporated SWCNTs in electrospun PCL to fabricate an electrically conductive nanoscale scaffold. They employed electrical stimulation to effectively differentiate human mesenchymal stem cells (hMSCs) into cardiomyocytes. The presence of CNTs resulted in elongated morphology and upregulation of cardiac markers like Nkx-2.5, Cx43, GATA-4, and cardiac troponin T (CTT) (59). Another study showed that MWCNT-doped PCL fibers can also enhance cardiac differentiation of hMSCs under electrical stimulation. The ionic resistance of doped fibers was measured through electrochemical impedance spectroscopy and the optimum amount of incorporated CNTs was determined by conductivity (70).

CNTs have also been integrated with hydrogels (71). Hydrogels and soft tissues have similar mechanical and structural properties. Typical hydrogels, such as gelatin methacryloyl (GelMA), are also biodegradable. In 2013, Shin *et al.* created controllable 3D biohybrid actuators for electrical stimulation of neonatal rat cardiomyocytes. They embedded aligned CNT (50–100 nm diameter) forest microelectrode arrays into hydrogel plates of GelMA (50 μm thickness) to construct scaffolds with anisotropic electrical conductivity. Engineered tissue showed better cell organization, higher cell-to-cell coupling, and an increase in HL-1 cell maturation. Synchronized beating improved and significant reductions in excitation thresholds were observed. In this study, expression of troponin I and Cx43 was increased and no toxic effects were observed for seven days (57). In 2015, Elkhenany *et al.* incorporated 2 and 5 nm diameter MWCNTs in GelMA to fabricate electrically conductive scaffolds for investigating cardiac cell behavior under electrical stimulation (1 Hz, 5 V, 50 ms pulse width). They observed that overexpression of sarcomeric α-actinin and Cx43 led to improved cell behavior (72). In another study, Pok *et al.* developed a scaffold containing subtoxic concentrations of SWCNTs (8 nm diameter × 262 nm length) in a gelatin-chitosan hydrogel. Nanobridges of SWCNTs between cardiac cells led to enhanced expression of cardiac markers (Fig. 3C.a), synchronous beating (Fig. 3C.b), electrical coupling, and normal function of cardiomyocytes. Excitation conduction velocities (Fig. 3C.c) of engineered tissues were similar to that of natural myocardial tissue at 22 ± 9 cm/s (69). Yu *et al.* incorporated carboxyl-functionalized MWCNTs into type I collagen hydrogels. They

demonstrated that the rhythmic contraction area of neonatal rat cardiomyocytes increased due to the addition of CNTs (73). In another study, Ahadian *et al.* fabricated a series of moldable elastomeric scaffolds by incorporation of MWCNTs into a polyester called poly(octamethylene maleate (anhydride) 1,2,4-butanetricarboxylate). Their study demonstrated that scaffolds composed of 0.5% CNTs could improve the excitation threshold in neonatal rat cardiomyocytes (74). Also, Ho *et al.* fabricated PCL/MWCNT composite scaffolds for cardiac tissue engineering using 3D printing techniques (75). This particular scaffold design offers selective treatments for complex cardiac tissues. In another attempt, Izadifar *et al.* fabricated hybrid cardiac patches by encapsulating human coronary artery endothelial cells in methacrylated collagen scaffolds with CNTs using a UV-integrated 3D bioprinting technique (76). Additional researchers attempted to build on this success by designing hydrogels with the same function for more specific applications. In this regard, Roshanbinfar *et al.* fabricated an injectable, thermoresponsive, conductive scaffold by adding MWCNTs to pericardial matrix hydrogel. Functionalized MWCNTs with carbodihydrazide improved electrical and mechanical properties of the hydrogel, leading to an increase in cell proliferation and expression of Cx43 (77). More recently, Cabiati *et al.* incorporated different concentrations of SWCNTs into gelatin-based genipin cross-linked scaffolds and observed overexpression of cardiac markers in cardiomyoblasts (78).

3.2 Carbon Nanofibers

Carbon nanofibers (CNFs) are hollow cylinders with diameters between 50 and 500 nm and length on the order of microns. Because of their high aspect ratio (length/diameter greater than 100), they have been utilized for numerous applications. They have many unique physical and mechanical properties including a tensile strength of approximately 3 GPa, Young's modulus of 500 GPa, thermal conductivity of 1900 $Wm^{-1}K^{-1}$, electrical conductivity of approximately 10^3 S/cm (79, 80), in addition to compatibility with organochemical modifications (81). CNFs have cup-stacked or platelet structures that are less uniform compared to the hexagonal network of CNTs (82, 83). CNFs are fabricated using one of two methods: catalytic thermal chemical vapor deposition growth or electrospinning followed by heat treatment. CNF-reinforced polymer scaffolds can also be fabricated by easily dispersing the CNF in a polymer matrix, followed by either melt mixing or sonication in low viscosity solutions (83). Several studies have mentioned applications of CNFs in neural (84, 85), bone (86–89), muscle (90), and cardiac regeneration (91–93).

Stout *et al.* investigated cardiomyocyte function on poly(lactic-co-glycolic acid) (PLGA) and CNF composites. Their results revealed that CNFs increased the conductivity and cytocompatibility of PLGA and promoted cardiomyocyte adhesion and proliferation. Also, the density of cardiomyocytes increased with CNFs (up to 25:75 wt% PLGA:CNFs). The electrical conductivity of PLGA/CNF composites increased by adding CNFs of any diameter (91). Meng *et al.* introduced injectable, biomimetic, electrically conductive scaffolds using CNFs, self-assembled rosette nanotubes (RNTs), and poly(2-hydroxyethyl methacrylate) (pHEMA) hydrogel for myocardial tissue engineering. As more CNFs and RNTs were incorporated into the pHEMA matrix, cardiomyocyte density in the hydrogel increased. Adding greater amounts of CNFs to the composites led to a decrease in tensile modulus and contact angle, but increased conductivity and surface roughness (92). In order to mimic

myocardial anisotropy, Asiri *et al.* created patterns (20 μm wide) of aligned CNFs (100 nm diameter) on the surface of PLGA (50:50 PGA:PLA weight ratio). The result showed that CNF alignment increased the density of cardiomyocytes in the scaffold. Also, aligning the CNFs in the PLGA scaffold increased the longitudinal (vertical) conductivity to 0.1 S/m and decreased the horizontal (transverse) conductivity to 0.0025 S/m compared to a scaffold with randomly oriented fibers; these conductivities are similar to those of natural heart tissue (93).

3.3 Graphene and its derivatives

Graphene is a freestanding, 2D active carbon allotrope. In graphene, the hexagonal aromatic structure is achieved by covalent bonds between each atom of carbon and three neighboring carbon atoms within the 2D crystal. The unique physical and electrical properties of graphene and its derivatives make it an ideal material for incorporation into composites to enhance desirable properties (94). Moreover, the high surface area of graphene facilitates the ability to load large quantities of bioactive compounds on its surface (95).

In vivo and *in vitro* biocompatibility of graphene and its derivatives has been reported in multiple studies (96, 97). Different approaches to improve biocompatibility such as oxidation, reduction, and functionalization, as well as controlling the size of the product, have been demonstrated (95, 98). Wang *et al.* found that cardiogenic differentiation of human iPSCs could be improved by using superconductive sheets of graphene (99). In a recent study, Smith *et al.* developed micro- and nano-patterned conductive hybrid scaffolds using graphene and polyethylene glycol (PEG). The anisotropic electrical conductivity and graphene-functionalized topography of these scaffolds led to an enhancement in myofibrils and sarcomeric structures in addition to an increase in electrical coupling of cardiac cells (100).

Graphene oxide (GO) is a highly oxidized form of graphene with colloidal stability that behaves as surfactant-like, amphiphilic sheets (101). GO and reduced graphene oxide (rGO) have been used in combination with different materials as tissue engineering scaffolds. rGO has great conductivity and can also increase the hydrophobicity of scaffolds (102). Additionally, the biocompatibility of rGO makes it a promising candidate for modifying bioprosthetic heart valves too (95, 102).

In one study, Shin *et al.*, incorporated GO into GelMA hydrogels for creating a cell-laden scaffold to investigate fibroblast behavior. Incorporation of GO significantly decreased the electrical impedance at low frequencies (103). In another study, the same group used GO-based thin films and fabricated a 3D nano-structure through a layer-by-layer (LbL) technique. GO sheets were coated with poly-L-lysine (PLL). Neonatal rat ventricular cardiomyocytes between PLL and GO under electrical stimulation showed strong, spontaneous beating, cardiac cell organization, maturation, and cell-to-cell electrical coupling (104). Also, the incorporation of rGO into GelMA hydrogels enhanced the electrical conductivity and mechanical properties of the material; the modified GelMA improved cardiomyocyte viability, proliferation, and maturation in addition to inducing increased spontaneous beating rates (105). Incorporation of graphene-based nanomaterials into hydrogels can improve both the mechanical and electrical properties. These

incorporations provide nanotopography similar to natural *in vivo* environments, resulting in better cell-to-cell signaling, and ameliorating signal propagation – all of which are essential parameters in cardiac tissue engineering (95). Table 1 gives a summary of carbon-based nanomaterials that have been applied to cardiac tissue engineering.

4. Gold nanomaterials

Gold nanoparticles (AuNPs) have been studied extensively for many biological and medical applications due to their controlled geometrical, optical, and surface chemical properties (107). Low cytotoxicity and biocompatibility of AuNPs are demonstrated in several studies (108, 109). AuNPs can be synthesized in different shapes including nanospheres, nanorods (110, 111), tripods (112), tetrapods (112), nanocubes (113), and nanocages (114). They also can be transformed into nanofibers, thin films, or nanoshells. Unique specific absorbance spectra have been reported corresponding to different shapes of AuNPs. A variety of geometries can be used for medical applications including diagnosis, sensing, molecular imaging, and stem cell tracking. Additionally, the nanoparticles can be used to enhance the electrical conductivity of nanocomposites. The ideal electrical conductivity, acceptable biocompatibility, ease of surface modification, nanotopography, and innate optic properties make AuNPs a desirable nanostructure candidate for cardiac scaffolds.

Shevach *et al.* have deposited AuNPs on decellularized omental matrix in order to make an electrically conductive scaffold for cardiac tissue engineering (Fig. 4A.a). Cardiac cells showed elongated and aligned morphology and increased Cx43 expression. These hybrid AuNP/omental patches demonstrated increased contraction force (Fig. 4A.b), lower excitation threshold, and boosted propagation of calcium signals (115).

In another study, Fleischer *et al.* integrated AuNPs into PCL electrospun fibers to fabricate an electroconductive nanocomposite scaffold for myocardium tissue engineering. Cardiomyocytes in the presence of AuNPs, exhibited aligned and elongated morphology, stronger contraction forces, and lower excitation thresholds in presence of electrical fields (116). Shevach *et al.* evaporated AuNPs (thickness of 2, 4, and 14 nm) to the surface of synthetic PCL-gelatin matrix nanofibers (250 nm diameter). This engineered hybrid nanocomposite enhanced cardiomyocyte elongation, alignment, cardiac sarcomeric α-actinin expression, and resulted in higher cell contraction amplitudes and rates (Fig. 4B) (117).

Cardiomyogenic differentiation of stem cells also has been studied in AuNP-loaded nanofibrous scaffolds. In 2014, Ravichandran *et al.* incorporated AuNPs into bovine serum albumin (BSA)/PVA hybrid nanofibers. By culturing hMSCs on an AuNP-loaded conductive nanofibrous scaffold with 5-azacytidine pre-treatment, cardiomyogenic differentiation of hMSCs was remarkably enhanced (Fig. 4C) (118). In another study, Sridhar *et al.* incorporated different materials such as AuNPs, vitamin B12, silk fibroin, and aloe vera in a series of PCL scaffolds in which they co-cultured cardiomyocytes and MSCs. The AuNP-blended scaffolds enhanced proliferation and cardiomyogenic differentiation of MSCs. Functionalizing the biomaterials with AuNPs enhanced the mechanical strength of nanofibrous scaffolds and resulted in better contractile characteristics for cardiac cells (119).

Hydrogels are also good candidates for integrating gold nanocomposites to create 3D scaffolds. You *et al.* incorporated AuNPs homogeneously into a thiol-HEMA/HEMA hybrid hydrogel to mimic physiological properties of natural myocardial ECM. Young's modulus of the composite gel is closer to the *in vivo* myocardium in comparison with naked polyaniline (PANI) and polypyrrole (PPy). AuNPs enhanced expression of Cx43 in neonatal rat ventricular cardiomyocytes (NRVC) in this hybrid scaffold (120). Naseri *et al.* incorporated silica-gold core-shell spheres into PCL composite films; the electrical conductivity of the scaffold was 1.51 S/cm. The particles were composed of 20 nm gold nanoshells covering silica microspheres (1.1 μm diameter) (121).

Dvir *et al.* demonstrated that the incorporation of gold nanowires (30 nm diameter) with alginate could upregulate electrical and mechanical coupling proteins (like Cx43) to make better 3D cardiac patches (122). Cardiomyogenic differentiation of stem cells has also been investigated in AuNP-incorporated hydrogels. In one study, Baei and colleagues dispersed AuNPs into thermosensitive chitosan matrices to make a conductive polymeric scaffold for cell stimulation. Their results revealed a comparable level of viability, metabolism, migration, and proliferation of bone marrow-derived MSCs and relatively high expression of cardiac-specific markers compared to chitosan hydrogel scaffolds without AuNPs. Also, electrical conductivity close to that of the native myocardium (around 0.13 S/m) was observed (123). Ganji *et al.* incorporated gold nanowire/nanotubes into porous polyurethane and fabricated biodegradable nanocomposites. Continuous electrical stimulation of H9c2 cells cultured on these scaffolds facilitated increased confluency and myocardial expression of Nkx-2.5, atrial natriuretic peptide, and natriuretic peptide precursor (124). In 2016, Navaei *et al.* fabricated a new class of scaffolds by incorporating gold nanorods into GelMA hydrogels. These scaffolds raised the expression of cardiac-specific markers including troponin I and sarcomeric actinin from neonatal rat ventricular cardiomyocytes (125). In another study, Li *et al.* developed a hybrid scaffold from natural collagen and AuNPs. They found that the presence of AuNPs regulated the assembly of intercalated discs in cardiomyocytes via the β1 integrin-mediated ILK/p-AKT/GATA4 pathway (126). To accommodate the use of new manufacturing technologies such as 3D printing, Zhu *et al.* developed a conductive bioink by incorporating gold nanorods into GelMA. These bioprinted constructs increased synchronized contraction and electrical propagation between cardiomyocytes (127).

Feiner *et al.* blended 3D nanocomposites with a complex electronic mesh device for online monitoring of engineered cardiac tissues. In order to sense the release of biomolecules and the electrical activity in cells and tissues, a gold electrode-based device was integrated with the electrospun scaffolds (Fig. 4D) (128). Table 2 shows different scaffolds incorporating AuNPs that have been fabricated for cardiac tissue engineering applications.

5. Electroactive polymers

Electroactive polymers (EAPs) are smart materials with controllable conductive properties suitable for fabrication of electrically conductive scaffolds. Their chemical, electrical, and physical properties can be tuned by incorporating antibodies, enzymes, and other biological components to meet the requirements of the specific application. Chemical and

electrochemical synthesis are two main methods of manufacturing conductive polymers (27). Many polymers are not conductive; therefore, they require a process called “doping” to transform into a conductive material. PPy, PANI, and polythiophene (PTh) are some important EAPs which have potential applications in cardiac tissue engineering.

5.1. Polypyrrole

PPy is one of the best-known conductive polymers. Stimulus-responsive properties, *in vitro* and *in vivo* biocompatibility (132), appropriate chemical stability, large specific surface area, and aptitude for surface modifications to incorporate bioactive molecules (27) make it an excellent candidate as a scaffold substrate for cardiac tissue engineering. In 2007, Nishizawa *et al.* electrochemically deposited PPy films onto polyimide microelectrodes. In this study, primary cardiomyocytes formed sheets on these electrodes and displayed synchronized beating upon non-invasive stimulation (133). Spearman *et al.* grew PPy films within PCL (treated with sodium hydroxide) films in order to form functional sheets of cardiac cells. Cardiomyocytes demonstrated an increase in Cx43 expression, faster calcium transfer, and lower calcium transient durations. The surface resistivity of the PCL/PPy film was 1.0±0.4 kΩ cm (134). In order to optimize PPy biomaterials for CPCs, Puckert *et al.* investigated the effect of surface properties on the viability of CPCs. The effect of different dopants on electroactivity of PPy was investigated using cyclic voltammetry (CV). The group established fabrication parameters to control the surface energy, morphology, and roughness of the materials (135).

In 2015, Gelmi and colleagues deposited chlorine doped-PPy on electrospun PLGA fibers to make 3D, electrically conductive scaffolds. Data confirmed biocompatibility of these scaffolds using cardiac progenitor cells, and iPSCs (136). Kai *et al.* demonstrated that PPy/PCL gelatin, electrospun, nanofiber scaffolds could not only improve the overall function of cardiomyocytes, but also increase the expression of cardiac-functional proteins (α-actinin, troponin T, Cx43). They also observed that incremental increases of PPy concentration could decrease nanofiber diameter and increase the tensile modulus of the scaffold. The nanofibers were measured to have an electrical conductivity between 0.01 – 0.37 mS/cm (137).

In 2015, Mihic *et al.* conjugated PPy to chitosan and developed a semi-conductive hydrogel (Fig. 5.A.a). *In vitro* cardiomyocyte studies demonstrated faster calcium transfer and lower calcium transient durations (Fig. 5.A.b and d). By increasing the amount of PPy in PPy-chitosan hydrogels, the electrical conductivity increased. *In vivo* studies showed a decrease in the QRS (one of three main waveforms in heart electrocardiograms) interval, an increase in the transverse activation velocity, and significantly higher action potential amplitudes compared to un-grafted chitosan (129). Recently, Wang *et al.* fabricated a conductive cryogel by integrating PPy nanoparticles, GelMA, and PEG diacrylate (PEGDA) using a mussel-inspired dopamine crosslinker. The *in vitro* and *in vivo* studies showed that migration of PPy nanoparticles from the scaffold to cardiomyocytes resulted in excellent synchronous contraction and a reduction in infarct size (138). In another study, Gelmi *et al.* coated PLGA fibers with PPy and made a biocompatible, electroactive scaffold for cardiogenic differentiation of human iPSCs under electromechanical stimulation (139).

5.2. Polyaniline

PANI is the oxidative polymeric product of aniline (140) and exists in different systems according to its oxidation level. Pernigraniline (fully oxidized base), emeraldine (half-oxidized base), and leucoemeraldine (reduced base) are some forms. Emaraldine is conductive and is the most stable form. PANIs are not only easy to synthesize, but also have good stability. Moreover, they are cost efficient and able to be both electrically conductive or resistant (27). Many synthesis methods for nano/micro-fabrication of PANI have also been published (141). However, PANI is not suitable for many biological applications as it is inflexible and biodegradable, making it difficult to integrate into soft cardiac tissue. Data from implantations that have been performed yielded reports of chronic inflammation in tissues due to PANI (142, 143). In addition to those discussed, many other studies have been conducted on cellular interactions with PANI in nerve, muscle, and cardiac tissue engineering (144–146).

In 2006, Bidez *et al.* investigated adhesion and growth and proliferation of cardiac H9c2 myoblasts cultured on PANI films for 200 hours. In the first 100 hours, the doubling time increased. Also, results showed that this scaffold, when in a physiological environment, maintained its conductivity for the first 100 hours; however, its conductivity gradually decreased over time (147).

To ameliorate the scaffold's biocompatibility, scientists have combined PANI with different biological materials. Li and his colleagues produced a nanofibrous blend based on co-electrospinning PANI and gelatin as a smart scaffold. PANI was doped with camphorsulfonic acid to form emeraldine PANI. Their results revealed that increasing the amount of PANI in the mixture led to reduced fiber diameter and increased tensile modulus. This biocompatible scaffold supports attachment, migration, and proliferation of cardiac myoblasts. Also, the conductivity of pure gelatin was determined to be 0.005 S/cm; however, by increasing PANI composition, the conductivity increased about four-fold (148). Fernandes *et al.* modified PANI nanofibrous scaffolds (69–80 nm diameter) with hyper-branched PLL dendrimers (4.5 nm diameter) (149). In this study, neonatal rat heart cells showed high biocompatibility and better proliferation with electrical stimulation in the scaffold. To improve the hydrophilic properties of PANI nanotubes, Moura *et al.* functionalized them with highly hydrophilic polyglycerol dendrimers (80–180 nm diameter); this modification allowed the scaffold to support cardiac cell proliferation and adhesion (150).

Hsiao *et al.* synthetized PANI-PLGA aligned fibers to develop a 3D environment for synchronous beating of cardiomyocytes (Fig. 5.B.a and b). Data showed that this scaffold increased the expression of gap junction protein (Cx43) and troponin T (Fig. 5.B.c). Cardiomyocytes formed isolated cell clusters and beat synchronously. HCl-doped PANI increased electrical conductivity, attracted positively charged cell membrane proteins, and improved adhesion (130). Borriello *et al.* used electrospun PANI with biocompatible PCL to make an electrically conductive nanocomposite scaffold. The scaffold promoted hMSC differentiation into cardiomyocyte-like cells (142).

Recently, attempts have been made to incorporate PANI into different hydrogels and polymers in order to yield electrically conductive properties. In 2013, Cui and his colleagues cultured cardiomyocytes, fibroblasts, and osteoblasts inside an injectable hydrogel made of a polylactide-poly(ethylene glycol)-polylactide (PLA-PEG-PLA) copolymer coated with Tetra aniline (with carboxylate modification). Electrical stimulation was applied directly to Tetra aniline conductive samples and enhanced proliferation of all three cell lines was observed (151). Qazi *et al.* produced a conductive cardiac patch by solvent casting PANI doped with camphorsulfonic acid and blended with PGS. The fabricated scaffold demonstrated good biocompatibility and supported attachment, growth, and proliferation of C2C12 myoblasts. After 4 days, the conductivity of the samples was similar to that of the native myocardium (152).

Baheiraei *et al.* embedded oligoaniline-polyurethane into PCL films to fabricate an electroactive biocompatible scaffold supporting cell proliferation and attachment. The electrical conductivity was on the order of 10^{-5} S/cm (153). In 2015, Baheiraei *et al.* also investigated cardiomyocyte behavior in this scaffold. They observed increased activity of cardiac-specific genes, actinin alpha 4 (Actn4) and troponin T-2, on the conductive substrate, even in the absence of electrical stimulation (154). Dong *et al.* fabricated antibacterial, self-healing, electroactive hydrogels by combining chitosan-graft-aniline tetramers with dibenzaldehyde-terminated PEG at physiological conditions. The data demonstrated that the fabricated electroactive hydrogel was biocompatible, injectable, and biodegradable. Additionally, the hydrogel was determined to have an electrical conductivity around 10^{-3} S/cm (155).

5.3. Piezoelectric polymeric materials

Piezoelectric materials generate electrical fields upon the application of mechanical stress and can apply mechanical force in the presence of an electric field (156). In piezoelectric materials, electric fields are created without an external power source; however, there are limitations on control over the stimulus (27). There are some studies on piezoelectric scaffolds in nerve (157), skeletal muscle (158), and cardiac tissue engineering. Weber *et al.* investigated *in vitro* cytocompatibility of piezoelectric, electrospun poly(vinylidene fluoride–trifluoroethylene) (PVDF-TrFE) scaffolds (159). Hitscherich *et al.* developed piezoelectric scaffolds by electrospinning polyvinylidene fluoride (PVDF) and PVDF-TrFE (Fig. 5.C.a). Mouse embryonic stem cell-derived cardiomyocytes adhered well to this scaffold and impulsively contracted, exhibited well-placed sarcomeres, and produced cardiac-specific markers including myosin heavy chain, CTT, and Cx43 (Fig. 5.C.b and c) (131). Table 3 provides a summary of scaffolds based on electroactive polymers applied to cardiac tissue engineering.

6. Biocompatibility of electrically conductive nanomaterials

Although each material offers suitable electrical properties for cardiac tissue engineering, the biocompatibility of the materials varies greatly. While the potential applications of carbon-based nanomaterials continue to expand, the ir biocompatibility may prevent their use. Several studies have been published showing mixed biological responses to the

materials. Lung toxicity to varying extents has been shown for both SWCNTs (54, 160) and MWCNTs (53). Each study found an inflammatory response to the CNTs in addition to granulation around the particles. It is believed that these inflammatory responses are due to long, biopersistent carbon nanotubes that are not completely cleared by the immune system (161). Other studies focusing on cytotoxicity have shown contrasting evidence. A study on human embryonic kidney cells reported toxicity as SWCNTs inhibited cell growth by reducing cell adhesion and inducing apoptosis (162). Cell cycle and biochip analyses showed that the nanotubes down-regulated the production of adhesion proteins (laminin, fibronectin, collagen IV) and increased expression of apoptosis-associated genes. However, another study by Tamura concluded that the cytotoxic effects were related strictly to the size of the nanoparticles, and that the CNTs are not inherently toxic (163). The study focused on neutrophil response to titanium oxide particles and CNTs in blood and concluded that toxicity is primarily related particle size under 3μm. The reason for the variation in these results likely lies within the broad range of sizes and concentrations of the nanotubes being studied; therefore, the toxicity of CNTs should be tested in each application prior to integration.

Another study compared the toxicity of carbon nanotubes to carbon nanofibers in human lung cancer cell lines (164). The team conducted in vitro analysis observing cell proliferation and morphology; they found that carbon nanofibers were significantly more toxic than nanotubes. Much of the research on carbon nanotube and nanofiber cytotoxicity has been performed on various models of the lungs as inhalation is a common method of exposure. Cardiac cells seeded with these nanomaterials in scaffolds may behave differently. Additionally, modulating the size and length of the materials is essential to achieving appropriate biocompatibility. Like other carbon-based nanomaterials, graphene has also been shown to have limited biocompatibility. A 2012 study by Li, *et al.* demonstrated the cytotoxic effects of pristine graphene on macrophages (165). Murine macrophage-like RAW 264.7 cells were cultured with various concentrations of dissolved, unmodified graphene. Strong dose-dependent toxicity was observed. Although unmodified graphene is limited, chemically modified graphene has been shown to improve the compatibility with cardiac tissue engineering by oxidizing (166–168), reducing (169), and functionalizing (170, 171) the surface.

Unlike carbon-based nanomaterials, materials using gold have shown remarkable compatibility in many studies. Shukla *et al.* showed that gold nanoparticles did not have any adverse biological impact and are biocompatible when studied with macrophages (172). The cytotoxicity of the nanoparticles on RAW 264.7 macrophages was studied with the MTT assay; the macrophages maintained viability after 72 hours. Additionally, Goodman and colleagues demonstrated the cytocompatibility of the nanoparticles with tethered ionic side chains (173). They found that cationic modifications increased cytotoxicity while anionic molecules showed little to no negative effects.

The biocompatibility of electroactive polymers varies significantly depending the specific polymer(s) used. Polymers such as PPy have been shown to be biocompatible with limited inflammatory response after implantation *in vivo* (132). The material was tested *in vivo* and *in vitro* on rat peripheral nerve tissue and was observed to be biocompatible and a viable

polymer for repairing nerve damage in rats. PANI has also been studied and has shown great biocompatibility with H9c2 cardiac myoblasts (147). While an initial reduction in growth and adhesion was observed, morphologically identical monolayers were formed on PANI-coated surfaces compared to polystyrene surfaces after six days. Additionally, the polymer maintained conductivity for 100 hours after coating. Another study on polylactide-aniline pentamer (PLAAP) copolymers also demonstrated excellent cytocompatibility with rat glioma cells (143). Cell viability (measured with the MTT assay) was highest for cells cultured on PLAAP compared to PLA and aniline pentamer (AP) individually. The last electroactive polymer discussed in this review was PVDF-TrFE; a study found that human skin fibroblasts proliferate normally on PVDF-TrFE in comparison to those cultured on treated polystyrene *in vitro* (159).

For these materials to be used in cardiac tissue engineering, it is imperative that they have the necessary physical, electrical, and biological properties. For some materials, such as graphene, simple modifications can be made to tailor the surface for the specific application; however, other materials may have inherent limitations that impair their utility as conductive materials for tissue engineering.

7. Concluding remarks and future challenges

Cardiovascular disease, involving the heart and/or blood vessels, is a primary cause of death in the 21st century. Cardiac tissue engineering has the potential to introduce suitable materials and procedures to serve as innovative alternative treatment strategies to heart transplantation. Despite the considerable achievements in recent years, scientists have faced many limitations in creating functional, engineered myocardium tissue at clinical levels (174). Promoting the electrical integration of an engineered tissue with the host myocardium can help restore functionality in a failing heart. Regulated beating of the heart is highly dependent on the structure and chemistry of the ECM. Engineered cardiac scaffolds require mimicked anisotropic structure of native myocardial ECM, electrical conductivity of the cardiac tissue (0.16 S/m longitudinally and 0.005 S/m transversely), and recreation of the unique mechanical properties of the myocardium (highly aligned collagen nanofibers 10–100 nm) that can be obtained by tuning the scaffold’s biochemical, biophysical, and topographical features. There have also been attempts to apply frequent, regular electrical stimulation to growing tissues, resulting more efficient engineered cardiac patches.

Tissue engineering scaffolds containing electrically conductive nanostructured materials are able to mimic the myocardial ECM (175). Moreover, they have been proven to support electromechanical integration of cardiomyocytes within the host myocardium after transplantation. There are a wide range of conductive nanostructured materials for cardiac tissue engineering; these include carbon-based nanomaterials (CNTs, CNFs, and graphene), gold-based nanomaterials, and electroactive polymers (such as PANI, PPy, and piezoelectric polymeric materials). Apart from developments in the chemistry of scaffolds, the fabrication techniques are also moving forward from conventional methods to innovative 3D manufacturing. Scientists from many disciplines are trying to facilitate cardiomyocyte communication through a myriad of strategies including electrically conductive scaffolds and gene transfer techniques. The ultimate goal in cardiac tissue engineering is to induce the

creation of specific cardiac gap junction proteins to enable the production of functional tissue constructs. Substantial interest in the scientific community has revolved around the use of electroactive nanostructured materials due to their great potential in cardiac tissue engineering. In addition, state-of-the-art fabrication techniques will assist electrically conductive scaffolds for improved functionality. Although nanostructured gold particles, carbon-family materials, and electroactive polymers have shed light on the preparation of promising scaffolds and patches, there are still unexplored biomaterials and fabrication strategies with potential to revolutionize the field. There are still many unanswered questions regarding different aspects of these biomaterials such as their cytotoxicity, biodegradability, injectability, and aptitude for surface functionalization. Moreover, it is important to better explore the effects of these biomaterials on differentiation of cardiomyogenic stem cells, their adherence, elongation, orientation, and functional properties as these properties relate to the development of functional cardiac tissue. Undoubtedly, more investigation on the use of electrically conductive nanostructured materials in cardiac tissue engineering must be pursued to answer the critical questions in the field. Due to the interdisciplinary nature of the field, materials scientists, biologists, engineers, and physicians should work together to develop new technology in the pursuit of surmounting the challenges of cardiac tissue engineering.

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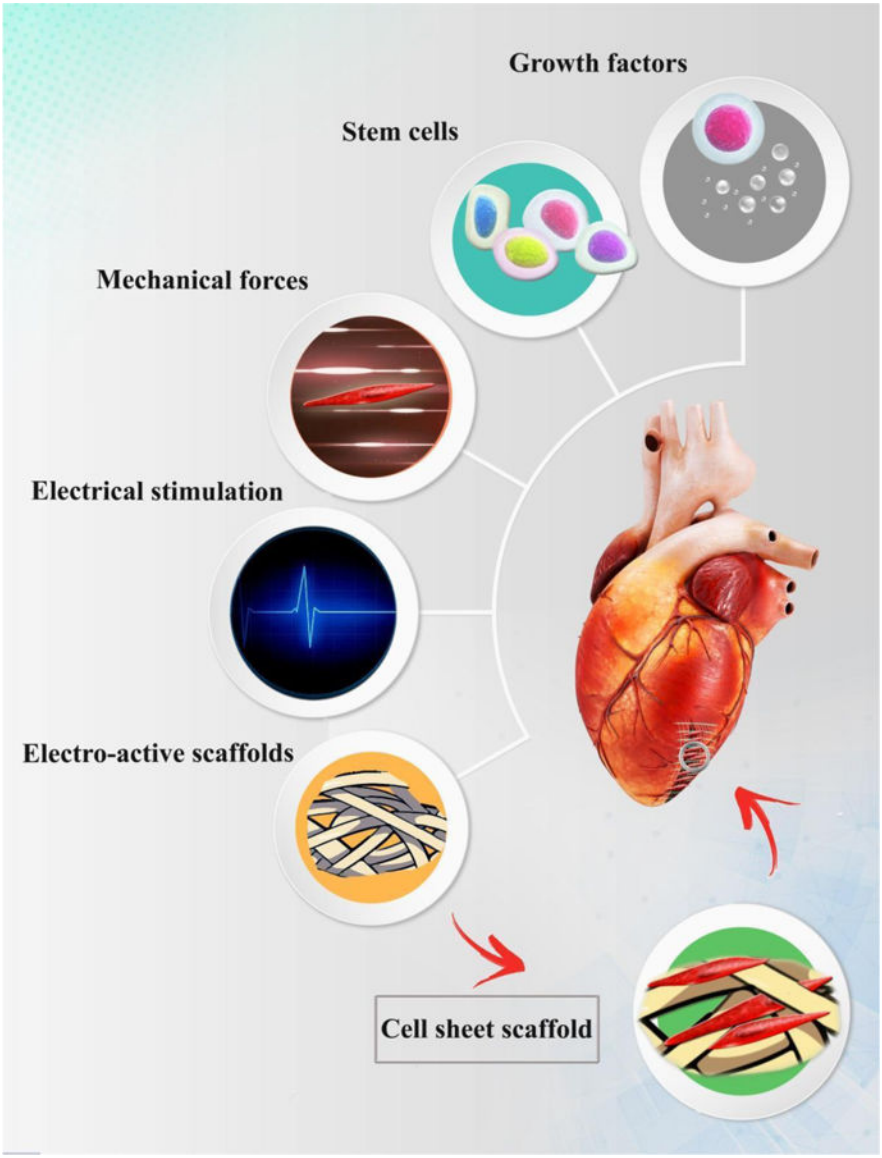

Fig. 1.

Representation of key factors for cardiac tissue regeneration. Induced pluripotent, mesenchymal, and embryonic stem cells have been used as cell sources for cardiogenic differentiation using various protocols and growth factors. Mimicking the native cardiomyocyte microenvironment is also crucial for functional tissue regeneration – this can be done by applying relevant mechanical and electrical stimulation through electrically conductive nanoscale scaffolds. Implementing these factors can help achieve dense populations of beating, functional cardiomyocytes embedded in scaffolds for cardiac regeneration.

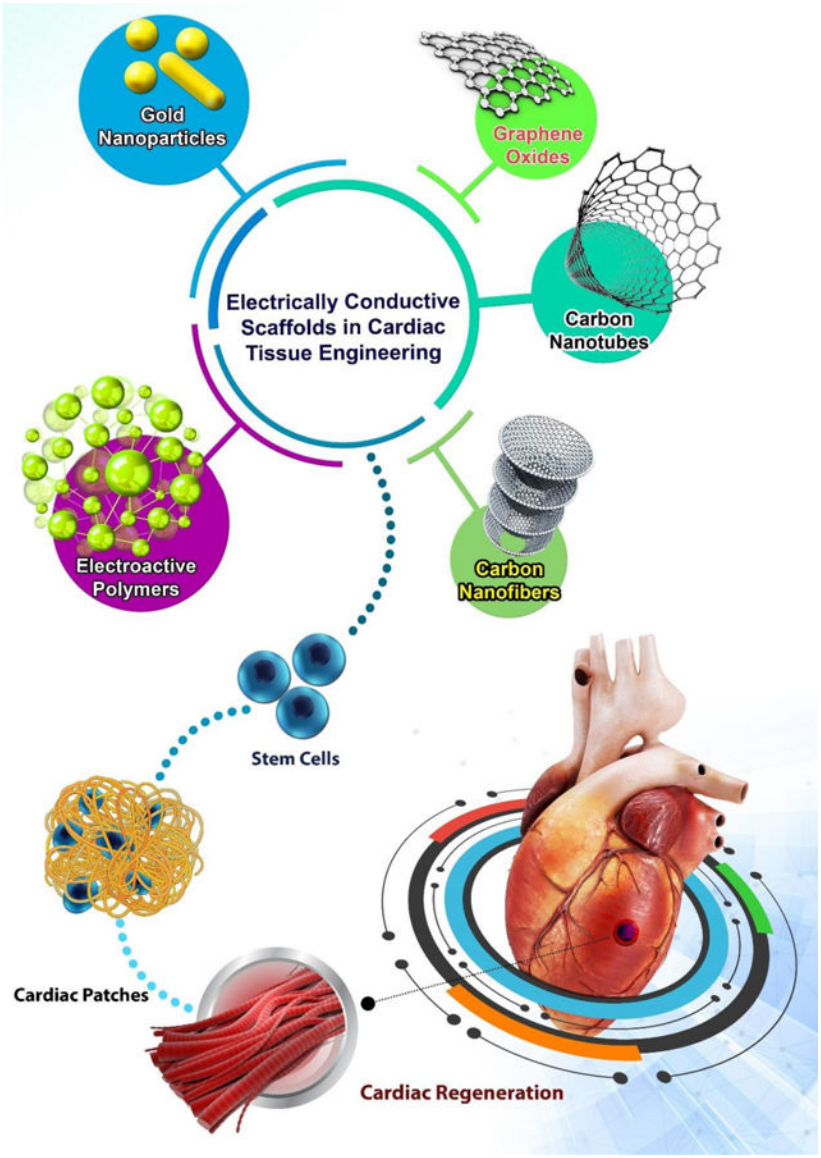

Fig. 2.

Different categories of nanomaterials utilized for the production of electrically conductive cardiac tissue engineering scaffolds.

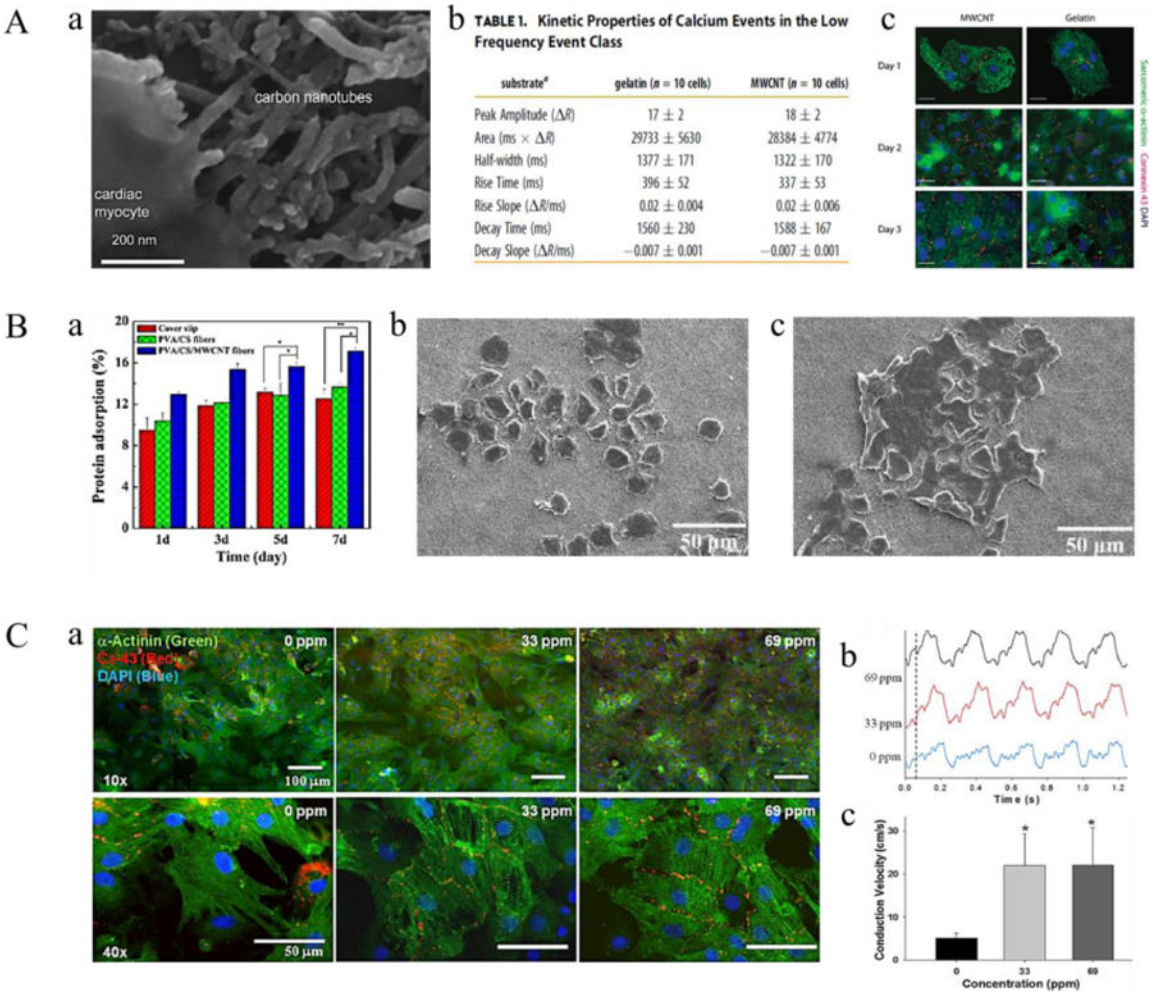

Fig. 3.

Carbon-based nanomaterials in cardiac tissue engineering. **(A)** Instructing physiological growth and functionally mature syncytia using 20–30 nm MWCNTs. (a) Scanning electron microscopy (SEM) image of cardiac myocytes and MWCNT (b) Reported data for the kinetic properties of calcium events of cells cultured on gelatin and MWCNTs. (c) Fluorescence images of cardiac myocytes, α-actinin (green), Cx43 (red) and nuclei (DAPI, blue). Distribution of Cx43 on the MWCNTs (left) and gelatin (right) scaffolds. Scale bars = 50 μm. Reprinted from (65). **(B)** Improved cellular response of cardiac cells on the MWCNT-incorporated PVA/chitosan scaffolds (a) Incorporation of MWCNTs improved nanofiber protein adsorption (b) PVA/chitosan nanofibrous mats without MWCNTs and (c) PVA/chitosan/MWCNTs nanofibrous mats. Reprinted from (66). **(C)** CNT-chitosan scaffolds. (a) Fluorescence images of ventricular myocytes cultured on CNT-chitosan scaffolds. Cells stained for sarcomeres: α–actinin (green), gap junctions: Cx43 (red), and DNA. SWCNTs addition enhanced synchronous beating (b) and conduction velocities (c). Reprinted from (69).

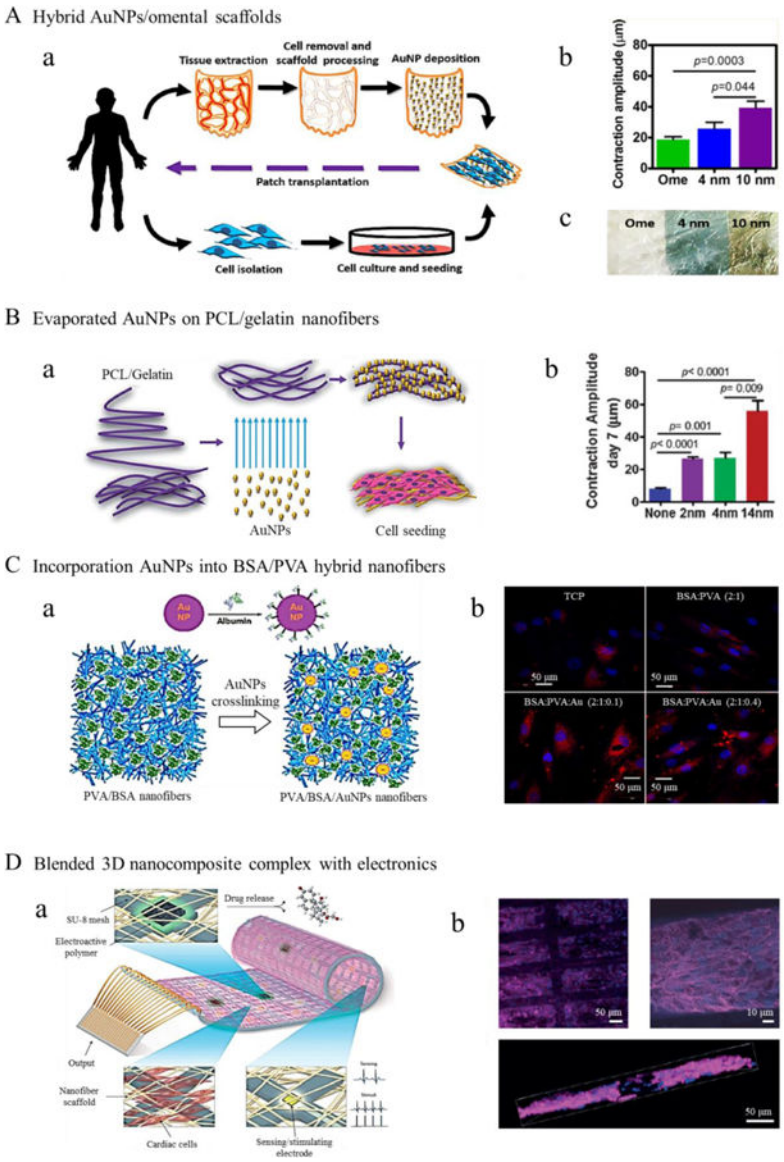

Fig. 4.

Gold nanomaterials in cardiac tissue engineering. **(A)** Modifying the electrical properties of omental matrix using AuNPs (a) Decellularized omental matrix was decorated with AuNPs using an e-beam evaporator. Cells were isolated from the same patient, placed on the scaffold for a personalized cardiac patch. (b) These hybrid scaffolds led to stronger contraction forces generated by cardiomyocytes. (c) Deposition of AuNPs, with thicknesses of 4 and 10 nm on the scaffold's fibers, caused a color change in the scaffold. Reprinted from (115). **(B)** Increasing the matrix conductivity of microporous scaffolds by incorporating AuNPs. (a) AuNPs (2, 4, and 14 nm) were evaporated on the surface of PCL-gelatin nanofibers (250 nm diameter). (b) Cardiac cells seeded in these scaffolds showed enhanced contraction amplitude and rate. Reprinted from (117). **(C)** AuNPs incorporated into BSA/PVA hybrid nanofibers remarkably enhanced cardiomyogenic differentiation of hMSCs. (a) The mechanism of crosslinking of AuNPs in bovine serum albumin (green) and

AuNPs (yellow). (b) Immunocytochemical results indicated gap junction protein expression, Cx43 (red), by differentiated contractile MSCs. DAPI: blue. Reprinted from (118). **(D)** (a) A blended 3D nanocomposite complex with embedded electronics for online monitoring of engineered cardiac patches. (b) Confocal microscope images of the assembled cardiac tissue within the biomaterial–electronics hybrid. Sarcomeric actinin is pink, nuclei are blue (Hoechst 33258). Reprinted from (128).

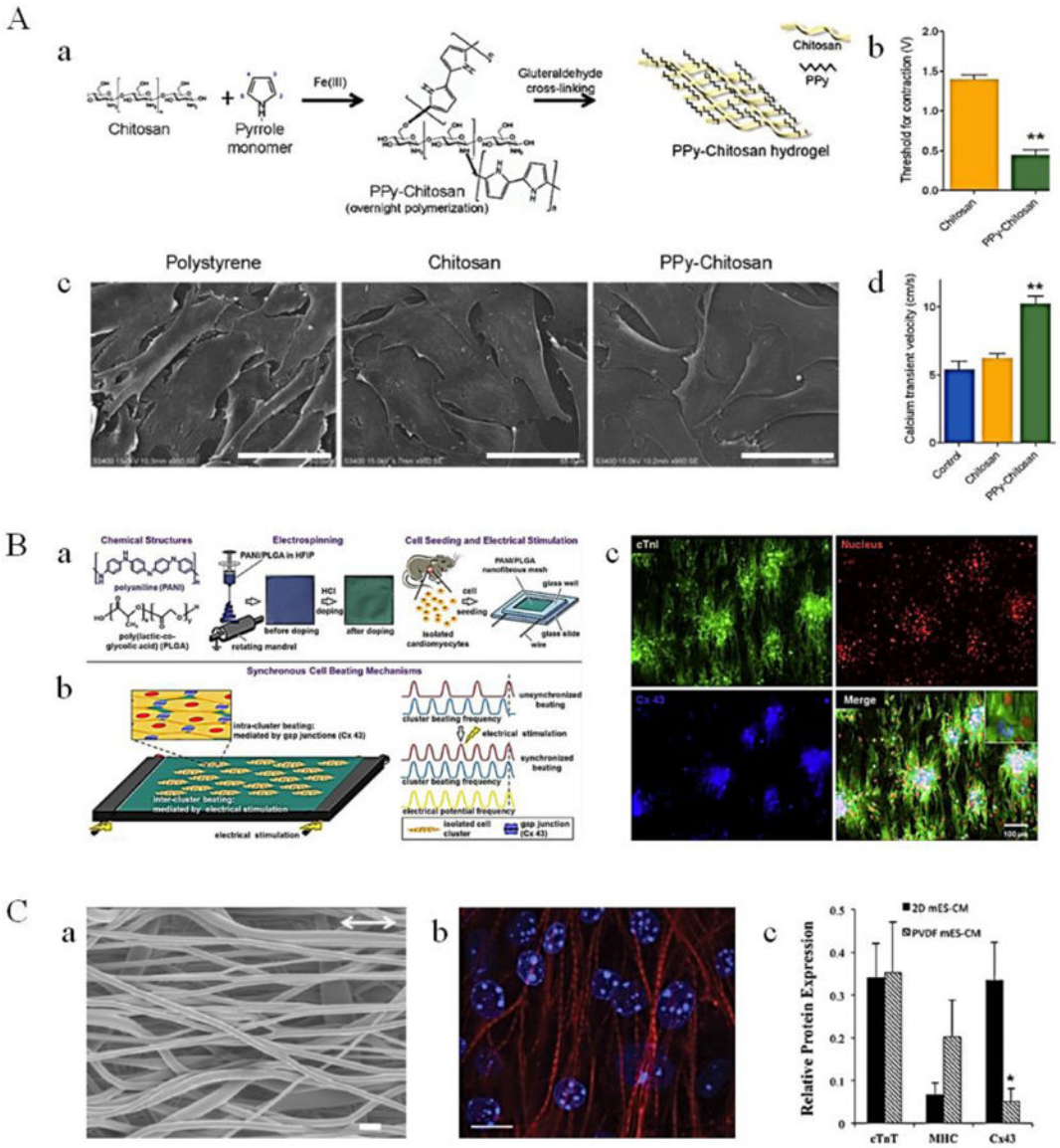

Fig. 5.

EAPs in cardiac tissue engineering. **(A)** PPy conjugated to chitosan formed a semi-conductive hydrogel to enhance Cx43 expression, faster calcium transfer, and lower calcium transient durations in cardiomyocytes. (a) PPy monomers grafting and cross-linking into a hydrogel. (b) Determination of the contraction threshold voltage was performed by anion-contact stimulation of a single skeletal muscle. PPy-chitosan has a lower threshold voltage than chitosan. (c) A normal morphology was observed in the SEM images of rat smooth muscle cells which were plated on polystyrene, chitosan, or PPy-chitosan. Scale bars = 500 μm. (d) Faster transient velocity was observed for calcium in rat neonatal cardiomyocytes which were plated on PPy-chitosan. Reprinted from (129). **(B)** PANI-PLGA aligned fibers develop a 3D environment for synchronous beating of cardiomyocytes and increasing expression of Gap Junction proteins. (a, b) Synthesis, seeding, and stimulation of the PANI/PLGA nanofibrous mesh for synchronous cell beating. (c) Fluorescence images of neonatal

rat cardiomyocytes cultured on meshes (cardiac troponin I is green, Cx43 is blue, and nuclei are red). Reprinted from (130). Scale bar = 100 μm. **(C)** Cardiomyocytes adhered well to piezoelectric scaffolds made by electrospinning PVDF and PVDF-TrFE. (a) SEM image of a PVDF-TrFE scaffold with aligned fibers. Scale bar is 2 μm. (b) Aligned actin filaments with well-placed sarcomeres. F-actin is stained red and DAPI-stained nuclei are blue. Scale bar = 10 μm. (c) Expression of cTnT, MHC, and Cx43 in cardiomyocytes cultured on PVDF-TrFE scaffolds compared to 2D cell culture. Reprinted from (131).

Table 1.

Carbon-based nanomaterials in cardiac tissue engineering.

Material	Electrical Cues	Scaffold	Cell	Results	Limitations	Year	Ref
Aqueous SWCNTs	Not mentioned	Pure CNTs/SWCNTs	H9c2	- No short-term toxicity - Biocompatible	Cell death due to physical interactions with SWCNTs	2005	**-63**
Precipitated MWCNTs on glass surface	More negative resting and action potential duration after 2–3 days	Pure MWCNTs (162 nm) deposited on glass	NRVC	- Improved viability and proliferation	Not suitability of glass surfaces for implantation	2012	**-64**
	Significant growth in resting membrane potential	Pure MWCNTs (20–30 nm) deposited on glass	NRVC	- Enhanced expression of terminal differentiation gene (myosin heavy chain [MHC], sarcoplasmic reticulum Ca^{2+} ATPase 2a) - Functional gap junctions were formed in syncytia		2013	**-65**
CNFs incorporated in nanofibers	Increased conductivity irrespective of CNFs diameter	PLGA/CNFs (100 and 200 nm)	Human cardiomyocytes	- Improved cardiomyocytes proliferation and density using 200 nm CNFs	Potential toxicity of CNFs during degradation	2011	**-91**
	Increased	pHEMA /CNFs (100 nm) / RNTs (inner/outer diameters of 1.1/3.5 nm)	Human cardiomyocytes	- Increased cardiomyocytes density		2012	**-92**
	Obtaining electrical resistance in horizontal and vertical direction with four-point probe method close to natural heart tissue	PLGA (100 nm)/ aligned CNFs	Cardiomyocytes	- Improved anisotropic mechanical and electrical properties		2014	**-93**
CNTs incorporated in nanofibers	Not mentioned	PVA/Chitosan (157 nm) / MWCNTs (70–30 nm × 100–400 nm)	L929	- Increased cell proliferation	- Potential toxicity of CNTs during degradation - May require additional manufacturing processes to develop 3D scaffolds - Low control on CNT dispersion in naofibers	2011	**-66**
	Not mentioned	PCL/thiophene/MWCNTs (15–7 nm × 2 μm)	CPCs	- CPCs induced to survive and differentiate - Proliferation was higher on the PCL/thiophene-CNT meshes		2014	**-67**
	Electrical field stimulation (biphasic square wave 5ms pulse / 0–7 volt / 1–3 Hz frequency)	PGS/gelatin (167 nm)/MWCNTs (30 nm diameter 20–50 nm length)	Cardiomyocytes	- Spontaneous and synchronous beating behavior observed - Resembling the myocardium anisotropic structure.		2014	**-68**

Material	Electrical Cues	Scaffold	Cell	Results	Limitations	Year	Ref
	Excitation threshold is 3.5 times less Maximum capture rate increased by 2.8 times			- Contractile properties of the cardiomyocytes were significantly improved			
	electrically stimulation (current of 0.15 V/cm and frequency of 1 Hz) for 14-days) Cells elongated and reoriented between 0–10 degrees with current	SWCNTs (two-pronged carbon nanotube)	hMSCs	- Upregulation of cardiac markers - 40 fold increase in cardiac myosin heavy chain [CMHC] - Upregulation of Nkx-2.5, GATA-4, CTT and Cx43		2012	**-59**
	Extrinsic electrically stimulation (current of 500 V/m, 5 ms duration, frequency of 1 Hz) for 4-days Optimum ionic resistance of scaffold was obtained in 3% CNTs incorporation	PCL/MWCNTs	hMSCs	- The *in vitro* cardiac cardiomyogenic differentiation of hMSCs promoted - Elongated morphology - Elevated expression of cardiac troponin T (cTnT), Nkx-2.5 and amyosin heavy chain		2013	**-70**
CNTs nanofibers incorporated in hydrogels/ polymers	Lower impedance on CNTs/Gel MA External electric field 1 V/cm at 1, 2 and 3 Hz	GelMA/MWCNTs (50–100 nm diameter)	NRVC	- Improved cell-cell coupling - Homogeneous cell organization and Cx43 distribution - Partial uniaxial alignment of sarcomeric structures	- Difficult to incorporate an ideal balance of materials to create the proper microenvironment - Low amounts of CNTs can be dispersed in hydrogels/polymers - Increase in electrical conductivity of scaffolds is not sufficient	2013	**-57**
	Electrical stimulation square wave 1 Hz at 5 V (50 ms pulse width) Conductivity of aligned CNTs was 12.1 S/cm	GelMA/MWCNTs (2 and 5 nm)	cardiac cells	- Homogeneous cell organization - Overexpression of sarcomeric α-actinin and Cx43		2015	**-106**
	Not mentioned	Collagen/Chitosan/SWCNTs (0.8 nm × 262 nm length)	cardiomyocytes	- Enhanced electrical coupling, synchronous beating, and cardiomyocytes function.		2014	**-69**
	Not mentioned	Type I collagen, MWCNTs (30 ± 15 nm × 5–20 μm)	Neonatal rat cardiomyocytes	- Improved cardiac cell functions		2017	**-73**
	Not mentioned	MWCNTs (40–90 nm × 10–20 μm), poly (octamethylene maleate (anhydride)	Neonatal rat cardiomyocytes	- Improved maturity and excitation threshold		2017	**-74**

Material	Electrical Cues	Scaffold	Cell	Results	Limitations	Year	Ref
		1,2,4-butanetricarboxylate)					
	PCL with 3% CNTs Conductivity of 2.2×10^{-7} S/cm) (PCL with 5% CNT (Conductivity of 1.2×10^{-6} S/cm)	PCL, MWCNTs (20–30 nm ×10–30 μm)	Rat H9c2 cells	- Myoblast cells attached to the scaffolds in a healthy condition for 4 days.		2016	**-75**
	The electrical conductivity of scaffold was 0.015 S/cm	MWCNTs functionalized with carbodihydrazide (≈166 nm), pericardial matrix hydrogel	HL-1 cardiomyocytes	- Increased expression Cx43 - Improved beating - Increased cellular viability		2017	**-77**
	Not mentioned	SWCNTs (average diameter of 50 nm), Gelatin	Rat H9c2 cells	- Increased proliferation, differentiation, and electrical conductivity of cells		2017	**-78**

Table 2.

Examples of the use of gold nanomaterials in cardiac tissue engineering.

Material	Electrical Cues	Scaffold	Cells	Results	Limitations	Year	Ref
Decellularized matrices + AuNPs	Lower excitation threshold and excitation threshold	AuNPs (4 and 10 nm) / decellularized omental matrices	Cardiac cells	- Elongated and aligned morphology - More Cx43 expression - Stronger contraction force - Faster calcium transients	- AuNPs may dissociate from the scaffold *in vivo* - Non-degradation of AnNPs - Mismatch between mechanical properties of decellularized ECM and AuNPs	2014	**-115**
Fibrous scaffold + AuNPs	In presence of external electrical field lower excitation threshold in AuNPs within the scaffold	gold (film)/PCL	NRVC	- Significantly higher aspect ratio and stronger contraction forces - Reaction to significantly lower electrical fields	- Non-degradation of AnNPs *in vivo* - Los dispersion of AuNPs in scaffolds	2014	**-116**
	Not mentioned	AuNPs (2, 4 and 14 nm) / PCL/ gelatin (250 nm)	NRVC	- Enhanced elongation and alignment, more cardiac sarcomeric α-actinin expression, higher contraction amplitudes and rates		2013	**-117**
	Not mentioned	BSA/PVA/ AuNPs(273±29 nm) BSA/PVA/AuNPs (278±22 nm)	hMSCs	- Cardiomyogenic differentiation - Multinucleated morphology - Improved cardiac protein expression (á-actinin, troponin T and Cx43)		2014	**-118**
	Not mentioned	PCL/Vit B12/Aloe Vera/Silk fibroin/ AuNPs (16nm)	hMSCs	- Cardiomyocy tes and hMSCs cocultured - Proliferation and cardiogenic differentiation enhanced		2015	**-119**
Hydrogel scaffold + AuNPs	Scaffold conductivity: 15.3±0.8 S/m Electrical stimulation (2mA rectangular pulses, 2 ms, 1 Hz, 5 V/cm) for 5 days	AuNPs (8.1 ± 0.9 nm and 4.4 ± 0.3 nm)/thiol-HEMA	NRVC	- Cx43 expression increased	- AuNPs may interfere with hydrogel crosslinking	2011	**99**
	Electrical conductivity of scaffold: 1.51 S/cm	1.1 μm beads (PCL/silica) / AuNPs (20 nm film)		Not mentioned		2014	**100**
	Electrical conductivity of scaffold was close to native myocardium 0.13 S/m	Chitosan/AuNPs (7.24 nm)	hMSCs	- Scaffolds supported viability, metabolism, migration and proliferation of hMSCs - Significantly increased expression		2016	**102**

Material	Electrical Cues	Scaffold	Cells	Results	Limitations	Year	Ref
				of α-myosin heavy chain (α-MHC) and Nkx-2.5			
	Not mentioned	GelMA hydrogel, gold nanorods (average aspect ratio of 3.15,16 ± 2/53 ± 4 nm width and length)	Cardiomyocytes	- High cell retention - Improved cytoskeleton organization - Enhanced expression of cardiac markers (troponin I, sarcomeric α-actinin)		2016	**104**
	Not mentioned	Collagen and AuNPs	Cardiac muscle cells	- AuNPs regulates the assembly of intercalated discs via the β1 integrinmediated ILK/p-AKT/GATA4 pathway		2016	**105**
		Gold nanorods (34 nm × 25 nm wide), GelMA	Neonatal rat ventricular cardiomyocytes	- Improved the electrical propagation between cardiac cells.		2017	**106**
Gold nanowire	Films showed low impedance at high frequencies (10 kHz)	Alginate/gold nanowire (30 nm)	Cardiac cells	- Thicker and aligned engineered tissues - Expression of Cx43 increased	- Gold nanowires can be entered the cell membrane and cause cytotoxicity	2011	**101**
	- Electrical stimulation: square pulse (1 V/mm amplitude, 2 ms pulse duration, frequency of 1 Hz) for 15 min.	Castor oil based polyurethane/gold	H9c2	- Increased cell confluency - Upregulation of myocardial functional gene expression: Nkx-2.5, atrial natriuretic peptide and natriuretic peptide precursor B		2016	**103**
New Devices	Provide information on the electronic devices that can control cell/ tissue functions	Gold used as electrodes		- Flexible cardiac patch which is freestanding in cardiac 3D scaffolds	- Proving safety and efficacy at low voltages is essential	2016	**107**

Table 3.

Summary of the electroactive polymer scaffolds used in cardiac tissue engineering.

Material	Electrical properties	Scaffold	Cell	Result	Year	Ref
Polypyrrole	Surface capacity of PPy thin films on the electrode substrate 5.8 C/cm2	Pt microelectrodes on polyimide (PI) surface/PPy film	Primary cardiac myocytes	- Adhesive strength of PPy film enhanced - Cells showed synchronized beating upon Stimulation	2007	**-133**
	The surface resistivity PCL/PPy 1.00±0.40 KΩ.cm	PCL films/PPy	HL-1	- Functional cardiac cell sheets formed - Increased in Cx43 expression - Faster Calcium transfer - Lower calcium transient durations	2015	**-134**
	Effect of different dopants on electroactivity of PPy though cyclic voltammetry (CV)	PPy/different dopants CS/PTS/DBS	CPCs	- Surface properties of conductive polymers controlled	2016	**-135**
	In CV, as more PPy deposited, the capacitance of PPy layer increases	Electrospun PLGA fibers/PPy (200 nm)	CPCs/iPSCs	- Confirmed biocompatibility	2015	**-136**
	Electrical conductivity 0.01 – 0.37 mS/cm	PCL/Gelatin fibers/PPy (216 ± 36 nm and 191 ± 45 nm)	New Zealand white rabbits Cardiomyocytes	- Improved attachment, proliferation, - Enhanced expression of cardiac-functional protein (α-actinin, Troponin T, Cx43)	2011	**-137**
	By increase the rate of PPy in PPy/Chitosan hydrogel, the electrical conductivity increase	Chitosan	Rat smooth muscle cells	- *In vitro*: increased Cx43 expression, faster calcium transfer and lower calcium transient durations. - *In vivo*: decreasing in the QRS interval, increasing in the transverse activation velocity	2015	**-129**
	Electrical conductivity was almost 0.0072 S/m	PPy nanoparticles (59 ± 6 nm) gelatin-methacrylate polyethylene glycol diacrylate	Neonatal Rat Ventricular Myocyte	- *in vitro*: higher level Cx43 expression, and α-actinin - *in vivo:* immobilizing cardiomyocytes into scaffolds for a long time, reduce in infarct size.	2016	**-138**
		PLGA fiber (2.27 μm) with a layer of PPy (320 nm and 0.49 μm)	human iPSCs	- With excellent cell viability, over expression of cardiomyocyte specific genes (Actinin, NKX2.5, GATA4, Myh6, c-kit)	2016	**-139**
Poly Aniline	Surface resistivity (non-conductive PANI) higher than 10 MΩ/square After partial de-doping, resistivity 2 kΩ/square	PANI	H9c2	- Enhanced cell attachment and growth on PANI films	2006	**-147**
	Conductivity of pure gelatin 0.005 S/cm By increasing PANI the conductivity increased four fold	Blend: Gelatin/PANI (61±13 nm fiber)	H9c2	- Biocompatible - Supporting migration, and proliferation	2006	**-148**
	Electrical current stimulation: voltage (10–40 V) 0.5 Hz, 5 ms pulses	Hyperbranched PLL dendrimers/PANI (69–80 nm)	Cardiomyocytes of rats	- Higher cell viability and proliferation	2010	**-149**
	Not mentioned	Polyglycerol dendrimers/PANI (80–180 nm)	Cardiomyocytes of rats	- Biocompatible - Supporting cardiomyocytes proliferation.	2011	**-150**

Material	Electrical properties	Scaffold	Cell	Result	Year	Ref
				- Microcurrent applied to stimulate the differentiation		
	Conductivity of mesh: 3.1×10−3 S/cm and ES: 1.25 Hz, 5 V/cm	PANI/PLGA fiber (184.7 nm and 101.7 nm)	Neonatal cardiomyocytes	- Elongated cardiomyocytes formed isolated cell clusters, beating synchronously, enhanced expression of Cx43	2013	**-130**
	PCL without incorporated PANI shows minimal conductivity (3×10−12 S/cm), by increase PANI in the film conductivity increases by up to seven orders of magnitude	PCL/PANI (50–100 nm)	hMSCs	- Cardiogenic differentiation of hMSCs into cardiomyocytes-like cells - Sarcomeric α-actinin of cardiomyocytes observed	2011	**-142**
	Electrical stimulation: square wave, frequency of 100 Hz and electrical potential of 0.5 V	Carboxyl-capped tetraaniline (approx. 265 nm)/(PLA-PEG-PLA)	Fibroblasts, cardiomyocytes, and osteoblasts	- *in vitro:* excellent cytocompatibility - *in vivo*: acceptable biocompatibility, injectable	2013	**-151**
	The conductivity close to native myocardium ranges	PGS	C2C12	- Cytocompatibility of the nanocomposites was confirmed	2014	**-152**
	electrical conductivity in 10–5 S/cm	Embedded oligoaniline-polyurethane into PCL films	- L929 mouse Fibroblast/ HUVECs	- Biocompatible - Supporting cell proliferation and attachment - Biodegradable	2014	**-153**
	- Scaffold's conductivity was 10−5 ± 0.09 S/cm	Aniline pentamer polyurethane/PCL (pore size (several μm to 150 μm))	Neonatal cardiomyocytes	- Cell produced more cardiac specific genes (Actn4 and troponin T-2) - Biodegradable	2015	**-154**
	- Conductivity of this cell delivery vehicles was ~10–3 S/cm	Chitosan-graft-aniline tetramer and dibenzaldehyde-terminated PEG	C2C12 myoblasts and H9c2 cardiac cells	- Biocompatible, injectable and biodegradable self-healing electroactive hydrogels	2016	**(155)**