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, and 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

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1. Introduction

In the United States cardiovascular diseases (CVDs) are responsible for one death every 40 s [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 [23, 24]. 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 immnosuppressive drug therapy after surgery to decrease the risk of transplant rejection [5]. The disadvantages of heart transplants 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 [17–19], nanoporous and composite materials [20], nanoparticles [21], and modified nano-patterned surfaces have been adopted. These technologies help to 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 (CNTs), 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 up-regulation 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 facilitating 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

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 CNTs 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, such as electric-arc technique [47] and laser ablation [48], and chemical methods, such as chemical vapor deposition [49].

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 to achieve dense populations of beating, functional cardiomyocytes embedded in scaffolds for cardiac regeneration.
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 3 days (short-term biocompatibility). However, the long-term toxicity became apparent as apoptosis occurred after 3 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 2–3 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. 3A.a) improved the electrophysiological characteristics of the cardiomyocytes, enhanced intracellular calcium signaling (Fig. 3Ab), 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. The 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 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 the 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 hybrid material resulted in strong and synchronized beating of cardiomyocytes. By incorporating the CNTs, the excitation threshold was 3.5 times lower and expression of Cx43 in cardiomyocytes was higher. In addition, the CNTs improved 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 such as Nkx2.5, 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 chosen using conductivity measurements [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 three-dimensional (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 \(\mu\)m thickness) to construct scaffolds with anisotropic electrical conductivity. The engineered tissues with the CNTs showed better cell organization, higher cell-to-cell coupling, and an increase in HL-1 cell maturation. Synchronized beating improved and significant reduction in excitation thresholds were observed. In the latter study, expression of troponin I andCx43 was increased and no toxic effects were observed for 7 days [57]. In 2015, Elkenany 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 \(\alpha\)-actinin andCx43 led to improved cell behavior [72]. In another study, Pok et al. developed a scaffold containing subtoxic concentrations of SWCNTs (5 nm diameter \(\times\) 262 nm length) in a gelatin-chitosan hydrogel. Nanobridges of the SWCNTs between the 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 the native myocardial tissue at 22 \(\pm\) 9 cm/s [69]. Yu et al. incorporated carboxyl-functionalized MWCNTs into type I collagen hydrogels. They demonstrated that 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-butadienecarboxylate). Their study demonstrated that scaffolds composed of 0.5% CNTs improved 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, Izadiar 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. The functionalized MWCNTs with carbodiimide 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\(^5\) 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 dispersing CNFs 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 the 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 \(\mu\)m wide) of aligned CNFs (100 nm diameter) on the surface of PLGA (50:50 PGA:PLA weight ratio). The results showed that the 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 the 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, 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 graphene, 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 myofibris and sarcomeric structures in addition to an increase in electrical coupling of cardiac cells [100].

Graphene oxide (GO) is an 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 high 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. The GO sheets were coated with poly-l-lysine (PLL). Neonatal rat ventricular cardiomyocytes between the PLL and the GO under electrical stimulation showed spontaneous beating, cardiac cell organization, cell maturation, and cell-to-cell electrical coupling [104]. Also, the incorporation of rGO into GelMA hydrogels enhanced 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 mechanical and electrical properties of hydrogels. These
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<tr>
<td>Aqueous SWCNTs</td>
<td>Not mentioned</td>
<td>Pure CNTs/SWCNTs</td>
<td>H9c2</td>
<td>- No short-term toxicity - Biocompatible</td>
<td>Cell death due to physical interactions with SWCNTs</td>
<td>2005</td>
<td>[63]</td>
</tr>
<tr>
<td>Precipitated MWCNTs on glass surface</td>
<td>More negative resting and action potential duration after 2–3 days</td>
<td>Pure MWCNTs (162 nm) deposited on glass</td>
<td>NRVC</td>
<td>Improved viability and proliferation</td>
<td>Not suitable of glass surfaces for implantation</td>
<td>2012</td>
<td>[64]</td>
</tr>
<tr>
<td>CNFs incorporated in nanofibers</td>
<td>Increased conductivity irrespective of CNFs diameter</td>
<td>PLGA/CNFs (100 and 200 nm)</td>
<td>Human cardiomyocytes</td>
<td>Improved cardiomyocytes proliferation and density using 200 nm CNFs</td>
<td>Potential toxicity of CNFs during degradation</td>
<td>2011</td>
<td>[91]</td>
</tr>
<tr>
<td>CNTs incorporated in nanofibers</td>
<td>Not mentioned</td>
<td>PVA/Chitosan (157 nm) / MWCNTs (70–30 nm × 100–400 nm)</td>
<td>L929</td>
<td>Increased cell proliferation</td>
<td>- Potential toxicity of CNTs during degradation - May require additional manufacturing processes to develop 3D scaffolds - Low control on CNT dispersion in nanofibers</td>
<td>2011</td>
<td>[66]</td>
</tr>
<tr>
<td></td>
<td>Obtaining electrical resistance in horizontal and vertical direction with four-point probe method close to natural heart tissue</td>
<td>PCL/thiophene/MWCNTs (15–7 nm × 2 μm)</td>
<td>CPCs</td>
<td>- CPCs induced to survive and differentiate - Proliferation was higher on the PCL-/thiophene-CNT meshes</td>
<td>- Spontaneous and synchronous beating behavior were observed - Resembling the myocardium anisotropic structure. - Contractile properties of the cardiomyocytes were significantly improved</td>
<td>2014</td>
<td>[67]</td>
</tr>
<tr>
<td></td>
<td>Electrical field stimulation (biphasic square wave 5ms pulse / 0–7 volt / 1–3 Hz frequency)</td>
<td>PGS/gelatin (167 nm)/MWCNTs (30 nm diameter 20–50 nm length)</td>
<td>Cardiomyocytes</td>
<td>- Upregulation of cardiac markers - 40 fold increase in cardiac myosin heavy chain - Upregulation of Nkx-2.5, GATA-4, CTT, and Cx43</td>
<td>Cardiac cardiomyogenic differentiation of hMSCs was promoted - Elongated cell morphology - Elevated expression of cardiac troponin T (cTnT), Nkx-2.5, and myosin heavy chain</td>
<td>2014</td>
<td>[68]</td>
</tr>
<tr>
<td>CNTs nanofibers</td>
<td>External electric field (1 V/cm at</td>
<td>GelMA/MWCNTs (50–100)</td>
<td>NRVC</td>
<td>- Upregulation of cardiac markers - 40 fold increase in cardiac myosin heavy chain - Upregulation of Nkx-2.5, GATA-4, CTT, and Cx43</td>
<td>Cardiac cardiomyogenic differentiation of hMSCs was promoted - Elongated cell morphology - Elevated expression of cardiac troponin T (cTnT), Nkx-2.5, and myosin heavy chain</td>
<td>2013</td>
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nanomaterials can also 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 electrical conductivity of nanocomposites. High electrical conductivity, acceptable biocompatibility, ease of surface modification, nanotopography, and innate optic properties make AuNPs a desirable nanostructure 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. 4Aa). Cardiac cells showed elongated and aligned morphology and increased Cx43 expression. These hybrid AuNP/omentum patches demonstrated increased contraction force (Fig. 4Ab), 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. deposited AuNPs (thickness of 2, 4, and 14 nm) on 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 has also been studied in AuNP-loaded nanofibrous scaffolds. For example, 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. Functionalized biomaterials with AuNPs showed high mechanical

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<td>incorporated in hydrogels/polymers</td>
<td>1, 2, and 3 Hz</td>
<td>nm diameter</td>
<td>Cardiac cells</td>
<td>- Improved cell-cell coupling</td>
<td>- 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</td>
<td>2015</td>
<td>[106]</td>
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Table 1 (continued)
strength 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 was closer to the in vivo myocardium in comparison with naked polyaniline (PANI) and polypyrrole (PPy). The AuNPs enhanced...
expression of Cx43 in neonatal rat ventricular cardiomyocytes (NRVC) in the hybrid scaffolds [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

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<th>Cells</th>
<th>Results</th>
<th>Limitations</th>
<th>Year</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decellularized matrices + AuNPs</td>
<td>Electrical stimulation</td>
<td>AuNPs (4 and 10 nm)/decellularized omental matrices</td>
<td>Cardiac cells</td>
<td>- Elongated and aligned cell morphology</td>
<td>- AuNPs may dissociate from the scaffold in vivo</td>
<td>2014</td>
<td>[115]</td>
</tr>
<tr>
<td></td>
<td>Fibrous scaffold + AuNPs</td>
<td>Gold (film)/PCL</td>
<td>NRVC</td>
<td>- Significantly higher aspect ratio and stronger contraction forces</td>
<td>- Non-degradation of AuNPs</td>
<td>2014</td>
<td>[116]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Not mentioned</td>
<td>AuNPs (2, 4 and 14 nm)/PCL/gelatin (250 nm)</td>
<td>- Enhanced elongation and alignment, more cardiac sarcomeric α-actin expression, higher contraction amplitudes and rates</td>
<td>- Mismatch between mechanical properties of decellularized ECM and AuNPs</td>
<td>2013</td>
<td>[117]</td>
</tr>
<tr>
<td>Hydrogel scaffold + AuNPs</td>
<td>Scaffold conductivity: 15.3 ± 0.8 S/m</td>
<td>AuNPs (8.1 ± 0.9 nm and 4.4 ± 0.3 nm)/thiol-HEMA</td>
<td>NRVC</td>
<td>- Cx43 expression was increased</td>
<td>- Non-degradation of AuNPs in vivo</td>
<td>2014</td>
<td>[118]</td>
</tr>
<tr>
<td></td>
<td>Electrical stimulation (2mA rectangular pulses, 2 ms, 1 Hz, 5 V/cm) for 5 days</td>
<td>Chitosan/AuNPs (7.24 nm)</td>
<td>hMSCs</td>
<td>- Scaffolds supported viability, metabolism, migration and proliferation of hMSCs</td>
<td>- Loss dispersion of AuNPs in scaffolds</td>
<td>2011</td>
<td>[99]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Not mentioned</td>
<td>GelMA hydrogel, gold nanorods (average aspect ratio of 3.15:16 ≥ 2/53 ± 4 nm width and length)</td>
<td>- Cardiomyocytes</td>
<td></td>
<td>2016</td>
<td>[102]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Not mentioned</td>
<td>Collagen and AuNPs</td>
<td>- Cardiac muscle cells</td>
<td>- AuNPs regulated the assembly of intercalated discs via the α-J integrin-mediated ILK/p-AKT/GATA4 pathway</td>
<td>2016</td>
<td>[104]</td>
</tr>
<tr>
<td></td>
<td>Gold nanowire</td>
<td>Collagen and AuNPs (34 nm × 25 nm wide), GelMA</td>
<td>Neonatal rat ventricular cardiomyocytes</td>
<td>- Cardiac cells</td>
<td></td>
<td>2017</td>
<td>[106]</td>
</tr>
<tr>
<td></td>
<td>Scaffolds showed low impedance at high frequencies (10 kHz)</td>
<td>Alginat/gold nanowire (30 nm)</td>
<td>Cardiac cells</td>
<td>- Thicker and aligned engineered tissues</td>
<td>- Gold nanowires can be entered the cell membrane and cause cytotoxicity</td>
<td>2011</td>
<td>[101]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Electronic stimulation: square pulse (1 V/mm amplitude, 2 ms pulse duration, frequency of 1 Hz) for 15 min.</td>
<td>H9c2</td>
<td>- Expression of Cx43 increased</td>
<td></td>
<td>2016</td>
<td>[103]</td>
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<tr>
<td></td>
<td>New Devices</td>
<td>Electronic devices that can control cell/tissue function</td>
<td>Gold used as electrodes</td>
<td>- Flexible cardiac patch, which is freestanding in cardiac 3D scaffolds</td>
<td>- Proving safety and efficacy at low voltages is essential</td>
<td>2016</td>
<td>[107]</td>
</tr>
</tbody>
</table>

Examples of the use of gold nanomaterials in cardiac tissue engineering.
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 a 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 (PTs) are some important EAPs, which have potential applications in cardiac tissue engineering.

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 a 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 (PTs) 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 PPy an excellent candidate as a scaffold for cardiac tissue engineering. In 2007, Nishizawa et al. electrochemically deposited PPy films onto polypyrrole microelectrodes. 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 Ca^2+ expression, faster calcium transfer, and lower calcium transient durations. Surface resistivity of 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 chlorinated-doped-PPy on electrospun PLGA fibers to make 3D and electrically conductive scaffolds. Their results confirmed biocompatibility of these scaffolds using cardiac progenitor cells and iPSCs [136]. Kai et al. demonstrated that electrospun PPy/PCL gelatin nanofibers could not only improve the overall function of cardiomyocytes, but also increase the expression of cardiac-functional proteins (α-actinin, troponin T, and Ca^2+). They also observed that incremental increases of PPy concentration could decrease nanofiber diameter and increase the tensile modulus of the scaffolds. The nanofibers had an electrical conductivity between 0.01 and 0.37 mS/cm [137].

In 2015, Mihic et al. conjugated PPy to chitosan and developed a semi-conductive hydrogel (Fig. 5A.a). In vitro studies demonstrated faster calcium transfer and lower calcium transient durations for cardiomyocytes in the conductive hydrogel (Fig. 5A.b and c). By increasing the amount of PPy in PPy-chitosan hydrogels, the electrical conductivity of gels was increased. 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 were observed for the cells in the PPY-chitosan gels 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. 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 and 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 of PANI. 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 either 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. Chronic inflammation in implanted tissues was reported due to PANI [142,143]. However, some 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, growth, and proliferation of cardiac H9c2 myoblasts cultured on PANI films for 200 h. In the first 100 h, the doubling time increased. Also, the results showed that this scaffold maintained its conductivity for the first 100 h in the physiological environment. However, its conductivity gradually decreased over time [147].

Scientists have combined PANI with different biological materials to enhance its biocompatibility. For example, Li and his colleagues produced a nanofibrous blend based on co-electrospinning PANI and gelatin. The 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 supported attachment, migration, and proliferation of cardiac myoblasts. Also, the conductivity of pure gelatin was determined to be 0.005 S/cm; however, the conductivity increased about four-fold by increasing the PANI concentration [148]. Fernandes et al. modified PANI nanofibrous scaffolds (60–80 nm diameter) with hyper-branched PLD dendrimers (4.5 nm diameter) [149]. Neonatal rat heart cells showed high biocompatibility and better proliferation with electrical stimulation in the scaffolds. 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. synthesized PANI/PLGA aligned fibers to develop a 3D environment for synchronous beating of cardiomyocytes (Fig. 5B.a and b). They showed that this scaffold increased the expression of gap junction protein (Cx43) and troponin T (Fig. 5B.c). The cardiomyocytes also formed isolated cell clusters and beat synchronously. The HCl-doped
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 for cardiomyocytes. (a) PPy monomers grafting and cross-linking into a hydrogel. (b) Contraction threshold voltage was measured using anion-contact stimulation of a single skeletal muscle. PPy-chitosan had 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 developed 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 3: Summary of the electroactive polymer scaffolds used in cardiac tissue engineering.

<table>
<thead>
<tr>
<th>Material</th>
<th>Electrical properties</th>
<th>Scaffold</th>
<th>Cell</th>
<th>Results</th>
<th>Year</th>
<th>Ref.</th>
</tr>
</thead>
</table>
| Polypyrrole               | Surface capacity of PPy thin films on the electrode substrate: 5.8 C/cm² | Pt microelectrodes on polyimide (Pt) surface/PPy film | Primary cardiomyocytes | - Adhesive strength of PPy film was enhanced  
- Cells showed synchronized beating upon stimulation  
- Functional cardiac cell sheets formed  
- Increased in Cx43 expression  
- Faster calcium transfer  
- Lower calcium transient durations | 2007  | [133] |
|                           | Surface resistivity PCL/PPy: 1.00 ± 0.40 kΩ/cm | PCL films/PPy                      | HL-1                  | Surface properties of conductive polymers controlled  
Confirmed biocompatibility                                                                 | 2015  | [134] |
| Dopants enhanced electroactivity of PPy though as measured by cyclic voltammetry (CV) |                           | CPCs                              | Primary cardiomyocytes | - Improved attachment and proliferation  
- Enhanced expression of cardiac--  
functional protein (α-actinin, Troponin T, and Cx43) | 2016  | [135] |
|                           | PPy increased the capacitance of scaffolds | Electropulent PLGA fibers/PPy (200 nm) | CPCs/IPSCs            | In vitro: enhanced Cx43 expression, faster calcium transfer, and lower calcium transient durations  
- In vitro: decreasing in the QRS interval, increasing in the transverse activation velocity  
- In vitro: higher Cx43 expression and α-actinin  
- In vitro: immobilizing cardiomyocytes into scaffolds for a long time, reduce in infarct size  
With excellent cell viability, over expression of cardiomyocyte specific genes (Actinin, Nkx2.5, GATA4, Myb6, c-kit) Enhanced cell attachment and growth on PANI films | 2015  | [136] |
|                            | Electrical conductivity: 0.01– 0.37 mS/cm | PCL/PPy fibers (216 ± 36 nm and 191 ± 45 nm) | New Zealand white rabbits cardiomyocytes | In vitro: increased Cx43 expression, faster calcium transfer, and lower calcium transient durations  
- In vitro: decreasing in the QRS interval, increasing in the transverse activation velocity  
- In vitro: higher Cx43 expression and α-actinin  
- In vitro: immobilizing cardiomyocytes into scaffolds for a long time, reduce in infarct size  
With excellent cell viability, over expression of cardiomyocyte specific genes (Actinin, Nkx2.5, GATA4, Myb6, c-kit) Enhanced cell attachment and growth on PANI films | 2011  | [137] |
| By increasing the ratio of PPy in PPy/chitosan hydrogel, the electrical conductivity increased |                           | Chitosan                          | Rat smooth muscle cells | Healthy blinded PPy/chitosan hydrogel scaffold (PPy/Chitosan 1:1) in the electroactive hydrogel Biocompatible | 2015  | [129] |
| Polyaniline               | Surface resistivity (non-conductive PANI) higher than 10 MΩ/square | PANI                              | H9c2                  | Biocompatible  
- Supporting migration, and proliferation  
Higher cell viability and proliferation  
- Biocompatible  
- Supporting cardiomyocytes proliferation  
- Microcurrent applied to stimulate the differentiation  
Elongated cardiomyocytes formed isolated cell clusters, beating synchronously, and enhanced expression of Cx43 | 2006  | [147] |
|                           | After partial de-sloping, resistivity: 2 kΩ/square | Blend: gelatin/PANI (61 ± 13 nm fiber) | H9c2                  | Biocompatible  
- Supporting migration, and proliferation  
Higher cell viability and proliferation  
- Biocompatible  
- Supporting cardiomyocytes proliferation  
- Microcurrent applied to stimulate the differentiation  
Elongated cardiomyocytes formed isolated cell clusters, beating synchronously, and enhanced expression of Cx43 | 2006  | [148] |
|                           | Conductivity of pure gelatin 0.005 S/cm | Hyperbranched PLL dendrimers/PANI (69–80 nm) | Rat cardiomyocytes    | Biocompatible  
- Supporting migration, and proliferation  
Higher cell viability and proliferation  
- Biocompatible  
- Supporting cardiomyocytes proliferation  
- Microcurrent applied to stimulate the differentiation  
Elongated cardiomyocytes formed isolated cell clusters, beating synchronously, and enhanced expression of Cx43 | 2010  | [149] |
|                           | Conductivity of mesh: 3.1 × 10–3 S/cm and electrical stimulation: 1.25 Hz, 5 V/cm | Polyglycerol dendrimers/PANI (80–180 nm) | Rat cardiomyocytes    | Biocompatible  
- Supporting migration, and proliferation  
Higher cell viability and proliferation  
- Biocompatible  
- Supporting cardiomyocytes proliferation  
- Microcurrent applied to stimulate the differentiation  
Elongated cardiomyocytes formed isolated cell clusters, beating synchronously, and enhanced expression of Cx43 | 2011  | [150] |
|                           | Conductivity of mesh: 3.1 × 10–3 S/cm and electrical stimulation: 1.25 Hz, 5 V/cm | PANI/PLGA fiber (184.7 nm and 101.7 nm) | Neonatal cardiomyocytes | Biocompatible  
- Supporting migration, and proliferation  
Higher cell viability and proliferation  
- Biocompatible  
- Supporting cardiomyocytes proliferation  
- Microcurrent applied to stimulate the differentiation  
Elongated cardiomyocytes formed isolated cell clusters, beating synchronously, and 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 increased by up to seven orders of magnitude | PCL/PANI (50–100 nm) | hMSCs                 | Biocompatible  
- Cardiogenic differentiation of hMSCs into cardiomyocytes-like cells  
- Sarcomeric α-actinin of cardiomyocytes was observed  
- In vitro: excellentcytocompatibility  
- In vivo: acceptable biocompatibility, injectable  
Cytocompatibility of the nanocomposites was confirmed  
Biocompatible  
- Supporting cell proliferation and attachment  
- Biodegradable  
- Cell produced more cardiac specific genes (Actinin, Nkx2.5, GATA4, Myb6, c-kit)  
Biocompatible, injectable and biodegradable self-healing electroactive hydrogels | 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 C2C12 | - Biocompatible  
- Supporting cell proliferation and attachment  
- Biodegradable  
- Cell produced more cardiac specific genes (Actinin, Nkx2.5, GATA4, Myb6, c-kit) | 2013  | [151] |
|                           | The conductivity close to the native myocardium ranges | PGS                               | L929 mouse fibroblast/HUVECs | - Biocompatible  
- Supporting cell proliferation and attachment  
- Biodegradable  
- Cell produced more cardiac specific genes (Actinin, Nkx2.5, GATA4, Myb6, c-kit) | 2014  | [152] |
|                           | Electrical conductivity in 10–5 S/cm | Embedded oligoaniline-polyurethane into PCL films | C2C12 myoblasts and H9c2 cardiocytes | - Biocompatible  
- Supporting cell proliferation and attachment  
- Biodegradable  
- Cell produced more cardiac specific genes (Actinin, Nkx2.5, GATA4, Myb6, c-kit) | 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 | - Biocompatible  
- Supporting cell proliferation and attachment  
- Biodegradable  
- Cell produced more cardiac specific genes (Actinin, Nkx2.5, GATA4, Myb6, c-kit) | 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 cardiocytes | - Biocompatible  
- Supporting cell proliferation and attachment  
- Biodegradable  
- Cell produced more cardiac specific genes (Actinin, Nkx2.5, GATA4, Myb6, c-kit) | 2016  | [155] |
PANI increased electrical conductivity, attracted positively charged cell membrane proteins, and improved cell adhesion [130]. Borriello et al. used electropositive 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 hydrogels. For example, Cui and his colleagues cultured cardiomyocytes, fibroblasts, and osteoblasts in an injectable hydrogel composed of a poly(lactide-co-glycolide-co-lactide) (PLG) copolymer coated with tetrani uniline (with carboxylate modification). Electrical stimulation was applied directly to cells on the tetrani uniline-coated samples and enhanced proliferation of all three cell lines was observed [151]. Qazi et al. fabricated 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, elongation, 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 and biocompatible scaffold supporting cell proliferation and attachment. The electrical conductivity of the films was on the order of $10^{-5} \text{ S/cm}$ [153]. In another work, Baheiraei et al. investigated cardiomyocyte behavior on the PCL films. They observed increased activity of cardiac-specific genes, actinin alpha 4 (Actn4), and troponin T-2 on the conductive substrates, even in the absence of electrical stimulation [154]. Dong et al. fabricated antibacterial, self-healing, and electroactive hydrogels by combining chitosan-graft-aniline tetramers with dibenzaldehyde-terminated PEG at physiological conditions. Their results demonstrated that the electroactive hydrogel was biocompatible, injectable, and biodegradable. Additionally, the hydrogel was determined to have an electrical conductivity around $10^{-3} \text{ S/cm}$ [155].

5.3. Piezoelectric polymeric materials

Piezoelectric materials generate electric field upon the application of mechanical stress and are able to induce 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 and electropositive polyaniline fluoride-trifluoroethylene (PVDF-TrFE) scaffolds [159]. Hitzschier et al. developed piezoelectric scaffolds by electrospinning polyvinylidene fluoride (PVDF) and PVDF-TrFE (Fig. 5C.a). Mouse embryonic stem cell-derived cardiomyocytes adhered well to this scaffold and impulsively contracted, exhibited well-organized sarcomeres, and produced cardiac-specific markers including myosin heavy chain, CTT, and Cx43 (Fig. 5C.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 electrically conductive nanomaterials offer suitable electrical properties for cardiac tissue engineering, the biocompatibility of these materials varies greatly. While the potential applications of carbon-based nanomaterials continue to expand, their 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]. These studies 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 and biopersistent CNTs 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 the 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, and collagen IV) and increased expression of apoptosis-associated genes. However, another study by Tamura concluded that the cytotoxic effects were significantly related to the size of CNTs [163]. The study focused on neutrophil response to titanium oxide particles and CNTs in blood and concluded that toxicity is primarily related to the particle size under $3\mu m$. 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 CNTs to carbon nanofibers exposed to human lung cancer cell lines [164]. The team conducted an in vitro analysis observing the cell proliferation and morphology. They found that the carbon nanofibers were significantly more toxic than the nanotubes. Much of the research on CNT and nanofiber cytotoxicity has been performed on various models of the lung as inhalation is a common method of exposure. Cardiac cells exposed to these nanomaterials in scaffolds may behave differently. Additionally, modulating the size and length of the materials is essential to achieve appropriate biocompatibility. Like other carbon-based nanomaterials, graphene has also been shown to have suitable biocompatibility. A 2012 study by Li et al. demonstrated the cytotoxic effects of pristine graphene on macrophages [165]. The murine macrophage-like RAW 264.7 cells were cultured with various concentrations of dissolved and unmodified graphene. A strong dose-dependent biocompatibility for the graphene was observed. Chemically modified graphene has been shown to improve compatibility with cardiac cells. The modification can be obtained using oxidizing [166–168], reducing [169], and functionalizing [170,171] of the graphene sheet. Unlike carbon-based nanomaterials, materials using gold have shown remarkable compatibility in many studies. For example, 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 MTT assay and the macrophages maintained viability after 72 h. Additionally, Goodman and colleagues demonstrated the cytocompatibility of gold nanoparticles with tethered ionic side chains [173]. They found that cationic modifications increased cytotoxicity while anionic molecules showed little to no negative effects on cell biocompatibility of the nanomaterials. The biocompatibility of electroactive polymers varies significantly depending on specific polymer(s) used. Polymers such as PPy have been shown to be biocompatible with limited inflammatory response after implantation in vivo [132]. PPy was tested in vivo and in vitro on rat peripheral nerve tissue and was observed to be biocompatible. PANI has also been studied and has shown great biocompatibility with H9c2 cardiac myoblasts [147]. While an initial reduction in the cell growth and adhesion was observed, morphologically identical monolayers were formed on the PANI-coated surfaces compared to polystyrene surfaces after 6 days. Additionally, the polymer maintained conductivity for 100 h after coating. Another study on polylactide-aniline pentamer (PLAAP) copolymers also demonstrated excellent cytocompatibility with rat glioma cells [143]. Cell viability (measured with MTT assay) was the 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 proliferated normally on PVDF-TrFE in comparison to those cultured on conventional polystyrene dish [159]. For these materials to be used in cardiac tissue engineering, it is imperative that they have required physical, electrical, and biological properties. For some materials, such as graphene, simple modifications can be made to tailor their surface for specific biomedical application.
7. Concluding remarks and future challenges

Cardiovascular diseases, involving the heart and/or blood vessels, are a primary cause of death in the 21st century. Cardiovascular 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 myocardial tissues 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 the 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 and regular electrical stimulation to engineered tissues, resulting more functional cardiac constructs.

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 polymers). Apart from developments in the chemistry of scaffolds, the fabrication techniques are also moving forward from conventional methods to innovative 3D manufacturing. Scientists from multiple disciplines have worked together 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-based 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 biocompatibility, 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 tissues. 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 surfacing the challenges of cardiac tissue engineering.

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improves cardiac function after implantation into myocardial infarct, Circulation 132 (8) (2015) 772–784.


