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Rational design of microfabricated electroconductive hydrogels for biomedical applications



Brian W. Walker^{a,1}, Roberto Portillo Lara^{b,c,1}, Emad Mogadam^{d,e}, Chu Hsiang Yu^b, William Kimball^b, Nasim Annabi^{a,f,g,*}

^a Department of Chemical and Biomolecular Engineering, University of California-Los Angeles, Los Angeles, CA, 90095, USA

^b Department of Chemical Engineering, Northeastern University, Boston, MA, 02115, USA

^c Tecnológico de Monterrey, Escuela de Ingeniería y Ciencias, Zapopan, JAL, Mexico

^d Department of Internal Medicine, Huntington Hospital, Pasadena, CA, 91105, USA

^e Department of Internal Medicine, University of Southern California, Los Angeles, CA, 90033, USA

^f Biomaterials Innovation Research Center, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA

^g Center for Minimally Invasive Therapeutics (C-MIT), California NanoSystems Institute (CNSI), University of California – Los Angeles, Los Angeles, CA, 90095, USA

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ABSTRACT

Electroconductive hydrogels (ECHs) are highly hydrated three-dimensional (3D) networks generated through the incorporation of conductive polymers, nanoparticles, and other conductive materials into polymeric hydrogels. ECHs combine several advantageous properties of inherently conductive materials with the highly tunable physical and biochemical properties of hydrogels. Recently, the development of biocompatible ECHs has been investigated for various biomedical applications, such as tissue engineering, drug delivery, biosensors, flexible electronics, and other implantable medical devices. Several methods for the synthesis of ECHs have been reported, which include the incorporation of electrically conductive materials such as gold and silver nanoparticles, graphene, and carbon nanotubes, as well as various conductive polymers (CPs), such as polyaniline, polypyrrole, and poly(3,4-ethylenedioxythiophene) into hydrogel networks. These electroconductive composite hydrogels can be used as scaffolds with high swellability, tunable mechanical properties, and the capability to support cell growth both *in vitro* and *in vivo*. Furthermore, recent advancements in microfabrication techniques such as 3D bioprinting, micropatterning, and electrospinning have led to the development of ECHs with biomimetic microarchitectures that reproduce the characteristics of the native extracellular matrix (ECM). The combination of sophisticated synthesis chemistries and modern microfabrication techniques have led to engineer smart ECHs with advanced architectures, geometries, and functionalities that are being increasingly used in drug delivery systems, biosensors, tissue engineering, and soft electronics. In this review, we will summarize different strategies to synthesize conductive biomaterials. We will also discuss the advanced microfabrication techniques used to fabricate ECHs with complex 3D architectures, as well as various biomedical applications of microfabricated ECHs.

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* Corresponding author at: Department of Chemical and Biomolecular Engineering, University of California-Los Angeles, Los Angeles, CA, 90095, USA.

E-mail address: nannabi@ucla.edu (N. Annabi).

¹ These authors are co-first authors and contributed equally to the preparation of the manuscript.

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1. Introduction

Hydrogels are three dimensional (3D) networks of hydrophilic polymers that can be formed through different mechanisms such as physical entanglement, electrostatic interactions, or covalent chemical crosslinking [1]. Hydrogels are remarkably suitable for a wide range of applications such as drug delivery, tissue engineering, and flexible electronics for biomedical devices, due to their high hydration, tunable physical properties, and porous architecture [2,3]. These characteristics also enable the diffusion of biomolecules, oxygen, and metabolic waste across the 3D structure of hydrogels, which is an important trait for substrates used in the physiological context [4,5]. Hydrogels can also be tuned to mimic biochemical, mechanical, and topographical cues of the native extracellular matrix (ECM), in order to modulate physiological responses in cells and tissues [6]. Therefore, several naturally-derived and synthetic-based polymers, as well as various fabrication methods have been reported for the design and manufacture of hydrogels with different physicochemical properties [7,8]. These polymers may be used individually or in combination with other polymers to yield composite hydrogels with enhanced functionality. Moreover, hydrogels may be further modified through the incorporation of chemically or biologically active moieties such as growth factors, cell binding and protease-sensitive sites, or other stimuli-responsive molecules [9]. Although hydrogels have been demonstrated to be highly versatile platforms for different biomedical applications, their insulating nature often limits their potential for the modulation of electrically-sensitive cells and tissues, such as cardiac and neural tissues [10].

In recent years, the development of advanced biomaterials combined with micro- and nanotechnologies improved the ability to control the properties and functionality of hydrogels for a wide range of applications (Fig. 1) [6]. For instance, the incorporation of inherently conductive materials to hydrogels via blending, doping or chemical modification have led to the development of new class of electroconductive hydrogels (ECHs) [11]. ECHs are composite biomaterials that combine the electroconductive capabilities of different materials with the intrinsic properties of crosslinked hydrogel networks [12]. Several strategies for the synthesis of ECHs have been reported such as the incorporation of conductive polymers (CPs) (e.g. polyaniline (PANI), polypyrrole (PPy), polythiophene (PTh), and poly(3,4-ethylenedioxythiophene) (PEDOT)) within a hydrogel network [12–16]. The organic nature of CPs greatly facilitates their chemical modification to incorporate dif-

Table 1

ECHs can be tailored to mimic the electrical properties of native tissues when used for cardiac and neural tissue engineering.

Native Tissue	Conductivity (S/cm)	Reference
Myocardium (transversely)	0.0016	[24,25]
Myocardium (longitudinally)	5×10^{-5}	[24,25]
Brain	0.0015 – 0.0030	[26]

ferent functional motifs into ECHs and provide them with high conductivity and processability [11]. ECHs can also be engineered by the *in situ* reduction of metal ions within the polymer network to form metallic nanoparticles (NPs) [17]. In this regard, different types of NPs have been used for the engineering of nanocomposite ECHs with tunable electrical, mechanical and optical properties [18]. For example, the incorporation of one dimensional carbon nanotubes (CNTs) and two-dimensional (2D) graphene has been shown to impart electrical conductivity to hydrogels and increased their mechanical strength [19]. In addition, our group has recently demonstrated the engineering of ECHs with intrinsic electrical conductivity through the functionalization of different hydrogels with a choline-based bio-ionic liquid (Bio-IL) [20]. This diverse range of synthesis methodologies has led to the development of ECHs which offer unique advantages for biomedical applications, such as tissue engineering, drug delivery, and engineering biosensors and medical devices [13]. Strategies for the design of therapeutic and diagnostic technologies based on ECHs rely on the accurate recapitulation of the electrical properties of the native tissues (Table 1).

Previous studies have demonstrated the ability of ECHs to mediate the adhesion, proliferation, migration, and differentiation of different cell types including cardiomyocytes (CMs), neurons, fibroblasts, endothelial cells, human mesenchymal stem cells (hMSCs), and preosteoblasts [21]. In addition, recent advances in hydrogel synthesis and fabrication techniques have led to the engineering of multifunctional ECHs that are able to sense and respond to different physicochemical stimuli [11]. This new class of smart ECHs have been increasingly used for a variety of applications, ranging from stimulating and recording electrodes, tissue engineered constructs, and electrically controlled drug release devices and biosensors [13]. Furthermore, the development of advanced micro-engineering techniques have allowed the accurate recapitulation of the complex microarchitectural features of physiological tissues [22]. In this regard, different patterning and templating approaches have been used to fabricate micro-scale structures using a broad

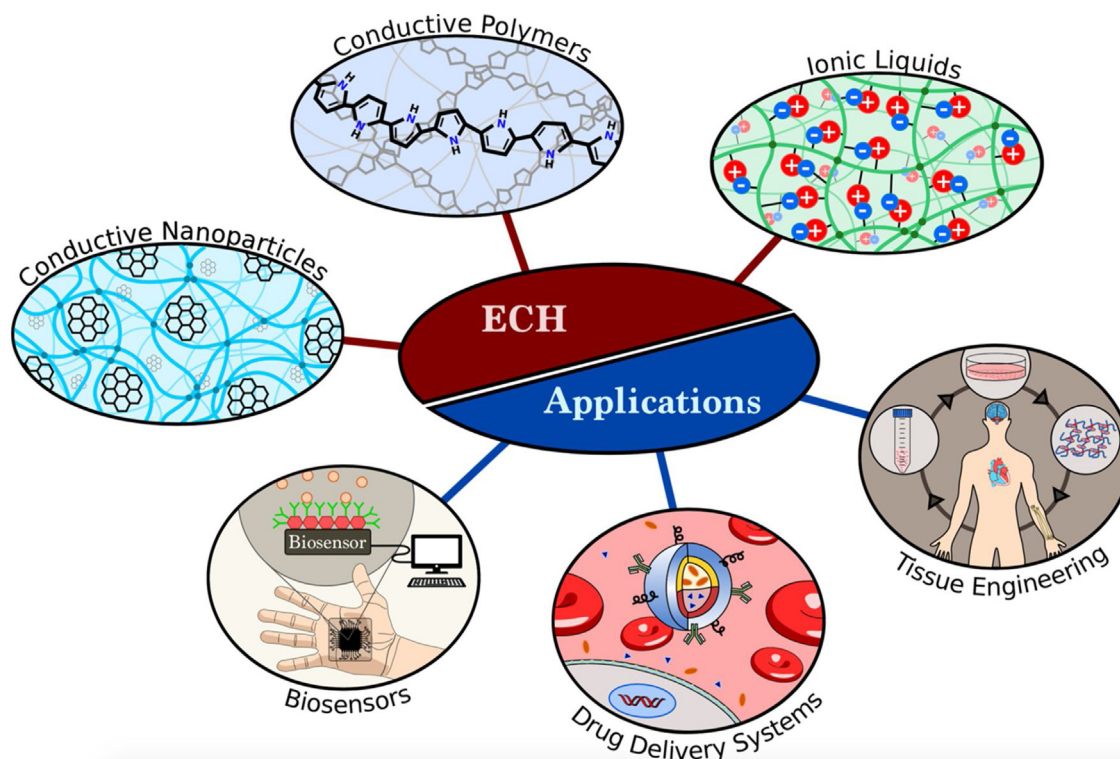


Fig. 1. Synthesis and applications of ECHs. ECHs can be formed by using different conductive materials including conductive nanoparticles, conductive polymers, or ionic liquids. ECHs have been utilized for different biomedical applications, including biosensors, drug delivery, and tissue engineering.

range of biocompatible and biodegradable materials [23]. With the advent of microengineering techniques such as 3D printing, electrospinning, and other lithography-based approaches, it is possible to exert precise control over the composition, geometry, and spatial arrangement of cells and biomolecules within ECHs. This unprecedented degree of customization holds remarkable potential for the engineering of smart interfaces and biomimetic scaffolds for fundamental research and clinical applications.

While previous review papers have mainly focused on either natural [12] or synthetic [13,27,28] systems, our review is inclusive to all major biomaterials used for the synthesis of ECHs. Here, we describe the most significant conductive materials incorporated into hydrogels to impart electroconductivity. Furthermore, while recent publications have detailed different fabrication strategies to form hydrogels with specific architectures [29–31], our review focuses on the latest microfabrication techniques used specifically in the fabrication of ECHs. These techniques include 3D printing, electrospinning, micropatterning, and self-assembly. We discuss the significance of these new and developing fabrication methods, and their ability to impart ECHs with new properties, such as self-healing, complex architecture, strong adhesion to native tissues, and the ability to respond to different stimuli. Lastly, we discuss recent strategies to tailor ECHs to specific biomedical applications, such as tissue engineering, drug delivery, and biosensing. In summary, this review explicitly elaborates on a) the key role of ECHs in the engineering of therapeutic and diagnostic systems, b) natural and synthetic materials used to develop ECHs, and c) state of the art biofabrication methods for engineering ECHs.

2. Conductive nanoparticle-incorporated ECHs

The design of ECHs often rely on conductive NPs, such as graphene or CNTs, conductive polymers, or ionic liquids to impart electroconductivity. These conductive biomaterials possess specific ranges of conductivity, which impart unique advan-

tages and disadvantages for various biomedical applications (Table 2).

2.1. Gold nanoparticles

Metallic NPs are colloids ranging from 1 to 100 nm in size featuring a high surface area-to-volume ratio [32], which exhibit chemical and physical properties different from that of bulk metals [33]. Gold NPs (AuNPs) and silver NPs (AgNPs) are of particular interest to engineer hydrogels with electroactive properties. In recent years, AuNPs have gained significant interest due to their unique conductive [34], optical [35–37], and magnetic properties [38,39]. These characteristics have been shown to be particularly advantageous for the development of biosensors [40], and drug delivery systems [41], as well as various tissue engineering applications [42]. Apart from their ease of synthesis, their high stability [40] and biocompatibility [36–38], the ability to fine tune the properties of AuNPs by varying their size and shape make them attractive candidates for the synthesis of ECHs. However, one drawback of incorporating AuNPs into ECHs is their tendency to generate reactive oxygen species (ROS). ROS are oxygen-derived small molecules that are naturally produced from several sources, including cellular respiration in the mitochondria and from an incomplete reduction of oxygen and NADPH in the plasma membrane [43]. At moderate concentrations, ROS play critical roles in the regulation of cell function, such as cell growth, migration, or apoptosis; however, high concentrations of these molecules can result in damage of proteins, lipids, and DNA [44,45]. Recently, ECHs have been designed to incorporate AuNPs for the purpose of generating high concentrations of ROS in order to eradicate diseased cells [46,47]. However, when developing ECHs for the regeneration of damaged tissues, it is important to note that AuNPs are capable of penetrating cell membranes and cause cellular dysfunction due to their small size. Therefore, significant efforts have been conducted to determine the ideal size and

Table 2
Advantages and disadvantages of common conductive biomaterials used to form ECHs and the conductivity of these systems.

Conductive materials	Advantages	Disadvantages	Conductivity (S/cm)	Refs
AuNPs/ polymer	<ul style="list-style-type: none"> • Tunable conductivity • Generally biocompatible 	<ul style="list-style-type: none"> • AuNP cytotoxicity is not fully understood • Synthesis of AuNPs may be difficult depending on target particle size • Possible generation of ROS 	8.0×10^{-4} – 1.0×10^{-2}	[34–3760,61]
AgNPs/ polymer	<ul style="list-style-type: none"> • High conductivity • Highly antibacterial 	<ul style="list-style-type: none"> • AgNPs increase brittleness of ECH • Possible generation of ROS 	1.0×10^{-4} – 5.8×10^{-1}	[51,62–67]
Graphene/ polymer	<ul style="list-style-type: none"> • High conductivity • Robust mechanical strength • Generally biocompatible 	<ul style="list-style-type: none"> • Complicated fabrication method for GO • rGO frequently aggregates during ECH synthesis • Cytotoxicity of GO and rGO is not fully understood 	4.0×10^{-5} – 5.8×10^{-1}	[58,68–71]
CNTs/ polymer	<ul style="list-style-type: none"> • High conductivity • Robust mechanical strength 	<ul style="list-style-type: none"> • CNTs frequently show aggregation during ECH synthesis • CNTs increase brittleness of ECHs • Cytotoxicity of CNTs is not fully understood 	5.0×10^{-5} – 9.0	[59,70,72,73]
PANi/ polymer	<ul style="list-style-type: none"> • Facile synthesis • Antimicrobial • Highly conductive • Facilitates cell proliferation 	<ul style="list-style-type: none"> • Fabrication requires harsh chemical environment 	5.0×10^{-4} – 1.2×10^{-2}	[13,74,75]
PPy/ polymer	<ul style="list-style-type: none"> • Facile synthesis • Biocompatible • Environmentally Stable 	<ul style="list-style-type: none"> • Poor solubility in polar solvents • Poor mechanical strength, brittle 	1.2×10^{-3} – 1.2×10^2	[76–79]
PEDOT/ polymer	<ul style="list-style-type: none"> • High conductivity • Facilitates cell proliferation • Biocompatible • High stability 	<ul style="list-style-type: none"> • Poor mechanical strength, brittle • Cytotoxicity of PEDOT is not fully understood 	6.7×10^{-4} – 1.0×10^{-1}	[80–84]
Bio-IL/ polymer	<ul style="list-style-type: none"> • High conductivity • Biocompatible 	<ul style="list-style-type: none"> • Variable cytotoxicity 	1.4×10^{-4} – 1.0×10^{-2}	[20,85–88]

shape of AuNPs, and to optimize their *in vivo* pharmacokinetics for therapeutic and clinical applications [48].

AuNP-incorporated ECHs have been used as drug delivery vehicles, by mediating the release of hydrophilic drugs encapsulated in their matrix. This is primarily due to their thermally responsive capabilities, which allow for dramatic phase changes through local changes in temperature that do not affect the surrounding tissues [49]. AuNPs also feature high absorption capacity and scattering power, which are highly advantageous for the development of drug delivery systems [50]. Current research has focused on studying the effect of the size and shape of AuNPs on drug release time, water absorbance, surface properties, as well as chemical and physical behaviors of AuNP containing biomaterials. AuNP-incorporated ECHs may also possess the ability to generate heat through the absorption of visible to near infrared (NIR) light, a trait which may be utilized in drug delivery systems [51]. Drug delivery strategies may take advantage of this phenomenon by loading drugs in AuNP-incorporated ECHs and stimulating a local region with light for controlled release of therapeutic molecules. For example, in a recent study, Strong et al. designed an ECH system composed of the thermally responsive polymer poly(N-isopropylacrylamide-co-acrylamide) (NIPAAm-co-AAm) and NIR absorbing silica-gold nanoshells with a lower critical solution temperature (LCST) just above physiological temperature at 40 °C [52]. The LCST refers to the temperature at which smart hydrogels physically shrink from a swollen to a collapsed state. The role of AuNPs in this context was to absorb NIR irradiation through external stimulation, which resulted in ECH deswelling and subsequent release of chemotherapeutic drugs. Their study investigated the ability for this AuNP-incorporated ECH system to initiate pulsatile drug release of either doxorubicin, or a DNA duplex. The efficacy of this drug delivery system was evaluated by culturing colon carcinoma cells on the surface of ECHs and comparing samples irradiated with light to non-irradiated samples. AuNP-integrated ECHs that were irradiated with NIR resulted in a 30% decrease in cell proliferation as compared with ECHs that had not been exposed with NIR [52]. It

was, therefore, proposed that these AuNP-integrated ECHs would be able to rapidly deliver chemotherapeutic drugs to the site of a tumor while minimizing the exposure of healthy tissues to the drug.

In another study, Das et al. developed an ECH as a drug delivery system by grafting hydroxypropyl methyl cellulose (HPMC) on polyacrylamide (PAM), and then coating the hydrogel with AuNPs [50]. *In vitro* biodegradation analysis carried over 21 days in phosphate buffered saline (PBS) demonstrated the progressive degradation of these ECHs at a constant rate. Furthermore, *in vitro* drug release kinetics for 5-ASA and ornidazole, showed that the amount of drug released was lower in ECHs with a higher concentration of AuNPs. This behavior highlighted the ability of AuNP-incorporated ECHs to be used for time-release strategies. Tissue regenerative strategies have also utilized AuNPs combined with hydrogels owing to their enhanced electrical properties, which may provide adequate coupling between adjacent cells [34]. ECHs formed based on AuNPs have been used for cardiac, bone, and nerve tissue engineering, due to their biocompatibility, and high mechanical strength and conductivity [53,54].

Gelatin methacryloyl (GelMA) is a photocrosslinkable biopolymer that has been widely used for tissue engineering applications, as it is capable of supporting cell adhesion due to the presence of Arg-Gly-Asp (RGD) motifs [55]. A recent study conducted by Navaei et al. demonstrated the effectiveness of gold nanorods (GNRs) embedded in GelMA hydrogels to develop cardiac tissue constructs [38]. Hydrogels containing a higher concentration of GNRs showed lower electrical impedance, compared with control samples containing pristine GelMA. This lower impedance reflected the high electrical conductivity of hybrid hydrogels embedded with GNR, which facilitated electrical propagation and promoted CM coupling [38]. The incorporation of GNRs also had a significant effect on the swelling ratio and the porosity of the hydrogels, which are key to mediate nutrient and gas exchange [38,56]. Further-

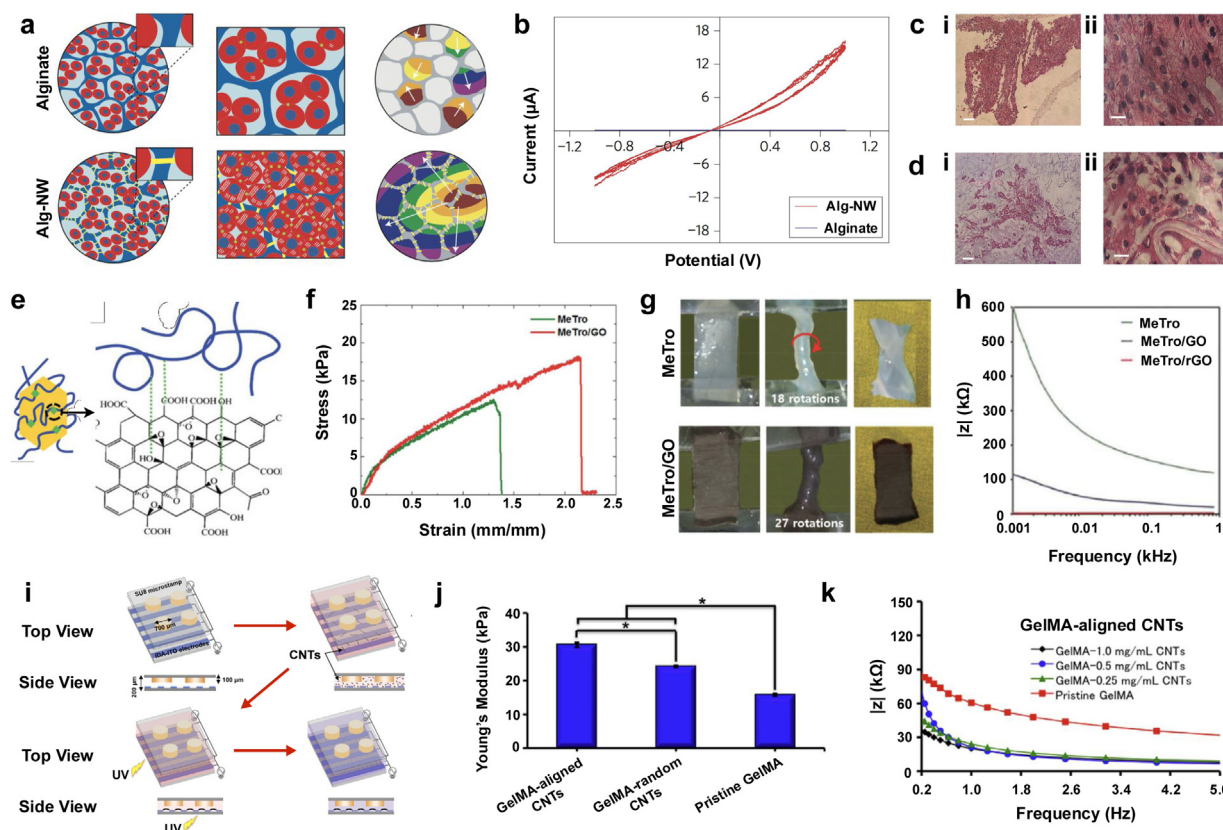


Fig. 2. Synthesis and applications of ECHs formed by using conductive NPs. Schematic for the formation of alginate hydrogels and gold nanowires (NW)-alginate ECHs. Cardiomyocytes cultured in alginate hydrogels formed small clusters and beated asynchronously. However, cardiomyocytes cultured in alginate-NW ECHs formed organized cardiac-like tissue and beat synchronously. Components of engineered cardiac tissue are shown: cardiac cells (red), alginate pore walls (blue), NW (yellow) (a). Current/Potential graph of alginate hydrogels and alginate-NW ECHs showing higher electrical conductivity exhibited by the ECHs (b). Hematoxylin and eosin (H&E) staining images based on *in vitro* studies showed thick tissue in the NW-alginate ECHs (c*i*, c*ii*), whereas the samples containing pure alginate showed non-continuous tissue separated by pore walls (d*i*, d*ii*). [57] Synthesis of ECHs by coating graphene oxide (GO) with methacryloyl-substituted tropoelastin (MeTro) (e). Elastic modulus of MeTro hydrogels and MeTro/GO ECHs demonstrating that the addition of GO significantly increased the elastic modulus (f). Torsion test on MeTro hydrogel and MeTro/GO ECH was conducted by twisting scaffolds for multiple rounds. Significant deformation was observed in MeTro hydrogel, however, MeTro/GO ECHs did not display any deformation (g). Overall impedance of MeTro hydrogels, MeTro/GO ECHs, and MeTro/reduced GO (rGO) ECHs shows that electrical resistance was the lowest for ECHs fabricated with rGO (h). [58] Schematic for the fabrication of CNT-embedded GelMA ECHs using dielectrophoresis force to align CNTs in GelMA. Highly aligned CNTs were observed in under 1 min, and the GelMA prepolymer was photocrosslinked using UV light (i). Young's modulus of GelMA hydrogels, and ECHs containing GelMA and both randomly arranged and aligned CNTs (j). Electrical evaluation of these ECHs demonstrated that the incorporation of CNTs into hydrogels resulted in lower impedance compared to pristine GelMA hydrogels. Further, the conductivity of these ECHs could be finely tuned by adjusting the concentration of CNTs in the system (k). [59] Scale bar = 200 μm (c*i*, d*i*), 20 μm (c*ii*, d*ii*) (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

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more, *in vitro* studies demonstrated that CMs seeded on the surface of GNR-embedded ECHs exhibited homogeneous distributions, as compared to control hydrogels without GNRs. Gold nanowires (GNWs) have also been incorporated into polymeric scaffolds for the synthesis of ECHs for tissue engineering applications. For instance, Dvir et al. reported the addition of GNWs to alginate scaffolds, to improve electrical communication between cells for engineering functional cardiac patches (Fig. 2a) [57]. By incorporating GNWs, these conductive scaffolds demonstrated increased electrical conductivity, enhancing the function of cardiac tissue constructs (Fig. 2b). CMs and fibroblasts were also seeded onto scaffolds under static and electrically stimulated conditions before implantation. Hematoxylin and eosin (H&E) staining revealed better-aligned CMs within GNW-embedded scaffolds after 8 days of culture (Fig. 2c i,ii), compared to a pure alginate matrix (Fig. 2d i,ii) [57]. These results demonstrated the potential of GNW-loaded

hydrogels for the development of devices that can be used to modulate excitable cells.

AuNPs have been used to engineer ECHs for different biomedical applications including biosensing, bio-imaging, and tissue engineering, owing to their high electrical conductivity, distinct optical behavior, and low cytotoxicity. These properties make AuNPs a useful tool as recognition elements for detection of specific biological analytes. AuNP-incorporated ECHs have also been used to develop drug delivery systems with the capability to trigger the release of loaded molecules. Further, these versatile biomaterials have demonstrated the ability to improve cell-cell coupling in ECHs developed for cardiac and neural tissue engineering. However, challenges still exist in the fabrication of AuNP-incorporated ECHs for biomedical applications such as their slow rate of clearance from the body. In addition, the preparation of AuNPs remains time consuming and costly, and multistep reactions may result in cytotoxicity. Another consideration is that AuNPs tend to aggre-

gate during synthesis of ECHs due to their large surface area. Nevertheless, AuNP-incorporated ECHs have shown promise to be successfully applied for future therapeutic and diagnostic innovations.

2.2. Silver nanoparticles

AgNPs have also been used in combination with several types of polymers and biomaterials to engineer ECHs with enhanced electrical conductivity and antimicrobial properties. The bactericidal properties of AgNPs are mainly due to the oligodynamic effect, a phenomenon characterized by the binding of small metal ions to reactive groups, which results in the denaturing of cellular proteins in bacteria [62]. AgNPs are highly active against many types of Gram-positive and Gram-negative bacteria, including antibiotic-resistant strains [62–64,89,90]. However, similar to AuNPs, AgNPs have the potential to generate ROS that are capable of damaging protein, lipids, and DNA [91]. Therefore, it is necessary to determine the cytotoxic effects of ECHs fabricated with AgNPs.

Recently, AgNPs have also been incorporated into hydrogels to impart electrical conductivity into these systems. The combination of antimicrobial and conductive properties makes AgNPs an attractive material for biomedical applications such as wound and burn dressings [92], coatings for surgical instruments [93], and biosensors [94]. In addition, AgNPs have also been used in a wide variety of industrial applications related to commercial sanitization, including areas of food packaging, textiles, plastics, soaps, and water treatment [95].

The incorporation of AgNPs into polymeric networks has been shown to influence the mechanical and swelling properties of ECHs. Previous studies have reported that the addition of AgNPs to polyvinylpyrrolidone (PVP) and polyvinylalcohol (PVA) hydrogels led to increased mechanical stiffness [65]. In addition, it was shown that AgNP-loaded ECHs often exhibited lower swelling ratios when compared to control hydrogels without AgNPs [51,66]. These lower swelling ratios associated with the incorporation of AgNPs have been directly correlated to increased electrical conductivity. When water uptake into a polymeric network occurs, this also increases the distance between the AgNPs, resulting in decreased conductivity. Therefore, polymer networks loaded with AgNPs must be designed to maintain adequate swelling to prevent loss of conductivity. In one study, Lee et al. found that the impedance of AgNP-loaded ECHs decreased 2 orders of magnitude compared to control hydrogels, reflecting the electroactive properties provided through the incorporation of AgNPs [66].

AgNP/polymer composite ECHs are also particularly advantageous to the field of biosensors. For example, Xiang et al. developed an AgNP-loaded ECH with homogeneous dispersion of NPs [96]. Hydrogels were rendered conductive by immersing the swollen poly(HEMA-PEGMA-MAA) (PHPM) scaffolds in aqueous 0.01 M AgNO₃, followed by reduction of Ag⁺ by submerging the hydrogels in 0.02 M NaBH₄. Their results showed that the conductivity of these ECHs was remarkably higher than that of control PHPM hydrogels, with values just under 600 μS cm⁻¹ [96]. Further, because the AgNPs had been anchored to deprotonated –COOH functional groups, the pH had a significant effect on the conductivity of these ECHs. This responsiveness to pH makes them attractive materials that could be further developed into biosensors or other biomedical applications.

Like AgNPs, silver nanowires (AgNWs) are another type of biocompatible materials that have been used to develop ECHs for flexible bioelectronics. Recently, Ahn et al. developed an ECH by incorporating AgNW-based microelectrodes into a PAM-based hydrogel using a photolithographic process [97]. These materials displayed excellent electrical properties, where the conductivity could be accurately tuned by controlling the AgNW width and the

spin coating speed [97]. Further, they possessed robust mechanical properties and excellent flexibility, which are key for the development of flexible bioelectronic devices.

Currently, most of the research involving AgNPs is largely focused on their antimicrobial properties. Nonetheless, AgNPs have been found to possess excellent conductive and optical properties, and incorporation of AgNPs into polymer-based hydrogels has little effect on their mechanical properties. Taken together, the properties of AgNP-incorporated ECHs make them suitable materials for a wide range of biomedical applications, including drug delivery systems, biosensors, and flexible electronics.

2.3. Graphene

Graphene is a 2D hexagonal lattice of carbon that possesses unique mechanical and conductive properties. Although pure graphene is extremely difficult to produce due to its atomic thickness, several methods have been reported for the synthesis of graphene derivatives [68–70]. One common derivative is graphene oxide (GO), where oxygen atoms form bonds with the carbon lattice and create small imperfections in the structure [68–70]. However, oxidized graphene exhibits poor electrical conductivity, and must be deoxidized to be used in the synthesis of ECHs. Oxidized graphene can be deoxidized through the repair of the sp² carbon bonds, which yields a conductive reduced graphene oxide (rGO) [68]. In addition, hydrothermal reduction of high concentration GO solutions can also be used to produce chemically converted graphene [69]. While graphene has been incorporated into many synthesis strategies to develop ECHs with robust electrical and mechanical properties, cytotoxicity remains to be a concern when these NPs are used for biomedical applications. The toxic effects demonstrated by graphene can be influenced by their size, shape, surface charge, surface area, and functional groups [98]. Future studies involving graphene NPs must carefully evaluate their *in vitro* and *in vivo* biocompatibility and potential cytotoxic responses before seeking approval from the Food and Drug Administration (FDA).

Conventional methods for the fabrication of ECHs using rGO are limited due to the poor water solubility of rGO NPs and their tendency to aggregate in solution [68]. This affects the homogeneity of ECHs, which could lead to anomalies in the conductivity of the hydrogel. Jo et al. reported that hydrogels containing homogenous rGO networks can be formed by first creating hydrogels containing GO, and then reducing it to rGO *in situ* [68]. In this study, ECHs consisting of GO and polyacrylamide (GO/PAAm) were reduced in an L-ascorbic acid solution. Scanning electron microscope (SEM) analysis of the reduced GO/PAAm ECHs revealed no significant clusters of rGO, which suggested that rGO was uniformly distributed [68]. Furthermore, *in vitro* studies using C2C12 myoblasts revealed that cell adhesion and proliferation were improved for GO/PAAm and rGO/PAAm ECHs, when compared to control PAAm hydrogels. In another recent study, we incorporated GO NPs inside a highly elastic methacryloyl-substituted tropoelastin (MeTro) hydrogel to form ECHs for cardiac tissue engineering [58]. The covalent bonds between polymeric chains, along with hydrophobic and electrostatic interactions between MeTro and GO yielded ECHs with excellent mechanics, electrical conductivity, and biocompatibility (Fig. 2e) [58]. ECHs containing GO NPs showed longer elongation at their breaking point as compared to pure MeTro hydrogels (Fig. 2f). MeTro-based ECHs possessed robust mechanical flexibility, making them ideal for the engineering of intrinsically dynamic tissues (Fig. 2g). In addition, MeTro/GO ECHs displayed significantly lower electrical resistance compared with pure MeTro hydrogels (Fig. 2h). Furthermore, these scaffolds supported the growth and function of CMs *in vitro* and elicited no inflammatory responses when implanted in rats [58].

There has also been an increased interest in the use of GO-loaded ECHs in drug delivery systems, owing primarily to their remarkable conductive [99] and magnetic [100] properties. In one recent study, Servant et al. synthesized stimuli-responsive hydrogels composed of poly(methacrylic acid) (PMMA) and ball-milled graphene (GBM) for the release of small molecules *in vivo*. Their results showed that the bulk resistance of these hydrogels decreased with an increasing concentration of GBM. These smart ECHs were evaluated for their ability to control the release of ^{14}C -sucrose through subcutaneous implantation into CD-1 mice. Mice were then electrically stimulated at 10 V for 1 min periods at 2 h intervals, which is a relatively low voltage applied for a short time period. It was found that PMMA/GO ECHs greatly outperformed control PMMA hydrogels, by releasing 5.5% of the ^{14}C -sucrose into the blood stream following electrical stimulation [99]. This work highlights the potential of GO for the development of advanced drug delivery systems for the release of different therapeutic molecules.

GO-incorporated ECHs have demonstrated their potential for different tissue engineering applications, such as cardiac and neural tissue engineering, owing to their excellent mechanical, and conductive properties. The amphiphilic nature of GO provides it with a structure that is deemed as biocompatible; however, more *in vitro* and *in vivo* investigations are required to evaluate the reaction these materials elicit to living tissues. In addition, the superior fluorescence quenching, and surface functionalization observed by GO make excellent material for use in biosensors, drug delivery systems, and other biomedical applications.

2.4. Carbon nanotubes

CNTs are nanostructures comprised of a cylindrical lattice of carbon atoms arranged either as single-walled or multi-walled nanotubes. CNTs have gained significant interest due to their unique properties, including high compressive and tensile strength, as well as high electrical conductivity [70,72,73,101–103]. CNTs have been shown to be useful for engineering ECHs due to their ability to reduce brittleness and significantly increase electrical conductivity. However, they can also be difficult to incorporate into ECHs due to their unique chemical structure, which is characterized by strong Van der Waals forces, high hydrophobicity, and low entropy [72]. The combination of these factors often leads to the aggregation of CNTs in solution, resulting in the formation of non-homogenous mixtures [72], which can be a challenge for the formation of ECHs.

Previous studies have described different methods for CNT dispersion into polymer solutions to engineer ECHs [72]. For instance, CNT-incorporated ECHs were formed by using pH-sensitive microgel particles containing high concentrations of $-\text{COOH}$ functional groups [72]. When the pH of a solution containing CNTs and pH-responsive microgel particles approached the pK_a value of the microgel particles, the swelling and attractive forces between the microgels and the CNTs could facilitate CNTs dispersion and minimize aggregation. These microgel/CNT ECHs exhibited elastic moduli suitable for tissue engineering applications, such as intervertebral disk repair [72]. In addition, these microgel/CNT composites exhibited a conductivity of 0.031 S cm^{-1} , which was significantly higher than other ECHs, particularly polyacrylamide/CNTs [72]. In addition, these ECHs were shown to be highly biocompatible *in vitro* by supporting the growth of adipose-derived hMSCs on the surface of these ECHs for 7 days.

The field of cardiac tissue engineering has also greatly benefited from the synthesis of CNT-embedded ECHs. A recent study conducted by Ahadian et al. used dielectrophoresis to align CNTs in a GelMA-based hydrogel (Fig. 2i), which enhanced the cardiac differentiation of embryoid bodies cultured in the microwells of patterned ECHs [59]. In addition, CNT-loaded ECHs exhibited higher

elastic moduli (Fig. 2j), lower electrical resistance, and enhanced cell beating, as compared to pure GelMA hydrogels (Fig. 2k). These results demonstrated that the engineered materials could be suitable for applications in regenerative medicine and cell therapy where the conductivity of the matrix plays an important role [59].

CNTs have also been incorporated into ECHs to form drug delivery systems. In a recent study, Cirillo et al. developed gelatin/acrylamide/polyethylene dimethacrylate ECHs containing varying concentrations of CNTs and investigated their effectiveness in modulating the delivery of curcumin (Cur). As expected, ECHs containing higher concentrations of CNTs exhibited lower electrical resistivity and enabled the controlled release of Cur following electrical stimulation. *In vitro* drug release studies conducted on ECHs with varying CNT concentrations showed that samples containing 1.25% (w/v) CNTs best met the therapeutic needs for Cur given as a topical wound dressing. Further, the rate of Cur released could be modulated by controlling the external voltage applied to these ECHs.

Incorporation of CNTs into polymeric networks constitutes an attractive strategy to produce scaffolds with optimal conductivity and mechanical strength for tissue engineering. However, future studies on CNTs incorporated ECHs should focus on *in vivo* evaluation to address biocompatibility concerns. If CNTs can be incorporated into hydrogels without toxicological or inflammatory issues, these NPs will be excellent candidates for current and future biomedical applications.

3. Electroconductive polymer-incorporated ECHs

3.1. Polyaniline

PANI is an electrically conductive polymer with a conjugated backbone that has been widely used for the synthesis of ECHs due its mechanical stability, ease of fabrication, and low manufacturing cost [74,104]. Previous studies have reported the use of several derivatives of PANi, including its most reduced form (i.e., leucoemeraldine), its fully oxidized form (i.e., pernigranalline), and a partially oxidized form (i.e., emeraldine) [28,105] for the formation of ECHs. Many natural and synthetic polymers have been used in conjunction with PANi to engineer ECHs. For instance, Xia et al. described the development of ECHs using PANi and polyacrylic acid (PAA), by reacting positively charged aniline monomers with the negatively charged COO^- functional groups in PAA [75]. Their results showed that the addition of PANi increased the electrical conductivity, as well as compressive and elastic moduli of the resulting ECHs. In particular, this improved electrical conductivity was likely due to PANi fibers filling in small pores of the PAA hydrogel [75]. A similar study conducted by Zhao et al. described the engineering of an injectable ECH using quaternized chitosan and PANi for tissue engineering [79]. The engineered ECH was shown to possess high antibacterial activity against Gram-negative and Gram-positive bacteria (Fig. 3a), as well as increased electrical conductivity (Fig. 3b) and swellability. In addition, *in vitro* studies using C2C12 myoblasts demonstrated that ECHs containing higher concentrations of PANi showed significantly increased cell proliferation as compared to chitosan hydrogels grafted with oxidized dextran as a control (Fig. 3c). This study introduced a new class of bioactive scaffolds that may be tailored for a variety of excitable tissue engineering applications, such as scaffolds for cardiac and neural tissues [79].

As a conductive polymer, PANi has gained significant attention due to its high electrical conductivity, stability, and unique redox properties [106]. However, limitations such as harsh processing conditions, toxicity, and non-biodegradability still limit the implementation of PANi in the biomedical field. Future investigations

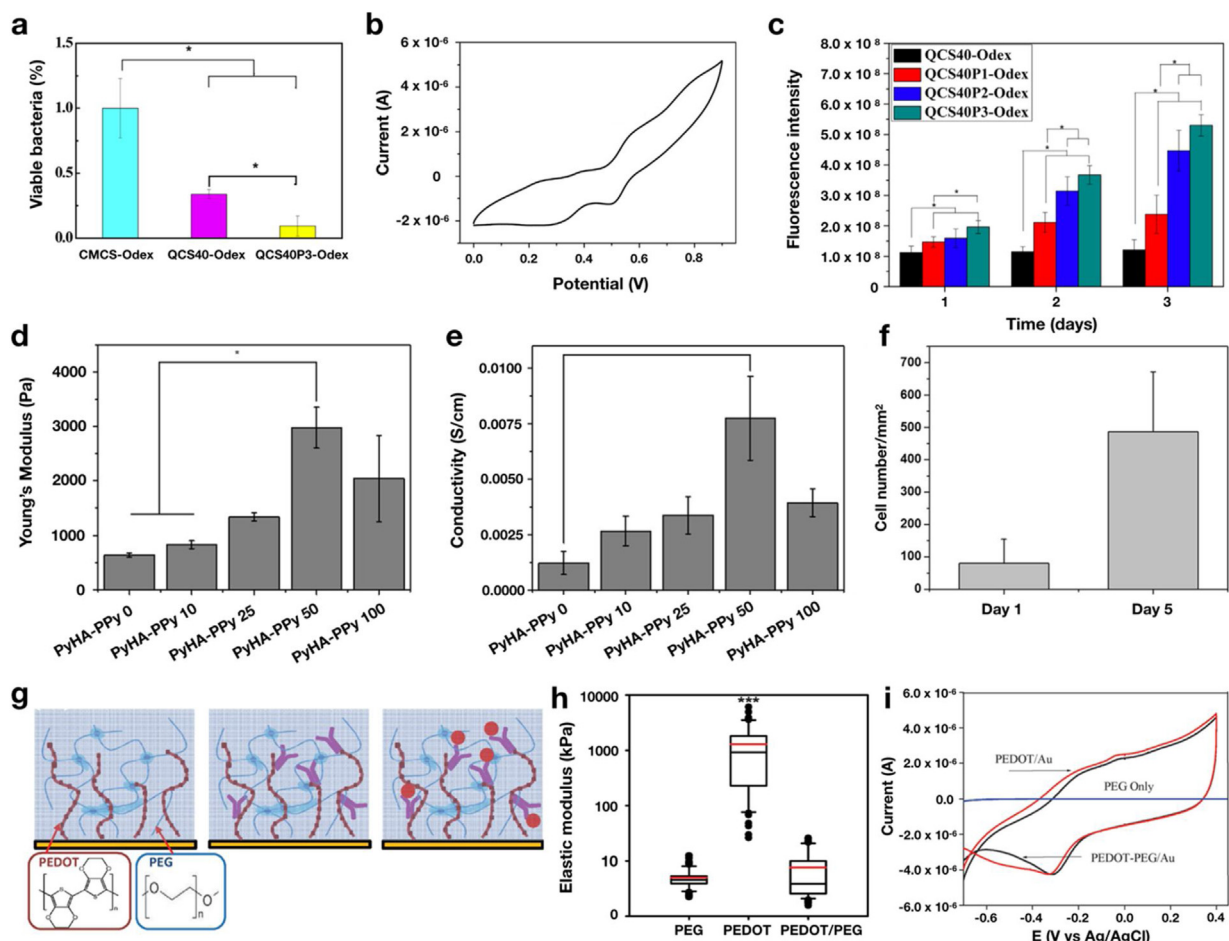


Fig. 3. Structure and properties of ECHs formed by using conductive polymers. *In vivo* antibacterial activity test using hydrogels formed with carboxymethyl chitosan (CMCS-Odex), quaternized chitosan (QCS40-Odex), and quaternized chitosan with 3% (w/v) polyaniline (PANI). Oxidized dextran (Odex) was used as a crosslinker to form these ECHs. Results showed that engineered PANi-incorporated ECHs exhibited high antimicrobial properties (a). PANi-incorporated ECHs also exhibited high electrical conductivity (b). These ECHs demonstrated increased C2C12 cell proliferation with an increasing concentration of PANi, after 3 days of culture (c). [79] ECH developed by crosslinking polypyrrole (PPy) with hyaluronic acid (HA). Mechanical testing of HA-based ECHs with varying concentrations of PPy results in higher elastic modulus (d). In addition, higher concentrations of PPy increased electrical conductivity up to samples containing 50 mM (e). Plot of the number of attached 3T3 cells seeded on the surface of HA-based ECHs containing 50 mM PPy. These results showed that HA/PPy ECHs supported cell adhesion and the proliferation of 3T3 cells up to 5 days (f). [107] Schematic for the synthesis of ECHs by adding PEDOT (red) and PEG (blue) to the surface of an electrode. Biorecognition molecules (purple) are added into the gel by attaching to –COOH groups of PEDOT. Capture of B-IFN- γ molecules (red dot) resulted in a change in the electrical signal of the ECH (g). Assessment of the mechanical properties of PEG hydrogels, as well as PEDOT and PEDOT/PEG ECHs. Here the results showed that by adding PEDOT to PEG did not significantly change the elastic modulus compared to pure PEG hydrogels (h). However, the addition of PEDOT resulted in a significant increase in electrical conductivity (i), which is necessary

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should evaluate PANi combined with other monomers to develop ECHs that possess enhanced conductivity, as well as biodegradability, and biocompatibility.

3.2. Polypyrrole

PPy is an electroconductive polymer that has been used for the synthesis of ECHs due to its facile synthesis, environmental stability, tunable mechanical properties, and biocompatibility [109–111]. PPy has also been used for other purposes, including drug delivery systems [78,112] and bio-electrodes [113,114], as well as the engineering of cardiac tissue constructs [115,116] and artificial muscles [117]. A recent study conducted by Yang et al. reported the combination of PPy with hyaluronic acid (HA) by conjugating N-(3-aminopropyl) pyrrole onto HA polymer chains (Fig. 3d) [107]. Their results showed that the elastic modulus and electrical conductivity of these ECHs increased concomitantly with

increasing concentrations of PPy up to 50 mM (Fig. 3e). The highest electrical conductivity exhibited by these PPy-incorporated ECHs was approximately 7.3 mS/cm, when fabricated with a 0.5 mM PPy concentration (Fig. 3f) [107]. *In vitro* studies conducted by seeding 3T3 cells on PPy-ECHs showed increased cell adhesion and proliferation, as compared to pure HA hydrogels. Taken together, these results demonstrated that PPy-incorporated ECHs could be used to develop tissue engineering scaffolds for excitable cells, as well as for future prosthetic devices.

The excellent redox properties of PPy have also made it an attractive material for development of patterned electrodes for the skin. In a recent study, Hur et al. developed a PPy-incorporated smart ECH using agarose as the polymeric network [76]. This strategy yielded ECHs that were not only highly conductive, but also possessed thermoplastic properties, which enabled thermal or light-assisted healing of the network. These ECHs exhibited

mechanical properties that were similar to that of human skin, with a Young's modulus of 27–46 kPa, while also possessing an electrical conductivity in the same range as other ECHs fabricated using CPs (0.35 S cm^{-1}) [76]. By developing ECHs with similar mechanical flexibility and self-healing properties, as well as high electrical conductivity, these materials are remarkably advantageous for applications involving flexible electronics and biosensors.

PPy has become a very popular CP for the fabrication of biocompatible ECHs with excellent stimulus-responsive properties and high thermal stability [28]. PPy may also be used for tissue engineering applications where the modulation of excitable cells is required, such as neural [118] and cardiac tissues [119]. However, the poor solubility of PPy in polar solvents, as well as the brittleness of ECHs containing PPy should be addressed to yield higher quality scaffolds. Future research into PPy-incorporated ECHs may focus on the incorporation of natural polymers with optimal biodegradation profiles and cell adhesion to be used for biomedical applications.

3.3. Polythiophenes / Poly(3,4-ethylenedioxythiophene) (PEDOT)

Polythiophenes (PThs) are another class of innately electroconductive polymers that have been used to impart electrical conductivity to ECHs due to their high electrical conductivity when they are present in a doped state [80]. However, the use of PThs for biomedical applications is often limited due to their increased weight and rigidity, which can potentially decrease the mechanical and electroactive performance of resulting ECHs [80,81]. Unlike PThs, its derivative PEDOT is commonly used in the synthesis of ECHs due to its biocompatibility, cost-effectiveness [120], and electrochemical stability in aqueous solutions [121–125]. While PEDOT itself is conductive, increased electrical and cationic conductivities are obtained when PEDOT is combined with poly(styrene sulfonate) (PSS) [126]. PEDOT:PSS also maintains suitable conductivity in the body and may be cleared by the kidneys, which has resulted in significant attention in a variety of biomedical fields [82,83].

Recent studies have shown that PEDOT-incorporated ECHs can be used to closely mimic the conductivity and mechanical properties of certain tissues, making them suitable to use as scaffolds for tissue engineering. A study by Kim et al. reported that native cardiac tissue has an elastic modulus within the range of 10–100 kPa, and has a conductivity ranging from 10^{-3} S/cm to 10^{-2} S/cm . In an attempt to engineer biomimetic biomaterials for cardiac tissue regeneration, Kim et al. reported the formation of an ECH based on a RGD-modified polyethylene glycol (PEG) containing PEDOT with an elastic modulus of $21 \pm 4 \text{ kPa}$ and a conductivity of $1.69 \times 10^{-2} \text{ S/cm}$ [82]. *In vitro* cell studies using electro-responsive H9C2 cells were conducted to determine the effect of PEDOT on cell growth. Their results showed that PEDOT-containing ECHs supported cell attachment and proliferation, without compromising the electrical and physiochemical properties of the hydrogel [82].

Another study that investigated PEDOT-incorporated ECHs for tissue engineering was recently conducted by our group. In this study, Spencer et al. developed ECHs containing GelMA and various concentrations of PEDOT:PSS [83]. Results showed that an increase in the concentration of PEDOT:PSS up to 0.3% (w/v) did not change the mechanical properties of the ECHs; however, the swelling ratio significantly decreased with higher concentrations of PEDOT. The results showed that ECHs that containing 0.3% PEDOT:PSS had significantly lower electrical resistance (261.0 kOhm) when compared to pure GelMA samples (449.0 kOhm) [83]. The biocompatibility of these ECHs was investigated *in vitro* via 3D encapsulating of C2C12 cells. High cell viability was obtained for ECHs containing up to 0.1% PEDOT:PSS [83]. Taken together, these results showed that PEDOT can be used to impart electrical conductivity to hydrogels without significantly altering their biocompatibility and mechanical properties.

PEDOT has also been used to develop ECHs for biosensor application. Recently, Shin et al. developed a PEDOT/PEG-based ECH to detect specific antigen molecules. The outer layers of these sensors were coated with the ECH loaded with a cytokine-specific antibody, which was attached to a PEDOT–COOH group. Once these biosensors come in contact with the IFN- γ analyte, the ECHs undergo a quantifiable decrease in electrical conductivity that would serve as the detection mechanism (Fig. 3g). Despite the high mechanical strength of PEDOT, PEDOT/PEG ECHs exhibited an elastic modulus of $7.58 \pm 0.84 \text{ kPa}$, which was similar to that of pure PEG hydrogels (Fig. 3h). These PEDOT/PEG ECHs exhibited remarkable electrical conductivities, and could be used to readily transduce the detection of different analytes into an electrochemical signal (Fig. 3i).

Advancements in the development of PEDOT-incorporated ECHs have led to promising innovations in areas including biosensors, implantable electrodes, and drug delivery systems. Researchers are able to take advantage of the high electrical conductivity, biocompatibility, as well as chemical and environmental stability exhibited by PEDOT. However, the high mechanical stiffness of this material could potentially trigger foreign body responses (FBRs) that may disrupt performance when PEDOT-incorporated ECHs are implanted into soft tissues, such as the brain. Moreover, long term *in vitro* and *in vivo* investigations are required to determine any potential cytotoxic response, before they can be transitioned into the clinical setting.

4. Ionic liquid (IL) conjugated ECHs

ILs have been implemented in a wide range of industrial applications, including fuel cells [85], solar cells [86], batteries [87], and sensors [127] due to their unique properties such as low volatility, non-flammability, high thermal stability, and high ionic conductivity [128–132]. ILs are liquids comprised completely of ions from salts, which have a melting point below 100°C . There are many sub-categories of ILs, including room temperature ILs, task-specific ILs, polyionic liquids, and supported IL membranes [131]. Previous studies have focused on the characterization of the physical structure and properties, nano-organization and self-assembly, and advanced chemical transformations of ILs [133]. Recently, it has been demonstrated that ECHs may be engineered by combining ILs with polymers for applications in tissue engineering, electrochemical biosensors, electro-stimulated drug release systems, and neural prosthetics [134,135].

ECHs can be formed by polymerizing monomers in ILs to increase the electrical conductivity of these scaffolds. This approach was demonstrated by Liang et al., using microcrystalline cellulose and PPy as polymers, to form a network in 1-butyl-3-methylimidazolium chloride (BMIMCl) IL [135]. BMIMCl was used as a solvent to dissolve cellulose while protecting its structure from degradation. The swelling of ECHs was found to be dependent on the microcrystalline cellulose concentration, with higher concentrations exhibiting lower swelling ratios. Furthermore, the mechanical properties were substantially improved with the incorporation of PPy, which increased from a maximum stress of 1.53 MPa for control samples, to 26.25 MPa for PPy-containing ECHs [135]. Their results also demonstrated that the composite hydrogels were suitable for the development of biological and semiconducting materials, as well as drug delivery systems and neural prosthetics [135]. In another study, Robinson et al. develop a synthetic sensing skin by 3D printing two inks; one that was ionically conductive and one that was electrically insulating [134]. For this, they used the conductive 1-decyl-3-methylimidazolium chloride IL, while the insulating material was a silicone elastomer. This approach was used to print a micropatterned material that could act as stretchable capacitive sensors. Both inks were extruded and

polymerized *in situ* to form a layer of ECHs, and a layer of insulating silicone. The resulting capacitive skin demonstrated excellent adhesion to actuation chambers, as well as the ability to detect a compressive force of approximately 2 N. The combination of an ECH with the insulating silicone led to the development of the first printable skin capable of tactile sensing and kinesthetic feedback [134].

Recently, our team demonstrated the conjugation of a choline-based bio-ionic liquid (Bio-IL) to hydrogel networks to form ECHs with controlled conductivity and physical properties for cardiac tissue engineering applications [20]. The difference between Bio-ILs and ILs are that Bio-ILs exhibit enhanced biocompatibility and thus may be attractive materials for biomedical applications. ECHs with tunable properties were synthesized by photocrosslinking various ratios of GelMA prepolymer to acrylated Bio-IL in the presence of Eosin Y photoinitiator and visible light. Our results showed that these composite hydrogels demonstrated tunable electrical conductivity that was in the range of native cardiac tissue (Fig. 4a). Biocompatibility of GelMA and GelMA/Bio-IL hydrogels were assessed by seeding CMs on the surface and inside of these materials. Both GelMA hydrogels and GelMA/Bio-IL ECHs demonstrated excellent cell viability throughout the culture (Fig. 4b). Further, immunofluorescent staining of sarcomeric α -actinin showed that GelMA control hydrogels exhibited an intermittent pattern of sarcomeric α -actinin (Fig. 4c), while GelMA/Bio-IL ECHs exhibited a homogeneous distribution of these protein (Fig. 4d). *In vitro* studies showed that by day 7 of cell culture, CMs seeded on GelMA/Bio-IL ECHs had a significantly higher beating frequency as compared to control GelMA hydrogels (Fig. 4e). This can be attributed to the high electrical conductivity observed in GelMA/Bio-IL ECHs. Taken together, these results demonstrated that the conjugation of Bio-ILs to polymeric networks is an effective approach to impart electrical conductivity to otherwise insulating hydrogels.

5. Microfabrication of ECHs

5.1. 3D printing

3D printing technology has significantly improved over the past few decades, enabling the creation of complex 3D structures that might otherwise be impossible to fabricate with traditional molding techniques or top-down milling procedures [136]. This technology has made its way into the biomedical field to form complex structures, which have great potential to contribute to our understanding of healthy and diseased states [137], to expand current treatment options [138], and to fabricate medical devices [139]. In addition, the turnover time for these structures or devices is appreciably less than many molding or fabrication processes, which can reduce lead time and accelerate clinical translation.

For many biomedical applications, engineering biomaterials with biomimetic mechanical properties has been a challenge. This mechanical mismatch has inspired widespread interest in the field of flexible electronics and electroactive tissue engineering [140]. To this end, the design of soft and conductive materials has been an emerging area of research in recent decades. Since the hydrostatic and mechanical properties of hydrogels are very similar to human tissues, they are an obvious choice as a material for fabricating complex 3D bioprinted structures. In this section, we will review recent work in the field of 3D printing of ECHs with their potential applications in the biomedical field.

One of the common applications of 3D printed ECHs is as pressure/motion sensors or as biosensors [141]. For these applications, tuning the geometrical or chemical structure of the ECHs can modulate its electrical properties and causes a proportional response,

signaling the occurrence of an event. In the case of pressure or motion sensors, the compression or extension of the hydrogel causes a corresponding change in electrical resistance and thus the generation of an electrical signal [142]. For biosensors, an analyte of interest binds to the conductive material and induces a change in its electrical properties, causing the signal to be generated [108]. Normally these sensors require advanced fabrication techniques to achieve the complex landscape for the sensor design. 3D printing can eliminate the need for costly tooling and equipment that is typically required to form these patterns and enables rapid formation of multiple designs at minimal cost and significantly reduced lead time. In some cases, elastomeric polymers, such as polydimethylsiloxane (PDMS), are utilized as printing substrates onto which the conductive hydrogel can be patterned with the printing device [143]. The PDMS helps to maintain the overall stability of the printed structure and provides a template for the sensing function of the device. While non-hydrogel conductive elastomers can be used for pressure or motion sensors [144], detection of water-soluble analytes might not be as sensitive or accurate if non-porous hydrophobic materials, such as PDMS, are used. For example, inkjet printing was used to pattern a nanostructured PANi hydrogel for multiplex detection of glucose, lactate, and triglycerides in real time selectively and with high sensitivity [145]. The use of printing for the devices enabled them to fabricate pages of sensor arrays with 96 electrodes in minutes. In comparison, traditional photolithography patterning methods would require pre-fabrication of photomasks and careful substrate preparation and washing/cleaning steps before the device could be realized. Pressure sensors were fabricated by printing a conductive self-healing hydrogel composed of polyacrylic acid and PPy (Fig. 5a), wherein changes in pressure caused a corresponding change in electrical resistance [146]. The devices were coated with a thin layer of PDMS to contain the conductive gel and pressed onto the wrist for taking blood pulse readings. These printed devices could be used as wearable sensors that conformed to the shape of the arm, finger, or wrist (Fig. 5b). Also, another group designed a photocurable conductive and elastic hydrogel that could be printed using digital light processing (DLP) stereolithography (SLA) [147]. The printed constructs were not tested for specific applications, but the formulation and printing technology hold great potential for applications where a transparent, elastic, and conductive hydrogel is required, such as optogenetics [148]. Careful deliberation of the materials and methods will undoubtedly improve the performance and capabilities of 3D printed conductive hydrogel-based devices in the near future.

Recapitulation of complex tissues *in vitro* is becoming more feasible as advanced microfabrication strategies evolve. 3D bioprinting has enabled the generation of features in an additional dimension compared to 2D patterning methods and has provided capacity to form biomimetic tissue structures *in vitro*. Ultimately, the goal of this technology is to generate full-scale tissues or organs that can replace organ donation and transplants [149]. Bioprinted hydrogels with electrical conductivity have been shown to improve the function of electroactive tissues, such as cardiac and neural tissue [57,150]. For example, PEGDA hydrogels mixed with various concentrations of amine-functionalized multi-walled carbon nanotubes (MWCNTs) was 3D printed into grid-like structures for nerve regeneration [151]. Results showed that neural stem cells (NSCs) proliferated more and differentiated early on scaffolds containing MWCNTs compared to controls without conductive nanotubes. In addition, exogenous electrical stimulation in the form of biphasic pulses enhanced neuronal maturity for structures containing the MWCNTs as confirmed by quantitative polymerase chain reaction (qPCR). A similar study targeting cardiac tissue utilized a bioink containing GNRs, GelMA, and sodium alginate with a co-axial printing system, where sodium alginate was used as a structural

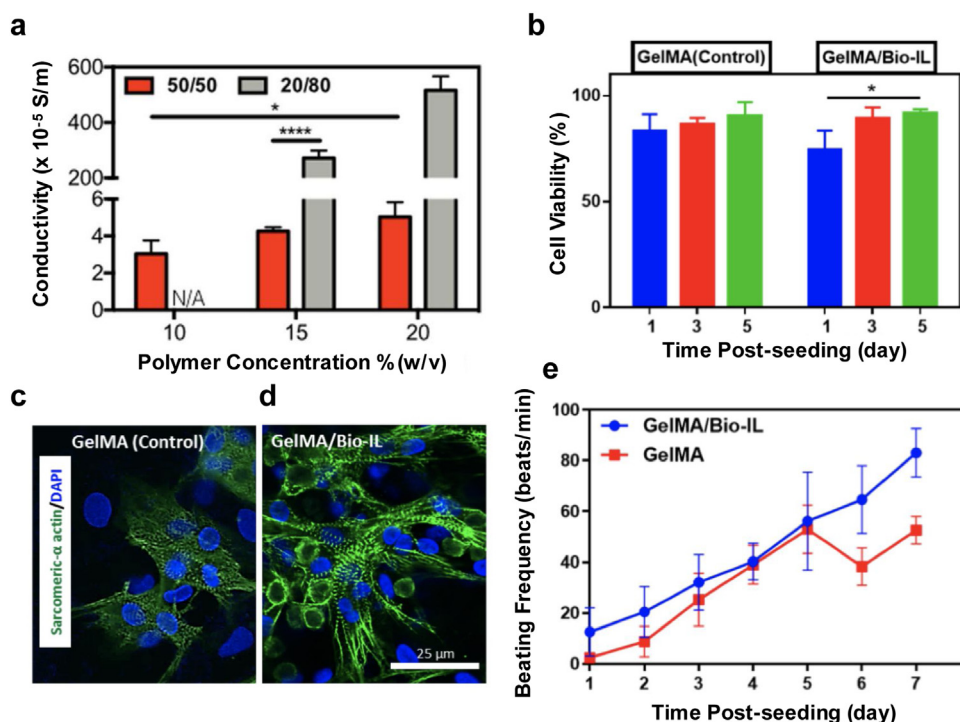


Fig. 4. Synthesis and characterization of ECHs engineered using Bio-ILs. Electrical conductivity assessment of ECHs formed with GelMA and bio-ionic liquid (Bio-IL) demonstrating that scaffolds fabricated with higher concentrations of Bio-IL exhibited greater electrical conductivity (a). Biocompatibility assessment using CMs seeded on the surface of GelMA hydrogels and GelMA/Bio-IL ECHs *in vitro* demonstrated that the incorporation of Bio-IL into hydrogels had no significant effect on cell viability after 5 days (b). Immunofluorescent staining for sarcomeric α -actinin (green) and DAPI (blue) for CMs seeded on control GelMA hydrogels (c) and GelMA/Bio-IL ECHs (d) at day 7. Characterization of synchronous beating of CMs seeded on GelMA hydrogels and GelMA/Bio-IL ECHs over 7 days of culture showing that the conductive GelMA/Bio-IL hydrogel exhibited higher beating frequencies compared to non-conductive GelMA hydrogels (e). [20], Copyright 2017. Reproduced with permission from Nature Publishing Group. Scale bars = 25 μ m (d) (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

material that was rapidly crosslinked as aqueous calcium chloride was extruded through the shell of the nozzle (Fig. 5c) [152]. CMs encapsulated in the 3D printed GelMA/GNR hydrogel structure expressed higher levels of Connexin 43 (Cxn43) cardiac junction protein and exhibited higher contraction rates than GelMA controls (Fig. 5d). These results show promise as cell-laden 3D printed structures with enhanced tissue function that may enable tissue replacement in the future.

3D printing has been widely used in the biomedical field. Its simplicity, low cost, minimization of waste, and ever-advancing capability have made this technique an extremely powerful tool. ECHs have proven to be very useful for applications where an electrically active, flexible, and hydrated material is required, such as in sensing devices and tissue culture. The combination of ECHs with advanced 3D printing has provided researchers with the means to create complex structures with unprecedented speed and precision. For 3D printed conductive hydrogel motion sensors, the capabilities tested thus far were limited to simple events, such as hand or arm movement [143]. More sophisticated devices capable of sensing motion and responding could be used as medical devices, such as a film that can sense cardiac beating and provide an electrical stimulation if arrhythmia is detected. An ongoing challenge in biosensors is improving selectivity [153]. Hydrogel compositions could be chemically modified to improve selectivity and prevent false positive or false negative responses. For 3D bioprinting, designing electroconductive bioink with biocompatibility and printability is a major limitation, especially in the case of printing complex cell-laden 3D structures. In addition, the ability to directly print integrated electrochemical probes into 3D printed devices would enable interrogation and stimulation of electroactive cells encapsulated in the structure [154]. Finally, the resolution of 3D printing is rapidly improving, and decreased feature size can

shrink the overall size of devices or sensors, increasing the fidelity of bioprinted structures that mimic native tissues.

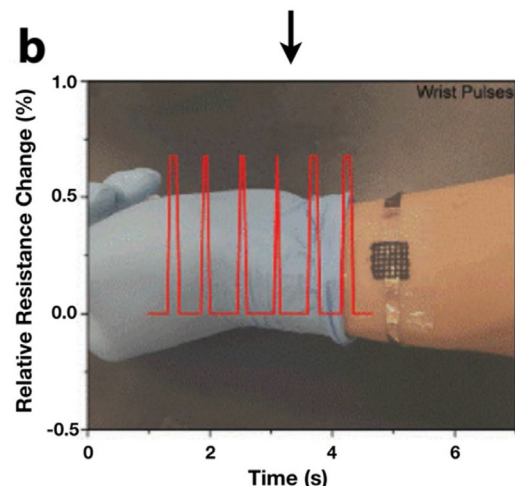
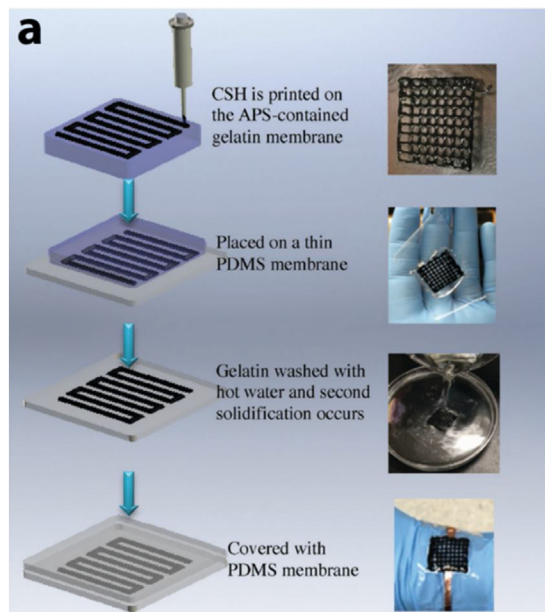
5.2. Electrospinning

The primary goal for the design of tissue engineered scaffolds is to develop materials that structurally and functionally mimic the native ECM. The ECM is a complex network of proteins, proteoglycans, and glycosaminoglycans that provides physical support for cells [155,156]. Furthermore, the ECM is responsible for the promotion of cell adhesion and migration, as well as proliferation, and function [157]. This is achieved, in part, due to the complex nanostructure of protein fibers, such as collagen and elastin [157], and the presence of specific ligands and growth factors [158,159]. Protein fibers may range in diameter from several tens to several hundred nm. In this regard, electrospinning has been increasingly used for the engineering of polymer fibers that resemble the fibrous architecture of the ECM.

Electrospinning systems are comprised of three main components; a high voltage supplier capable of generating 10–20 kV of potential, a small diameter metal nozzle, and a metal collector (Fig. 6a) [160–162]. The generated electrical field drives the polymer feed to the grounded metal collector in a process that is referred to as a whipping mode, which is characterized by increased acceleration and oscillation [160,163,164]. The solvent used in the polymer feed is mainly evaporated or solidified in the electrospinning process, and trace amounts of solvent in the fibers may be removed using vacuum after synthesis.

Electrospun conductive fibrous scaffolds have been widely used in tissue engineering and biomedical applications due to the promotion of favorable cellular responses, such as increased adhesion and proliferation [165–167]. CPs such as PANi, PPy, and PTh have

3D Printed Conductive Hydrogels as Sensing Devices



3D BioPrinted Conductive Hydrogels for Tissue Culture

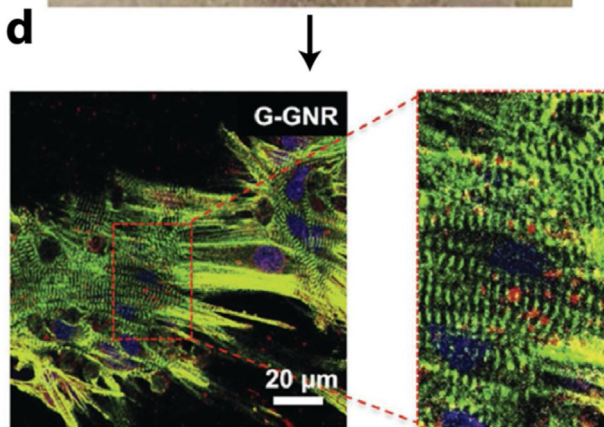
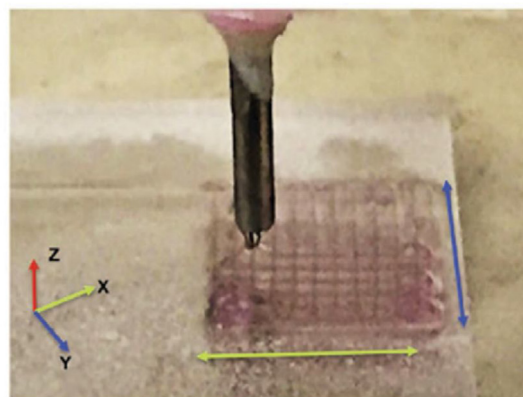
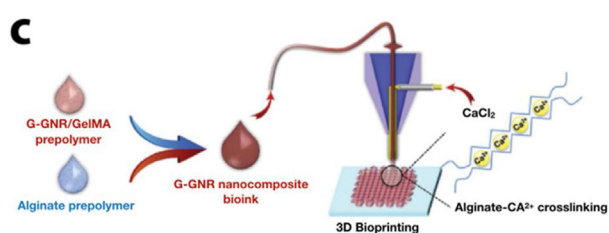


Fig. 5. 3D printing of conductive hydrogels for different biomedical applications. Pressure sensors were fabricated by 3D printing a conductive self-healing (CSH) hydrogel composed of PPy-grafted chitosan and poly(acrylic acid), and coating them with a thin layer of PDMS (a). When applied to the wrist, the sensor could detect blood flow pulses in the veins below the skin in real-time (b). [146] Hydrogel prepolymer solution containing sodium alginate, gold nanorods, and GelMA was bioprinted via a co-axial nozzle, in which the alginate prepolymer solution in the core was crosslinked by calcium ions flown in the sheath (c). Lattice-like structures could be printed, and hydration was maintained by the continuous flow of the aqueous sheath solution. CMs printed in these structures expressed higher levels of Cxn43 cardiac junction protein and exhibited higher contraction rates compared to controls (d). [152].

[146] [152]. Copyright 2018, 2017, respectively. Reproduced with permission from John Wiley and Sons Inc.

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been electrospun into composite fibers with non-conductive polymers to form hydrophilic scaffolds for cardiac, neural, and skeletal regenerative tissue engineering [166,168]. The combination of CPs with natural polymers provides good electrical conductivity while also improving the swellability, biodegradation, and biocompatibility of these hydrophilic scaffolds. For example, an electrospun PANi-gelatin fiber blend has recently been engineered for cardiac and neural tissue engineering [169]. These PANi-gelatin scaffolds demonstrated a uniform distribution of polymers, which exhibited high biocompatibility, and were able to promote the adhesion and proliferation of cardiac myoblasts [169]. Furthermore, mechanical characterization of PANi-gelatin fibers showed an increase in the elastic modulus, and a reduction in fiber size with increasing PANi concentrations.

Another recent study by Malki et al. investigated the formation of ECHs by electrospinning of albumin and then absorbing AuNRs

into the fibers to engineer cardiac patches capable of cardiac tissue regeneration following myocardial infarction (MI) [170]. A porous albumin scaffold composed of ribbon-like fibers with a thickness of 0.5 μm was developed by using electrospinning [170]. Further, the incorporation of AuNRs to these cardiac patches improved electrical conductivity that could assist in the cell-cell interactions. The AuNR-incorporated cardiac patches were evaluated *in vitro* through CMs encapsulation within these hybrid scaffolds to analyze the expression of the gap junction protein Cxn43. Their results showed that after 7 days of culture, there was a significant expression of Cxn43, as well as pronounced actinin striation [170]. The electrical enhancement of these electrospun scaffolds yielded cardiac patches which were able to support CM contraction and may be used to improve the function of cardiac tissue following MI.

In another study, Liu et al. developed ECHs with architecture that closely resembled the cardiac microenvironment

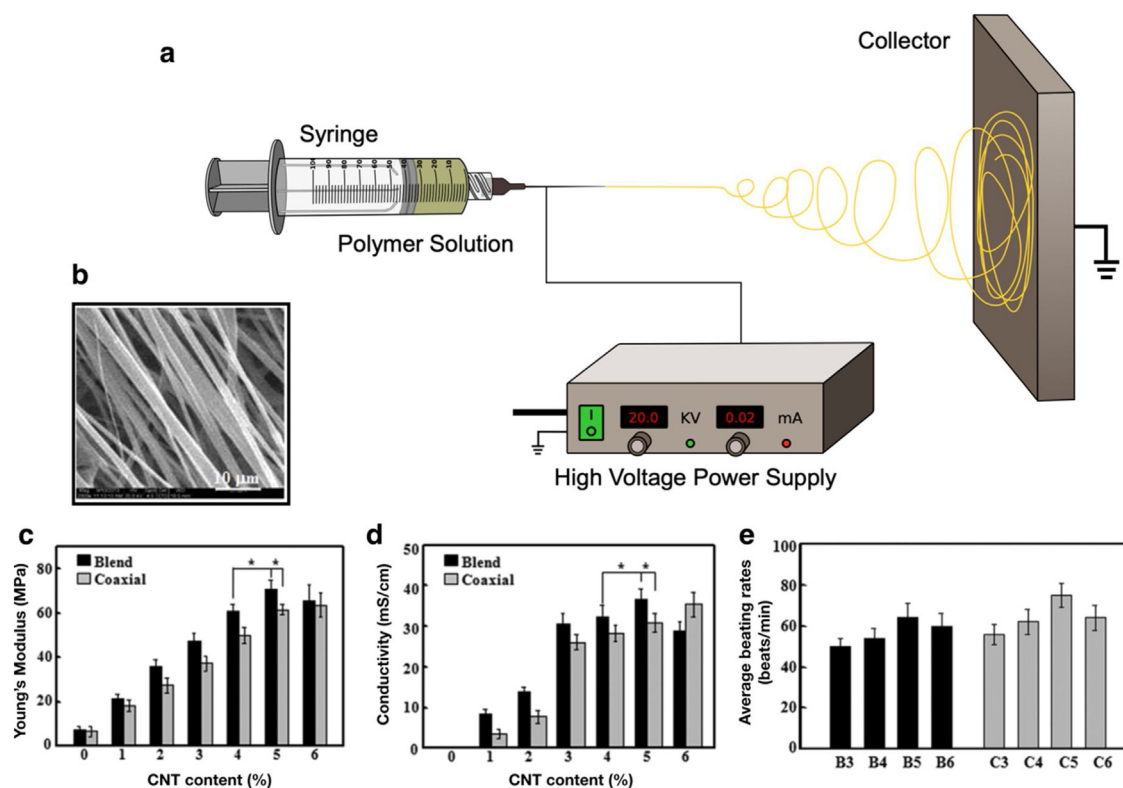


Fig. 6. Electrospinning of ECHs for different biomedical applications. Schematic for an electrospinning setup consists of a polymer dissolved in solvent being injected out of a metal nozzle (a). A high voltage power supply is connected to a metal nozzle and a metal collector creating an electrical field. A polymeric solution is then slowly pumped out of the syringe and spun onto the metal collector. Representative SEM image of a blend electrospun PELA/CNT fibrous scaffold with a 5% CNT concentration showing high alignment of fibers with 2 μm fiber diameter (b). Mechanical study of electrospun PELA/CNTs demonstrated that ECHs fabricated with higher concentrations of CNTs resulted in a higher Young's modulus (c). Electrical evaluation of electrospun ECHs showed that these fibrous scaffolds exhibited higher conductivity when fabricated with a higher concentration of CNTs in both blended and coaxial electrospun PELA/CNT scaffolds (d). Beating rate of CMs when seeded on PELA/CNT fibrous scaffolds and cultured for 10 days. The ECHs coaxially electrospun with a 5% CNT concentration (C5) achieved an average beating rate of 70–80 times/min, which was similar to that rate of CMs seeded on other non-conductive hydrogels (e). [171], Copyright 2016. Reproduced with permission from Elsevier Inc.

through electrospinning poly(ethylene glycol)-poly(D,L-Lactide) (PELA) copolymers with CNTs onto a spinning mandrel [171]. Their approach investigated the efficacy of both blend electrospinning and coaxial electrospinning techniques. Blend electrospinning involved mixing CNTs and PELA into a polymer solution. Coaxial electrospinning followed the same principles, however, two separate solutions, one containing PELA and the other CNTs, were coaxially and simultaneously electrospun through different capillary tubes into the same nozzle. Electrospinning PELA/CNT onto a rotating mandrel resulted in aligned fibers with diameters between 2–3 μm (Fig. 6b). These blended and coaxial electrospun scaffolds demonstrated that higher CNT concentrations resulted in higher mechanical stiffness, concomitantly (Fig. 6c). Further, scaffolds fabricated with higher concentrations of CNTs yielded higher electrical conductivity (Fig. 6d). The beating rates of CMs when seeded on the engineered fibrous mats were evaluated for up to 10 days. Results showed that CMs seeded on electrospun scaffolds fabricated with 5% CNTs had significantly higher average beating rates (70–80 beats/min) when compared to scaffolds fabricated with 3%, 4%, and 6% CNT concentrations (Fig. 6e). CMs seeded on all PELA/CNT electrospun scaffolds showed synchronous beating, except for those fabricated with 6% CNTs. The engineered fibrous scaffolds in this study could mimic the organized cardiac muscle fibers of the heart.

Electrospinning is a facile method that allows precise control over many parameters of the synthesis process, such as voltage, distance from the nozzle to collector, and flow rate. In addition, metal collectors may be static, generating randomly arranged fibers, or rotating, which results in highly aligned fibers. By adjusting these parameters, it is possible to engineer ECHs with finely tuned fiber

morphology and geometry. Using advanced electrospinning setups, ECHs with enhanced microarchitecture, porosity, mechanical properties, and electrical conductivity have been fabricated with unique properties for biomedical applications. Further, electrospinning provides a method to optimize the patterning of cells or bioactive ligands in a way that is biomimetic to the structure and morphology of native tissues. Further characterization including *in vivo* studies, however, will be required for electrospun ECHs before they can be used for clinical applications. In addition, future studies should seek to improve cost-effectiveness and increase the yield of fibrous scaffolds. Nonetheless, modern electroactive and fibrous scaffolds, with excellent scalability, reproducibility, and consistency show promise for use in future biomedical applications such as cardiac and neural tissue engineering.

5.3. Micropatterning of ECHs

Micropatterned hydrogels have been used in a wide range of biomedical applications, such as drug delivery [172,173], and tissue engineering [174]. Some of the most commonly used techniques for micropatterning of hydrogels include microfluidics [175], magnetic [176] and acoustics guided hydrogel assembly [177]. However, micropatterned non-conductive hydrogels could impede electrical cell-cell coupling and lead to signal deferment inside the scaffolds [57]. This, in turn, could potentially limit their application in the context of physiological environments where excitable cell types are present such as nerve and cardiac tissues. Therefore, micropatterning of ECHs has gained significant attention, and different techniques have been investigated to generate unique

architectures of micropatterned ECHs for biomedical applications [178]. For example, in a study by Kim *et al.*, conductive PEDOT-incorporated ECHs were patterned to develop flexible electrodes [179]. Briefly, a PEDOT film was prepared by reacting a liquid phase PEDOT monomer casting with the oxidant Fe(III) tosylate. A UV-induced photopolymerization of PEG was then performed using a photolithography technique at the PEG/PEDOT interface using a photomask. The PEG hydrogel was then peeled off removing the region of PEDOT film that was exposed to UV irradiation. Finally, a second PEG gelation step was performed on the remaining patterned PEDOT film, which left it embedded in the hydrogel. Micropatterned PEDOT-embedded ECHs exhibited high electrical conductivity coupled with high flexibility, which demonstrate their potential for biomedical applications such stimuli-responsive drug delivery systems and growth factor delivery systems [179].

In another study, Wu *et al.* reported the engineering of conductive GelMA-PANi ECHs in patterned hexagonal geometries, by utilizing digital projection stereolithography [180]. This was achieved by injecting GelMA-PANi precursor solution in a chamber and crosslinking it in organized patterns using a computer-aided design-based digital mask. GelMA/PANi ECHs demonstrated remarkably lower impedance ($2.9 \pm 0.3 \text{ k}\Omega$) compared to control GelMA hydrogels ($6.9 \pm 0.7 \text{ k}\Omega$), at physiologically relevant frequencies. In addition, *in vitro* cell studies were used to investigate the effect that these micropatterned ECHs on the morphology of adhered cells. This was done by culturing C3H/10T1/2 murine mesenchymal progenitor cells (10T1/2s) cells on the surface of GelMA and GelMA/PANi samples for 5 days. Results showed that 10T1/2s cells seeded on GelMA/PANi ECHs demonstrated better adhesion and viability after 5 days compared to pristine GelMA [179]. Further, while 10T1/2s cells adhered exclusively to GelMA control samples, migration between the hexagonal patterns was observed only in GelMA/PANi ECHs.

More recently, Navaei *et al.* described a technique for micropatterning CMs onto ECHs by incorporating conductive GNRs into GelMA hydrogels to create microgrooved architectures [181]. Their approach utilized a micro-mold composed of PDMS with a microgrooved topography. A prepolymer solution containing GelMA and GNRs was pipetted on a 3-(Trimethoxysilyl)propyl methacrylate (TMSPMA)-coated slide, and the PDMS micromold was placed on top (Fig. 7a). The solution was then photopolymerized through exposure to UV irradiation. The resulting ECH constructs possessed microgrooves that were $50 \mu\text{m}$ in width. GelMA/GNR ECH constructs also exhibited significantly lower impedance ($1.35 \pm 0.36 \text{ k}\Omega$) as compared with pristine GelMA ($15.58 \pm 9.18 \text{ k}\Omega$) samples. In addition, cellular viability and morphology were investigated *in vitro* by seeding CMs between the microgrooves of GelMA-GNR and GelMA constructs. Their results showed that there was an enhanced organization and spreading of CMs on GelMA/GNR ECHs (Fig. 7b), when compared to pure GelMA hydrogels (Fig. 7c) after 7 days of culture. This study demonstrated the efficacy of micropatterning techniques to fabricate ECHs with highly organized structures that could be used to develop native-like cardiac tissues.

Micropatterning of ECHs has also been performed to enhance therapies designed to stimulate nerve tissue regeneration. In a recent study, Lee *et al.* developed an ECH composed of PED and AgNWs with parallel microridges using a standard soft lithography process [182]. Briefly, a microgrooved PDMS stamp was fabricated and placed on a polyethylene terephthalate (PET) film. A prepolymer solution containing PEG and AgNWs was then injected between the grooves of the stamp and the PET film. The prepolymer solution was then photopolymerized using UV irradiation, and the PDMS stamp was peeled from the ECH. Electrical characterization of these ECHs was conducted using Comsol Multiphysics software to simulate the current at the wall of PEG/AgNW and PEG

samples when a voltage of 10V was applied. Results of this simulation showed that PEG/AgNW exhibited a significantly higher current as compared to pure PEG hydrogels due to the higher conductivity [182]. To determine if these materials could support the differentiation of NSCs into neurons and guide neurite outgrowth, NSCs were incubated and grown into neurospheres, then seeded on PEG/AgNW and PEG samples. Following 1 day of culture, an intermediate electrical stimulus of 5, 10, and 20 V was applied. Results showed that neurite growth was much higher in the PEG/AgNW ECHs as compared to PEG hydrogels [182]. Further, the neurite growth followed the microgrooves of the ECH, while neurite growth on PEG hydrogels appeared more randomly dispersed. The combination of high conductivity, robust mechanical properties, and micropatterns that support the differentiation of NSCs into neurons suggests that these ECHs are suitable for tissue engineering applications involving excitable cell types.

Overall, micropatterning of ECHs is a facile and precise method to control the 2D arrangement in a way that mimics the native ECM. Advanced micropatterning techniques can also be used to guide cellular interactions thus influencing the function of tissues. However, the use of micropatterning ECHs does have some limitations. For example, one challenge is determining an appropriate UV exposure time that would photocrosslink hydrogels, but not affect cellular viability. Another limitation is the inability to micropattern 3D constructs that closely represent the microenvironment *in vivo*. ECHs with micropatterned surfaces shows potential in developing future 2D modeling systems that emulate human physiological functions. Taken together, micropatterned ECHs holds great potential for the development of stimuli-responsive systems for biomedical innovations, and to facilitate the development of new therapeutics aimed towards cardiac and neural tissue regeneration, as well as biosensors and drug delivery systems.

5.4. Self-assembly / self-healing

Combining conductive components such as CPs and NPs into polymeric networks has been regarded as the most straightforward approach to synthesize ECHs [185–187]. However, conventional approaches for fabricating ECHs yield matrices with randomly suspended conducting components, limiting the ability to impart morphologies that enhance cell-material interactions [188]. To address this issue, self-assembly approaches have been introduced as an efficient strategy to develop a new class of smart materials with the ability to spontaneously form complex structures without external participation [189,190]. Self-assembled hydrogels are characterized by the interaction of weak noncovalent bonds to form networks, such as hydrogen bonds, ionic interactions, van der Waals forces, and π - π stacking [191]. However, the absence of covalent bonds in these networks may result in low mechanical properties, making them unsuitable for some biomedical applications. Therefore, it is often necessary to engineer self-assembling ECHs with either strong noncovalent bonds (*e.g.* ionic interactions, metal-ligand coordination), or multiple weak interaction sites (*e.g.* hydrogen bonds, VDW forces) [192].

Due to its unique structure, graphene has become a popular biomaterial capable of imparting self-assembling properties to ECHs. Self-assembled graphene hydrogels (SGH) have been increasingly used in different applications such as drug delivery systems [193], supercapacitors [194], and devices for human motion detection [195]. In one study by Xu *et al.*, 3D SGHs were fabricated by using a single-step hydrothermal approach [189]. This was achieved by heating 2 mg/mL of a homogenous GO solution to 180°C for 12 h using an autoclave. This treatment hydrothermally reduced GO creating graphene sheets, which self-assembled into ECHs *via* π - π stacking interactions. Characterization of these SGHs showed that these materials exhibited high electrical conductivity (5×10^{-3}

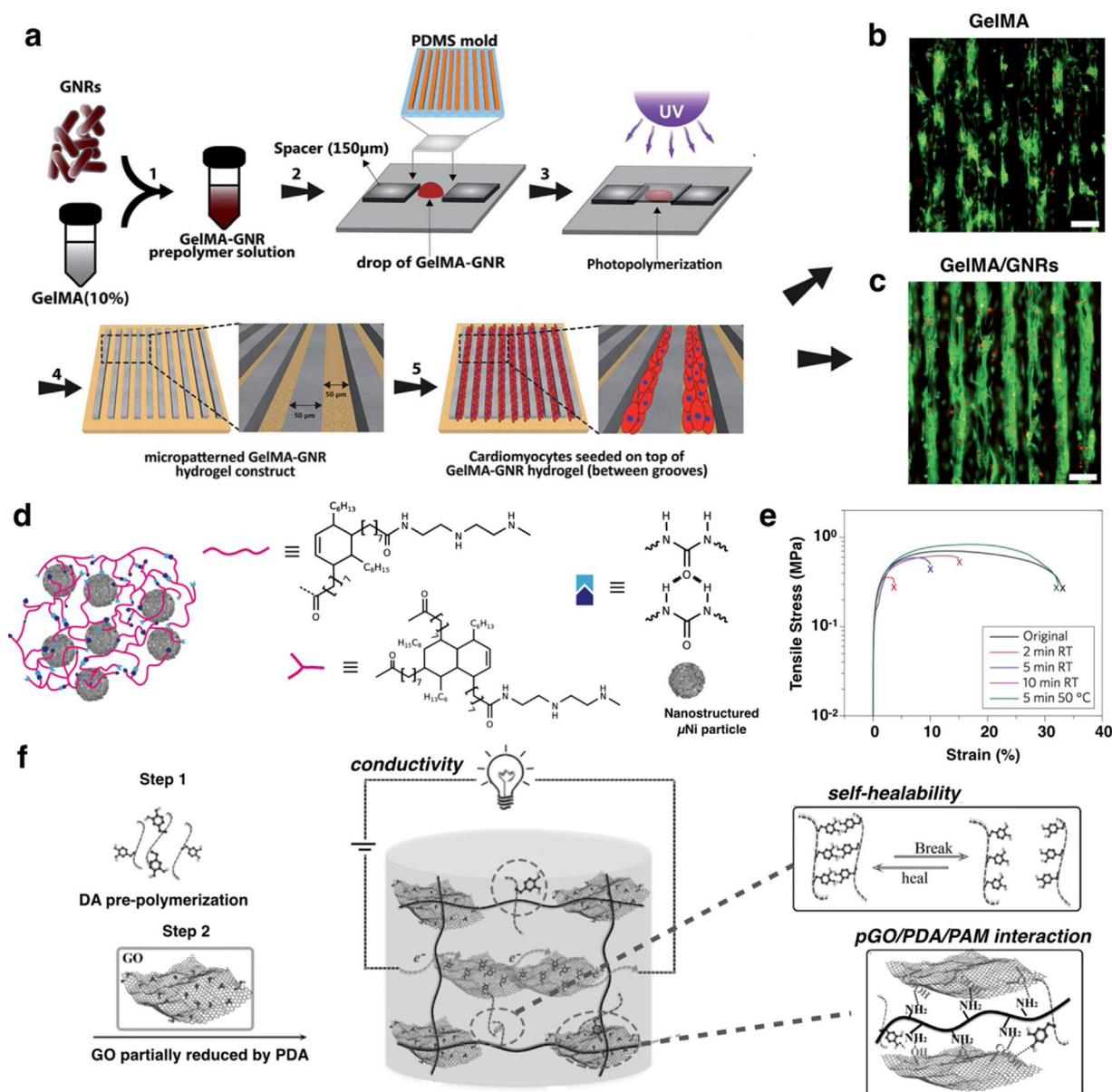


Fig. 7. Micropatterning and self-assembly of ECHs for different biomedical applications. Schematic of GelMA-GNR ECHs that were photocrosslinked in the presence of UV light in a microgrooved PDMS mold. The resulting ECH was seeded with CMs, which aligned within the microgrooves (a). Live/dead assay performed on CMs seeded on pure GelMA hydrogels (b) and GelMA-GNR ECHs (c). Live cells were stained in green, while dead CMs were stained in red, confirming high cell viability after 7 days of cell culture. [181] Schematic of supramolecular organic-inorganic composite capable of self-healing via interactions between oligomer chains (pink lines) with micro nickel and urea groups (blue and purple shapes) (d). Representative stress-strain curves for ECHs containing 31% micro nickel volume ratio under different healing conditions showing that healing efficiency was found to be a function of time (e). [183] Schematic of composite ECHs constructed with partially reduced GO using polydopamine, crosslinked with polyacrylamide (f). [184] Scale bars = 100 μ m (b, c). Scale bars = 100 μ m (b, c) (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

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S/cm), high storage modulus (450–490 kPa), and high thermal stability [189]. It was, therefore, proposed that these self-assembling ECHs were suitable for biomedical applications, such as drug delivery and tissue engineering.

ECHs with self-healing properties have also gained significant attention due to their ability to spontaneously restore the original functionality of ECHs after being damaged, which can prolong service life and avoid failure in therapeutic or diagnostic biomaterials [196]. This new class of smart material relies on dynamic

and reversible noncovalent bonds, such as hydrophobic interactions, host-guest interactions, or hydrogen bonding to autonomous crosslink polymeric networks following physical, chemical, or mechanical damage to the ECHs [184]. Advanced self-healing smart materials can be used for biomedical applications such as wound healing, where cuts, scratches, and breaks are common. The use of these systems may improve performance and lower the overall cost [196].

A study by Tee et al. described the development of biomimetic electronic skin sensors with enhanced mechanical sensing and self-healing properties [183]. This composite ECH was formed by using a supramolecular polymer capable of forming hydrogen bonds with itself, coupled with micro-nickel particles with nanoscale surface modifications (Fig. 7d) [183]. The resulting ECH was able to self-heal by forming new hydrogen bonds in response to mechanical trauma, which in turn restored the structural integrity of the hydrogel. An important feature of these supramolecular polymeric networks was their glass transition temperature, which was below the room temperature, allowing for better movement of polymer chains after damage. These ECHs exhibited high electrical conductivity (40 S/cm), and higher elastic moduli with increasing concentrations of micro nickel particles. In addition, the engineered ECHs were able to electrically heal within 15 s after damage occurred (Fig. 7e), which could enable the engineering of electric skin sensors, as well as flexible biosensors.

In another study, Han et al. developed a mussel-inspired self-adhesive, self-healing, stretchable ECH as implantable and wearable bioelectronics [184]. These ECHs were fabricated in three steps. Briefly, dopamine was prepolymerized to form poly-dopamine (PDA). Next, partially reduced GO (pGO), where both GO and rGO were present, was achieved by exposure to PDA. Lastly, pGO and PDA were polymerized in the presence of acrylamide monomers, forming PDA/pGO/PAM composite ECHs (Fig. 7f). The resulting pGO and PDA chains interacted with PAM, forming hydrogen bonds and π - π stacking between catechol groups [184]. This ECH exhibited high conductivity, the ability to electronically and mechanically heal after damage, as well as high stretchability and toughness. Furthermore, the ECH possessed excellent attachment to native skin without the use of additional adhesives. Both *in vitro* studies using bone marrow stem cells and *in vivo* studies in a rabbit model demonstrated the high biocompatibility of the engineered ECHs.

Smart ECHs capable of self-assembling have been utilized for various biomedical applications. Further, self-healing ECHs have been engineered to autonomously adapt to dynamic environments and lengthen the service life of the implantable gels. These materials rely on weak noncovalent bonds to assemble and repair polymeric networks. However, due to the absence of stronger covalent bonds, these self-assembling and self-healing ECHs often lack the proper mechanical stiffness necessary for use as tissue regenerative therapies or drug delivery systems. In addition, the rational design of such complex structures is a challenge and prediction of ECH behaviors is often based on empirical observations. Regardless, the benefits of these smart ECHs make them attractive materials for many biomedical related applications where self-regulation and durability are required.

6. Biomedical applications of ECHs

ECHs constitute an emergent class of multifunctional and smart materials that possess technologically relevant properties for different biomedical applications. These properties stem mainly from their mechanical strength, high water uptake, tunable microarchitecture, and electrical conductivity, which are highly advantageous in the engineering of electrochemical biosensors, drug delivery systems, and excitable tissue constructs [11]. In addition, ECHs can be engineered to possess physicochemical characteristics that favor their implantation into the body and minimize the FBR, which could lead to fibrous encapsulation and implant failure [197]. These characteristics have allowed the engineering of biomedical devices that sense and modulate a wide array of electrically active physiological tissues such as nerve, as well as cardiac and skeletal muscle.

6.1. Biosensors and electrode coatings

The engineering of low-cost, versatile, and scalable biosensors for the accurate and rapid detection and quantification of different metabolites and biomarkers, is of great interest for the pharmaceutical and healthcare industries [198]. One of the most widely reported application for ECHs is the engineering of electrochemical biosensors for the detection and quantification of different clinically relevant molecules. Electrochemical biosensors are composed of biological sensing elements (e.g., enzymes) that interact with a given analyte, producing a signal that is transduced by the ECH and transformed into an electrical signal (Fig. 8a) [199]. Despite the remarkable potential of CPs as coatings for biomedical devices, they are often brittle when deposited as thin films, and are susceptible to the unspecific deposition of cells and proteins (i.e., biofouling) [197]. Their incorporation into ECHs effectively addresses these limitations since hydrogels can be molecularly engineered to prevent biofouling and possess increased mechanical resilience [108]. The mechanism by which ECHs transduce different physicochemical stimuli, as well as the different types of electroactive polymers used for this application have been reviewed elsewhere [14,200–203].

Monoclonal antibodies (mAbs) could also be bound to ECHs to engineer immunosensing interfaces for the detection of biomarkers associated with different pathologies or cancer (Fig. 8b). Wang et al. recently reported the engineering of an ECH composed of 1,3,5-benzenetricarboxylic acid and Fe^{3+} , and electrochemically deposited AuNPs [204]. This ECH was then deposited on a glassy carbon electrode (GCE) to develop a highly sensitive label-free electrochemical immunosensor for the detection of neuron-specific enolase (NSE), a biomarker associated with lung cancer. In another recent study, Shin et al. reported the engineering of a biosensor based on the incorporation of mAbs to a PEG/PEDOT ECH. This approach was used for the detection of bovine-interferon- γ , an inflammatory cytokine that can be used for diagnosing tuberculosis in cows [108]. In addition to enzymes and antibodies, biomolecules such as DNA (Fig. 8c) and even whole cells (Fig. 8d) can also be employed as biological recognition elements. For instance, Gao et al. reported the engineering of an electrochemical biosensor based on immobilized *Saccharomyces cerevisiae* cells on a chitosan hydrogel film with boron-doped nanocrystalline diamond particles, electrodeposited onto a GCE [205]. Their results demonstrated that this whole-cell biosensor could be used to assess the acute toxicity of waste water samples in a highly sensitive, integrated, and miniaturized fashion. Taken together, these studies demonstrate the potential of ECHs to engineer conductive and anti-fouling interfaces for biosensors, which allow electrochemical detection in complex media such as whole blood.

A different class of biosensors have been engineered through molecularly imprinted polymers (MIPs), which are artificial macromolecular networks with high affinity towards a specific template molecule (Fig. 8e) [206]. This approach was used by Bayer et al. for the detection of specific protein biomarkers, since charged amino acids cause changes in the electroconductivity of an MIP upon binding of the target protein [206]. Furthermore, ECHs have also been explored for the engineering of stimuli-responsive devices that can sense changes in temperature. For instance, Shi et al. recently reported the combination of conductive PANi and PPy CPs, with a thermally responsive poly(*N*-isopropylacrylamide) (PNI-PAM) hydrogel [207]. Using this approach, they engineered a smart material with high thermo-responsive sensitivity and electrical conductivity, as well as enhanced mechanical properties for stimuli-responsive electronic devices, and self-adaptive and flexible bioelectronics [207].

The use of ECHs as electrode coatings has also been explored for the engineering of tissue interfaces with enhanced biocompatibility

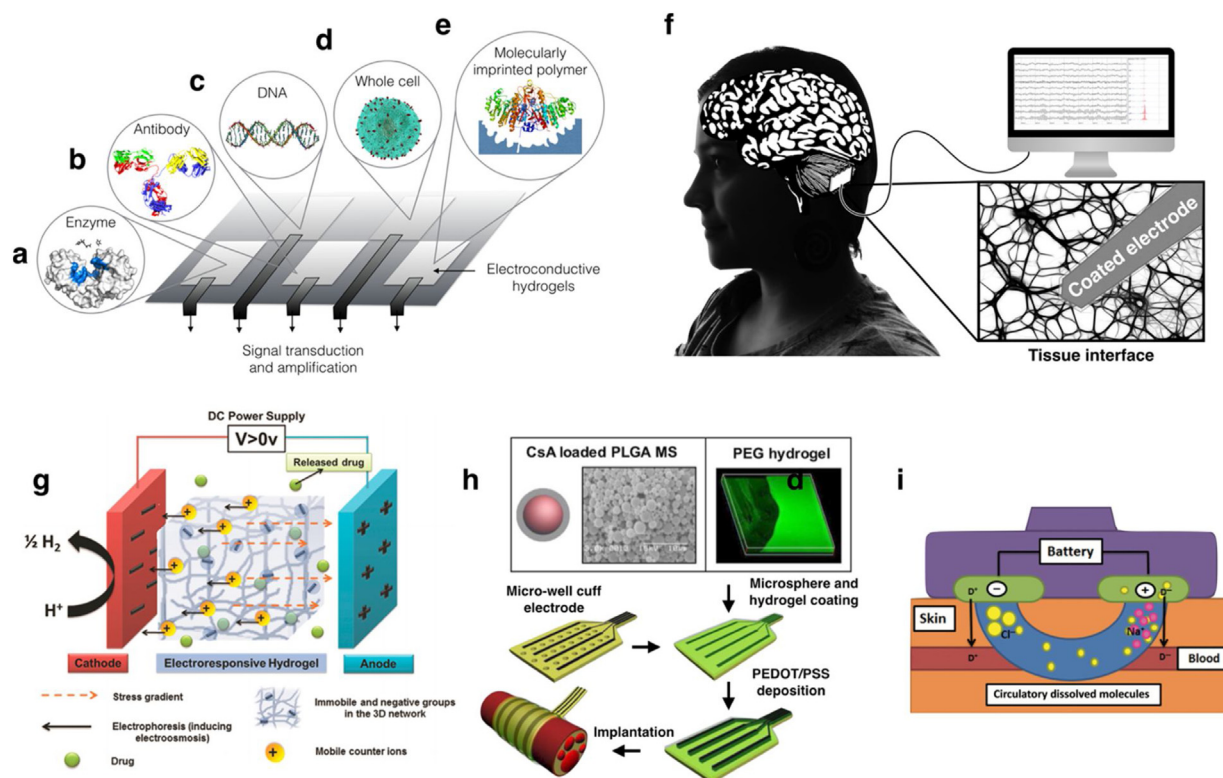


Fig. 8. ECHs for tissue engineering applications. The addition of electrically conductive materials (red circles) can be used to provide conductive properties to intrinsically insulating hydrogels (a). These ECHs could be used to deliver relevant biophysical stimuli to cardiomyocytes *in vitro*, such as mechanical and biological cues, or electrical stimulation/pacing. These maturation cues have been shown to trigger phenotypical changes that ultimately lead to fully mature and functional cardiomyocytes. ECHs have been used to develop conductive nerve conduits as an alternative to nerve autografts for nerve regeneration and repair (b). [220]. ECHs could provide physiological stimuli that mimic native nerve tissues and induce the differentiation of progenitor cell types to neural lineages (c). [224] ECHs deliver biomimetic topographical and electrical cues *in vitro* to form highly oriented cellular constructs with tissue-level functionality (d). [181] Scale bars = 100 μm (d upper image), 20 μm (d lower image) (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

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for bionic implants (Fig. 8f). The engineering of biomimetic coatings could theoretically reduce the effect of strain mismatch at the tissue interface, which would minimize the deposition of fibrous scar tissue and improve device function [14]. In this regard, Mario Cheong et al. described the covalent incorporation of bioactive molecules within a conducting poly(vinyl alcohol) (PVA)-heparin hydrogel with PEDOT [211]. To improve the biological interaction of the engineered ECH, sericin and gelatin were covalently attached to the matrix *via* methacrylate crosslinking. Their results showed that the composites could be used to deliver nerve growth factor (NGF) to target cells and could be further modified to incorporate bioactive motifs and deliver water-soluble drug molecules. A similar approach was recently reported by Goding et al., for the engineering of PEDOT/PVA-aurine composites as soft, hydrogel-based electrodes for low impedance neuroprosthetic devices [212]. In this study, they demonstrated the development of a novel ECH system that enabled the controlled incorporation of covalently attached conductive polymer dopants directly to the polymer backbone. Their results provided insight into the role of immobilized dopants in the fabrication of interpenetrating networks in ECHs, as well as in the morphological, electrochemical, and mechanical properties of the composites [212].

Conventional environmentally responsive hydrogels can respond to various cues such as temperature, pH, and ionic strength. More recently, stimulus-responsive ECHs have emerged as a new class of smart materials with high affinity towards spe-

cific physicochemical cues [200]. This high specificity is achieved through the incorporation of biomolecules with intrinsic molecular recognition ability (e.g., peptides, nucleic acids, mAbs, enzymes, etc.) into the polymer network. They can be engineered to possess different mechanisms of molecular recognition, and to trigger different responsive behaviors upon activation. In addition, the conductive matrixes provided by ECHs allowed the collection and rapid propagation of electric charge across large surface areas with high biocompatibility. Therefore, biosensors based on smart ECHs hold significant potential in different areas of clinical diagnostics and therapeutics, as implantable interfaces to sense and modulate different electroactive tissues.

6.2. Drug delivery systems

Recent advancements in nanotechnology and smart biomaterials have led to the engineering of systems that not only sense physiological stimuli, but could also respond to deliver a therapeutic output [201]. The delivery of therapeutic biomolecules to the implant site could, in turn, trigger specific tissue responses, or prevent the development of infection or inflammation [203]. Despite their intrinsic insulating nature, the porous architecture and high-water content of hydrogels provide the unique ability to load bioactive molecules or drugs into the polymer network. Moreover, ECHs respond to applied electrical fields either by swelling, shrinking, bending, or other structural changes due to minor differences

in the electric potential across the hydrogel [200]. Previous studies demonstrated that HA-, alginate-, and chitosan-based hydrogels possess intrinsic electroactive properties due to the presence of acidic or basic ionic groups throughout their polymer networks [41]. However, their low electrical responsiveness and poor mechanical performance limit their application for implantable smart systems for long-term drug delivery [12]. More recently, the engineering of composite ECHs with enhanced mechanical and electroconductive properties has allowed the development of electrically controlled drug release devices (EDRDs) (Fig. 8g) [208]. Moreover, the modulation of the electrical stimulus could be used to achieve different release profiles (e.g., burst release, slow-elution, etc.) due to combinatorial electrophoretic and electroosmotic effects [12]. The different mechanisms by which small biomolecules could be loaded into ECHs, as well as the various strategies used to trigger and modulate their release have been reviewed previously in the literature [200,201,208].

ECHs have also been used to deliver antimicrobial agents to prevent the development of infection at the implant site. For instance, Paradee et al., reported the engineering of a benzoic acid-loaded PEDOT-alginate composite for transdermal drug delivery [213]. Their results demonstrated that the release profile and the rate of diffusion of the drug were dependent on the crosslinking ratio, PEDOT polymer size, strength of the electric field, and the polarity of electrodes. ECHs could also be used to deliver anti-inflammatory drugs to improve the long-term biocompatibility of implanted biomedical devices. In this regard, Heo et al. recently reported the incorporation of cyclosporine A (CsA)-loaded PLGA microspheres into a PEDOT:PSS/PEG hydrogel (Fig. 8h) [209]. The formation of microwells and chemical treatment of the surface of the electrode led to increased surface area for loading of high doses of the drug, as well as enhanced adhesion of the hydrogel to the electrode. Moreover, *in vivo* experiments showed that delivery of CsA resulted in a significant decrease in fibrous tissue deposition and increased axonal density [209]. In another study by Pairatwachapun et al., the diffusion coefficient of acetylsalicylic acid from carrageenan hydrogels was shown to be significantly enhanced by incorporating the CP PTh, followed by an applied electric field [214]. Another potential application for ECHs in EDRDs, is the development of platforms for transdermal patient-controlled analgesia. In recent years, the engineering of patch-like devices that possess both the microelectronic-processing mechanism along with the drug of interest in a small wearable device has been actively pursued (Fig. 8i) [210,215]. The engineering of iontophoretic patches for transdermal delivery would eliminate the need for conventional intravenous or epidural administration, resulting in lower costs and improved patient acceptability [210]. However, the passive diffusion of the drug into the aqueous physiological environment, as well as the precise modulation of the release profile and pharmacokinetics of the drug still constitute significant technical challenges [12]. Recent advancements in material science and other emerging areas such as gene editing technologies, could be integrated into EDRDs to engineer bioelectronics with enhanced therapeutic function [216]. These hybrid systems could one day monitor multiple parameters continuously and respond with the precise release of therapeutic molecules in real time.

6.3. Tissue engineering

Polymeric hydrogels have been extensively used as scaffolds to mimic biological functionality and induce the proliferation, differentiation, and migration of cells, both *in vitro* and *in vivo* (Fig. 9a). Cells in physiological environments are exposed to various types of physicochemical cues that modulate the development and regeneration of tissues, including endogenous electric fields from excitable cell types. Therefore, ECHs have been increasingly explored for the

engineering of functional tissue constructs, to control the behavior and promote the regeneration of excitable tissues [12]. To this end, the use of ECHs for the engineering of scaffolds for neural cell encapsulation, nerve conduits, and electrode coatings for neural tissue interfaces has been widely reported (Fig. 9b). Previous studies have shown that neuroblastic PC-12 cells under electrical stimulation *in vitro* exhibit increased proliferation and differentiation [217–220], as well as a higher number of neurites with a greater overall length [28]. This behavior has been mainly associated to enhanced fibronectin adsorption onto the ECHs, coupled with the direct effect of the electrical field on the integral membrane proteins of these cells [221,222]. In a different study by Yow et al., hMSCs growing on PPy-collagen hydrogels exhibited neuronal-like morphology and upregulation of noggin, MAP2, neurofilament, β tubulin III, and nestin neural markers [223]. Using a similar approach, Yang et al. recently demonstrated that PPy-alginate hydrogels promoted the adhesion and growth of hMSCs, as well as overexpression of Tuj1 and MAP2 neural differentiation markers (Fig. 9c) [224]. In addition, the engineered PPy-alginate hydrogels were shown to possess high biocompatibility *in vivo*, as demonstrated by subcutaneous implantation experiments. These studies demonstrate that ECHs are remarkably advantageous to study the effects of electrical fields on stem cells and/or neural cells *in vitro*, and to engineer heterocellular neural tissue constructs and interfaces for *in vivo* implantation.

The complex organization and microarchitecture of the myocardium, along with the electrical and mechanical coupling between CMs, are critical for the maintenance of the synchronous contractility of the heart [225,226]. In recent years, the engineering of cardiac tissue constructs using hydrogel-based biomaterials with biomimetic physicochemical cues have demonstrated great promise for cardiac tissue regeneration and repair [6,227]. For instance, Dong et al. recently described the engineering of an injectable, self-healing ECHs based on chitosan-graft-aniline tetramer (CS-AT) and dibenzaldehyde-terminated poly(ethylene glycol) (PEG-DA) [228]. Their results demonstrated that the engineered ECH could be used to deliver C2C12 and H9c2 myoblasts *in vivo*, and that they possessed high biocompatibility and biodegradability. In another study by Baei et al., thermosensitive chitosan-AuNPs ECHs were shown to support the proliferation, migration, and differentiation of hMSCs, as shown by the expression of the α -myosin heavy chain (α -MHC) and Nkx-2.5 cardiac markers [34]. Furthermore, in addition to electrical stimulation, the delivery of mechanical and topographical cues could also be incorporated into multifunctional ECHs. In this regard, Gelmi et al. recently reported the use of a PLGA-PPy hydrogel as an electromechanically active scaffold to promote the engraftment, proliferation, and differentiation of induced pluripotent stem cells (iPSCs) into CMs [229]. The mechanical actuation of the scaffold enabled individual microfiber actuation to provide encompassing, coherent physiological strain to individual cells, which mimicked the native mechanical flow and force in the heart. In another study by Navaei et al., 50 μ m microgrooves were incorporated into gelatin GelMA hydrogels with electrically conductive GNRs (Fig. 9d) [181]. Fluorescent images revealed uniform, dense, and highly aligned cellular organization, as well as enhanced cytoskeletal alignment and cellular connectivity.

In addition to cardiac and neural cell types, ECHs have also been shown to modulate the proliferation and differentiation of pre-osteoblasts MC3T3-E1 for bone tissue engineering [230], as well as human primary skin fibroblasts for wound healing [231,232]. However, conventional methods for engineering ECHs through the incorporation of conductive nanomaterials and polymers are often associated with poor solubility, processability, biodegradability, and biocompatibility [233]. To address these limitations, our group recently reported a new method to engineer electro-

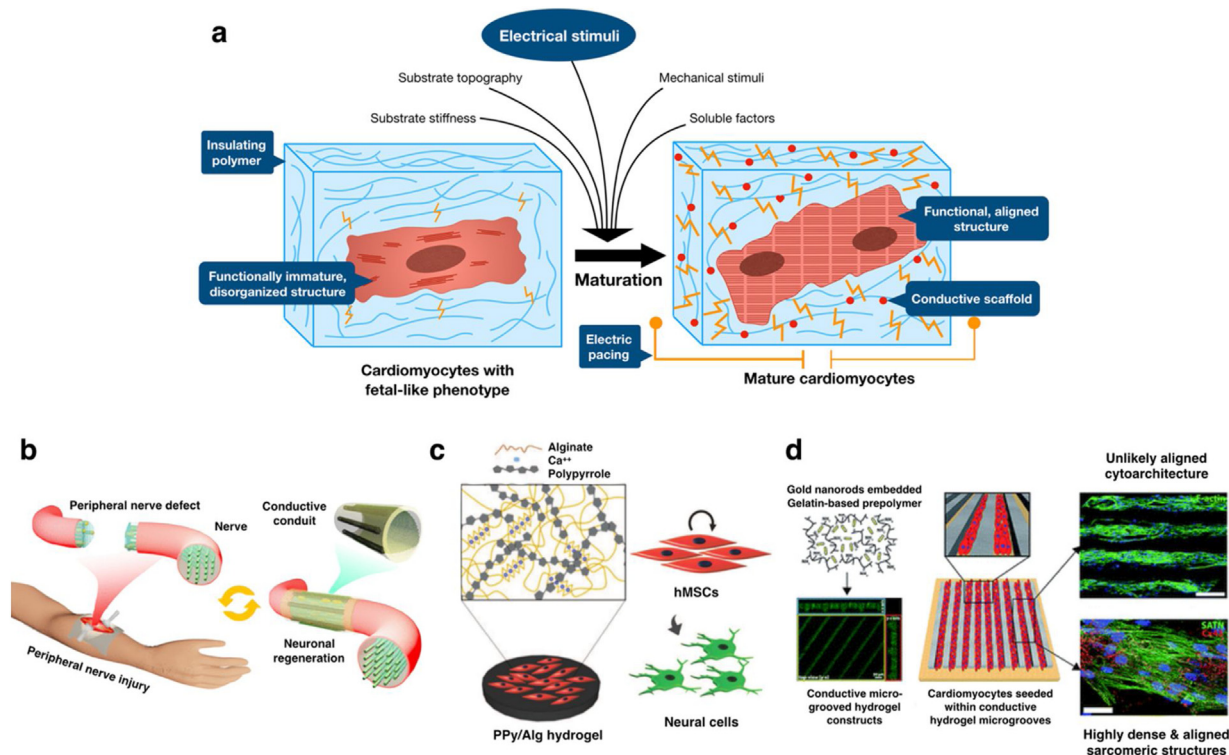


Fig. 9. ECHs for tissue engineering applications. The addition of electrically conductive materials (red circles) can be used to provide conductive properties to intrinsically insulating hydrogels (a). These ECHs could be used to deliver relevant biophysical stimuli to cardiomyocytes *in vitro*, such as mechanical and biological cues, or electrical stimulation/pacing. These maturation cues have been shown to trigger phenotypical changes that ultimately lead to fully mature and functional cardiomyocytes. ECHs have been used to develop conductive nerve conduits as an alternative to nerve autografts for nerve regeneration and repair (b). [220], Copyright 2017. Reproduced with permission from American Chemical Society. ECHs could provide physiological stimuli that mimic native nerve tissues and induce the differentiation of progenitor cell types to neural lineages (c). [224], Copyright 2016. Reproduced with permission from John Wiley and Sons. ECHs deliver biomimetic topographical and electrical cues *in vitro* to form highly oriented cellular constructs with tissue-level functionality (d). [181], Copyright, 2017. Reproduced with permission from the Royal Society of Chemistry. Scale bars = 100 μm (d upper image), 20 μm (d lower image) (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

conductive materials from inherently non-conductive polymers, through the conjugation of a choline-based Bio-IL [20]. Our results demonstrated that GelMA/Bio-IL ECHs could be used to modulate the proliferation and contractile function of primary rat CMs *in vitro*. Furthermore, as current understanding of biological processes increases, and new multifunctional smart biomaterials are developed, novel applications of ECHs in tissue engineering will be explored. For instance, ECHs could be used to engineer bioactuators that transduce electrical stimuli into mechanical force, which in turn could be used as biomimetic muscle fibers [203]. ECHs could also be used to develop transparent and deformable touch sensors for emerging applications, such as bionic skin, as well as flexible/wearable biomedical devices [234].

7. Concluding remarks and future perspectives

Herein, we have discussed several strategies used to impart electrical conductivity into hydrogels, such as the incorporation of metal NPs, graphene, or CNTs, as well as several innately CPs such as PANi, PPy, or PEDOT. The combination of these synthesis methods and biomaterials has enabled the development of ECHs that demonstrate remarkable potential for biomedical applications, including tissue engineering, drug delivery systems, flexible electronics, and biosensors. However, some of the major obstacles for these biomaterials lies in addressing issues related to their processability and cytotoxicity. Further, research has primarily focused on the *in vitro* biocompatibility of these materials. Future research must investigate the biocompatibility of these materials *in vivo* using animal studies before clinical trials. In addition, merging ECHs with advanced microfabrication techniques have allowed the accu-

rate reproduction of the structural properties of the native ECM, which in turn enhances the functionality of ECHs in physiological environments. Future research on ECHs should focus on addressing the limitations of current fabrication techniques, such as producing self-healing ECHs with more robust mechanical properties for tissue engineering. In addition, 3D bioprinting technologies will continue to improve the resolution, speed, and compatibility with other biomaterials. It will be essential to develop ECH-based bioinks that may be 3D printed into constructs with suitable mechanical and electrical properties, and integrity for biomedical applications. In addition to 3D printing, other microfabrication techniques have been used to develop ECHs with precise structure and function and will undoubtedly be instrumental in the development of future translational biomedical technologies.

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