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Review

Endogenous Electric Signaling as a Blueprint for Conductive Materials in Tissue Engineering

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Abstract

Bioelectricity plays an important role in cell behavior and tissue modulation, but is understudied in tissue engineering research. Endogenous electrical signaling arises from the transmembrane potential inherent to all cells and contributes to many cell behaviors, including migration, adhesion, proliferation, and differentiation. Electrical signals are also involved in tissue development and repair. Synthetic and natural conductive materials are under investigation for leveraging endogenous electrical signaling cues in tissue engineering applications due to their ability to direct cell differentiation, aid in maturing electroactive cell types, and promote tissue functionality. In this review, we provide a brief overview of bioelectricity and its impact on cell behavior, report recent literature using conductive materials for tissue engineering, and discuss opportunities within the field to improve experimental design when using conductive substrates.

Keywords: bioelectricity, conductive polymers, tissue engineering, endogenous electric field, electrical stimulation

Introduction

Bioelectricity is a term that describes voltage-mediated communication inherent to all cells and tissues. Bioelectricity plays a major role in cell behavior during development and tissue homeostasis, but is understudied within tissue engineering. The development and application of biomaterials for tissue engineering are broadly focused on providing mechanical and chemical cues in their scaffolds to influence cell behavior (e.g., survival, migration, differentiation, etc.), yet few seek to incorporate electrical cues.[1]

Bibliometric analysis using PubMed illustrated that, from 2000 to 2019, there were 10 times more publications in the field of tissue engineering and regenerative medicine related to the influence of mechanical (greater than 2 million publications) or chemical cues (more than 3 million publications) than those related to electrical cues (200,000 publications). By acknowledging and catering to the electrical aspects of tissues and organs, the potential to improve communication between engineered and endogenous tissue will be increased, which could improve clinical translation.

Nerve cells and cardiomyocytes consistently exhibit improved growth and differentiation when seeded on conductive substrates, even in the absence of electrical stimulation (ES).[2–14] While possible mechanisms for this phenomenon are explained in later sections of this review, it grossly appears that these materials support the function of electroactive cell types by capturing and disseminating electrical signals.

Synthetic polymers, including polypyrrole (PPy), polyaniline (PANI), and poly(3,4-ethylenedioxythiophene) (PEDOT), or carbon-based materials, such as carbon nanotubes (CNTs) and graphene oxide (GO), are frequently used to increase conductivity of biomaterials.[15–20] Although these materials provide at least physiologically relevant, and in some cases, metallic like,[21] conductivity to a system, they also face a number of disadvantages. Most conductive polymers are hydrophobic, which is beneficial for protein adsorption, but leads to poor cell adhesion. Some materials (e.g., PANI) trigger an immune response. Also, most synthetic conductive materials are neither degradable nor resorbable, and the effects of their permanent presence in the body is damaging or unknown.[3,22–24]

The mechanism of conductivity of these synthetic materials comes from electron transfer, whereas in the body, conductivity arises from the movement of ions. While this has not impeded encouraging results, the gap in mechanistic understanding surrounding these materials is a roadblock for optimal design. Given these drawbacks, there is an important need for approaches that use natural biomaterials to interact with endogenous tissues and confer physiological conductive signals.

This review summarizes recent work using synthetic and naturally derived conductive materials to aid in tissue regeneration. It contextualizes the use of conductive materials in tissue engineering and postulates the future direction of the field.

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Origin and Endogenous Effects of Bioelectricity

Endogenous electric fields

Bioelectricity was first described in the late 1700s by Luigi Galvani, while experimenting with frogs. Bioelectricity remains a topic of great importance to biologists, as it is a key player in regulating many cell and tissue behaviors.[25] On the cellular level, bioelectricity is derived from differences in the endogenous membrane potential of each cell. The transmembrane potential is generated by the separation of charges by transmembrane pumps, transporters, and ion channels and results in a resting potential between −90 and −50 mV for most cells.[26] Membrane potentials give rise to endogenous electric fields, which then guide cell behavior and may even override chemical and topographical cues.[26–28]

Charge gradients (i.e., electric fields) are also created when ions and other charged molecules pass from cell to cell through gap junctions.[26] On the cellular level, endogenous electric fields are involved in orientation, migration, adhesion, proliferation, and differentiation.[29,30] On the tissue and organismal level, electric fields play a major role in development, wound healing, and healthy tissue function (Fig. 1).[28] Many literature reviews exist on bioelectricity and provide further detail on its role in development and homeostasis.[1,26,27,31] Given the mounting evidence that electrical signals can influence cell behavior, there is great interest in developing techniques to electrically stimulate injuries and repair tissue to improve healing.

Electrical stimulation

ES refers to an externally generated electric field applied by electrodes to influence cell or tissue response. In tissue injuries, application of an external electric field has enhanced anatomical and behavioral recovery.[32,33] ES can alter cell behaviors such as migration, adhesion, proliferation, and differentiation. However, the specific molecular mechanisms of ES on cell behavior remain elusive, preventing optimal design of materials for clinical use.[33] While most ES protocols set key parameters, including field strength (0.00048–6000 mV/mm), current density (0.015–5 A/m^2), and frequency (usually under 100 Hz),[34] within previously reported ranges, the variation in setup between studies limits the ability to directly compare results and draw conclusions about the effects of ES as a whole.[33]

Bioelectric signaling at the cellular level

Numerous reports describe the role of endogenous or applied electric fields at physiological levels on cellular migration, adhesion, proliferation, and differentiation during development and wound healing.[27,35,36] Generally, applied electric fields affect cell surface receptors, enzyme activity, charge distribution throughout the cell membrane, and membrane protein conformation.[28,37,38] It is believed to trigger similar responses cells would have to other chemical or physical stressors (e.g., fluid shear stress[39]) that also promote cell survival.[40–42] Upstream signal transduction pathways and

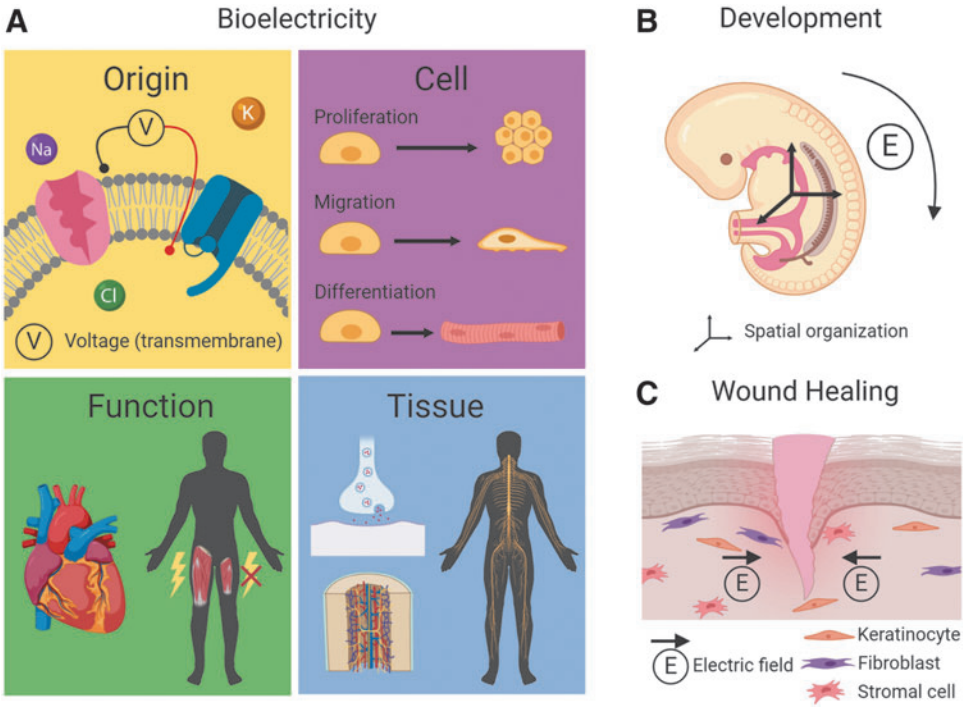

FIG. 1. Summary of the effects of bioelectricity on the cell, tissue, and organismal level, as well as its role in development and wound healing. **(A)** Bioelectricity originates from the separation of charges across the cell membrane, generating a voltage, and can influence cell behaviors, including proliferation, migration, and differentiation. Tissue development and homeostasis are also dependent on bioelectric signaling, even if those tissues are outside of the nervous system. Many tissues (e.g., cardiovascular and musculoskeletal) are highly dependent on electrical signals and are disrupted in their absence. **(B)** During development, electric fields are critical for proper morphogenesis and spatial organization of organ systems, as well as directing stem cell differentiation. **(C)** Endogenous electric fields arise from wounds and recruit cells to accelerate healing.

calcium ion flux mediate many of the cell behaviors listed, but electric fields also affect cells by stimulating cytoskeletal reorganization, surface receptor redistribution, ATP synthesis, heat shock protein activation, and reactive oxygen species and lipid raft formation.[33] ES elevates the activity of mitogen-activated protein kinase, which initiates multiple signaling pathways, each associated with different cell behaviors related to migration, adhesion, proliferation, and differentiation.[33,37,43]

ES can also influence cell migration by causing lipids to accumulate into rafts.[44] Lipid rafts are believed to be the principal sensors of electric field within cells, and their formation can activate integrins and other membrane proteins involved in directional cell migration.[45] During development, endogenous electric fields are key players in initial cell polarization and provide cues to guide long-distance migration of neurons and neural stem cells throughout the central and peripheral nervous systems.[27,46] When ES is applied, most cell types preferentially travel toward the cathode and change directions when the field direction is switched.[36] Some cell types exhibit accelerations in migration speed as a function of field strength,[35] whereas others do not.[36] In the context of wound healing, endogenous electric fields recruit stem cells to wound sites and direct fibroblasts, keratinocytes, and other cell types within the wound to promote healing.[29,47–49] When combined with topographical cues, ES caused a synergistic directional migration of corneal epithelial cells mediated by upregulated MMP-3 activity.[50,51]

Cell adhesion is a foundational event that is influenced by electric signals and must be considered when developing new materials for tissue engineering applications. When a cell is triggered by an electric field, cells arrange their cytoskeletal elements to shape to the trigger.[33,52] For example, $\alpha 2\beta 1$ integrins of ligament fibroblasts polarized and clustered after the cells were electrically stimulated. Integrin clustering led to intracellular RhoA polarization, which is directly involved in cell membrane protrusion and migration.[53] Conductive materials can also affect cell adhesion, even in the absence of an electric field.[54] One possible explanation of this observation is that increased electrostatic interactions characteristic of conductive substrates cause cells to strongly adhere without forming focal adhesion complexes (FACs). Because this adhesion is not derived from FACs, growth arrest occurs, which ultimately leads to decreased cell proliferation (Fig. 2A).[55] Increases in seal resistance that arise between a cell and a conductive substrate may also contribute to increased cell adhesion (Fig. 2B).[56] Seal resistance originates from the collection of ionic solution in the cleft between the cell membrane and the surface and can be physically considered as the adhesion strength between the cell and the surface. ES may increase extracellular matrix protein adsorption to substrates, providing additional sites for integrin-ligand interactions (Fig. 2C).[57] The variation within these explanations and their conjectural nature highlight the need for more mechanistic studies of how cells and conductive substrates interact.

Proliferating cells are more depolarized than nonproliferating cells, indicating that electroactivity varies throughout the cell cycle. Compared to that of quiescent cells, proliferating cells have a membrane potential between −30 and −10 mV.[26] Potassium and chloride channels are key regulators of ion flow (i.e., endogenous electric fields) that can affect proliferation. This relationship could be used to promote cell growth in tissue engineering applications or leveraged to inhibit cell growth (e.g., developing chemotherapeutics).[27,55,58] As with other cell behaviors, all cell types may not behave similarly, given the same inputs. For example, cardiomyocytes grown on a conductive surface without ES showed increased proliferation, yet fibroblasts in the same system exhibited no proliferative response.[59]

Electrical signaling can initiate differentiation *in vivo* and influence cell fate during development and tissue homeostasis.[60]

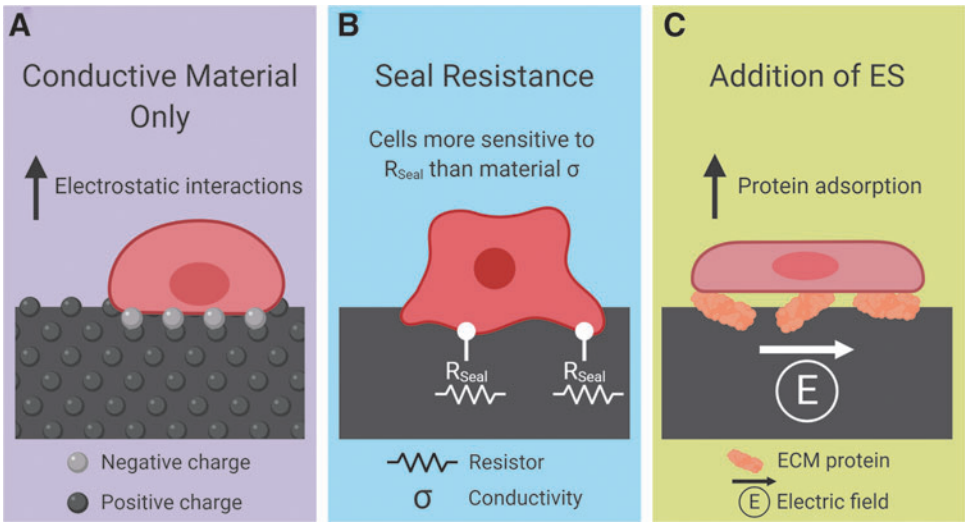

FIG. 2. Cell interactions with conductive materials with and without electrical stimulation. **(A)** Conductive materials present more electrostatic charge, which increases electrostatic interaction with cells. **(B)** Conductive materials promote cellular attachment through increased R_{seal}. R_{seal} originates from the collection of ionic solution in the cleft between the cell and the surface and can be considered as the adhesion strength between the cell and the substrate. **(C)** Protein adsorption is enhanced by applying an electric current to a conductive substrate, facilitating cell adhesion. ECM, extracellular matrix; ES, electrical stimulation; R_{seal}, seal resistance.

Endogenous currents also arise from wounds and signal to begin the differentiation process of depolarized, undifferentiated cells toward a reparative phenotype.[27,32] Altering the transmembrane potential of a variety of stem cells with ES can influence their differentiation fate and has been demonstrated in neural, hepatic, and mesenchymal stromal cells (MSCs), as well as in cancer.[27] Applied electric fields can increase cellular uptake of calcium ions and generate reactive oxygen species,[61] both of which are linked to stem cell differentiation toward the neurogenic and osteogenic lineage.[61–64] For instance, ES caused bone marrow-derived MSCs to express neural markers, including Nestin and MAP2.[65,66] Human neural progenitor cells undergoing ES on a conductive substrate showed increased *MMP-9* gene expression and VEGF-A secretion, indicating increased capacity for angiogenesis and survival.[67] ES also induced chondrogenesis of human MSCs without exogenous growth factors[68] and enhanced calcium deposition by adipose-derived human MSCs.[69]

Bioelectric signaling at the tissue/organism level

In the developing embryo, endogenous electric fields play an important role in orchestrating organ shape and in anterior/posterior and left/right patterning, which is important for the development of asymmetrically spaced organs such as the heart, organs in the digestive tract, and liver. By depolarizing or hyperpolarizing the membrane potential, electric fields can induce the expression of signaling factors that influence morphological patterns (e.g., folding, proliferation, and migration of cell groups).[70] Transfer of bioelectric information between cells in both the embryo and adult organism may occur by gap junctions, tunneling nanotubes, nonsynaptic neuronal (i.e., ephaptic) field effects, transepithelial potentials, and transfer of ion channels through exosomes.[26,28] Electric field patterns also precede and even pinpoint major morphological events in development, such as limb bud development[26,28]

Endogenous bioelectric signaling plays a key role in many behaviors and functions at both the cell and tissue level. When ES is combined with other inputs, whether mechanical or chemical, synergistic effects are generally observed. However, given conflicting reports about ES,[71] the variation in application parameters, and the overall mechanistic knowledge gaps, more studies are necessary before ES becomes common clinical practice.[33] There is also great opportunity to use biomaterials as a means to magnify, leverage, or mimic the influence of bioelectric signaling.

Conductive Materials for Tissue Engineering

Synthetic materials with enhanced electrical properties have great potential for numerous biological applications. Comprehensive reviews[72–76] and articles detailing the use of conductive polymers,[73,77–80] nanoparticles,[81–83] and carbon-based[84] and metal-based structures[81,85,86] for use in nerve[77,84,87–89] and cardiac[85,90] tissue engineering have become increasingly prevalent over the last decade. A variety of additives have been used to tune the conductivity of biomaterials (Fig. 3), and those used in the most recent reports are summarized in Table 1. Conductive additives incorporated into hydrogels (Table 2) result in scaffolds that better approximate endogenous tissue (Table 3). The following sections summarize and provide critical analysis of the most up-to-date research using these materials.

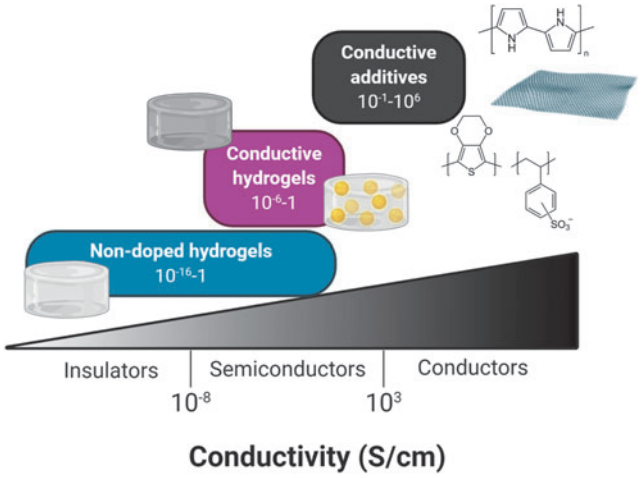

FIG. 3. Electrical properties of biomaterials with conductive additives used in tissue engineering. Nondoped hydrogels have reduced conductivity, ranging from 10^{-16} S·cm^{-1}, observed in polyacrylamide, to 1 S cm^{-1}, observed in alginate. Other unmodified hydrogels within this electroconductive range include collagen type I, PEGDA, and chitosan. Conductive additives, including polymers like PPy, PANI, and PEDOT, CNTs, and AuNPs, have much higher conductivity ($\sim 10^{-1}$–10^{6} S·cm^{-1}) and are used to enhance the conductivity of hydrogels. AuNPs, gold nanoparticles; CNTs, carbon nanotubes; PANI, polyaniline; PEDOT, poly(3,4-ethylenedioxythiophene); PPy, polypyrrole.

Synthetic conductive polymers

Electrically conductive synthetic polymers were first reported in 1977 by Heeger, MacDiarmid, and Shirakawa using polyacetylene. Their fabrication of a ''conductive plastic'' with metallic-like electroactivity was a major breakthrough in the field and resulted in the 2000 Nobel Prize in Chemistry.[91,92] Since then, over 25 types of conductive polymers have been developed, the most common of which are illustrated in Figure 4A.[93] The mode of conductivity for all of these polymers arises from the freedom with which electrons move within and between their polymer chains.[94] Conductive polymers contain moieties that consist of alternating single and double bonds (i.e., conjugated double bonds). The double

Table 1. Electrical Properties of Synthetic Conductive Materials

Material	*Conductivity* ($S \cdot cm^{-1}$)	*Reference*
PPy	$0.02–7.5 \times 10^{3}$	21,94
PANI	$0.11–10^{5}$	22,158
PEDOT	$0.4^{94}–500^{22}$	22,94
Pristine PEDOT:PSS	$0.2–1^{159}$	159 and Sigma Aldrich
Pure PEDOT:PSS hydrogel	20–40	111
CNTs	$10^{4}–10^{5}$	22,160
Single-layer graphene	$2000–10^{6}$	17,160
MOGS	675 ± 22	17

CNTs, carbon nanotubes; MOGS, mildly oxidized graphene sheets; PANI, polyaniline; PEDOT, poly(3,4-ethylenedioxythiophene); PPy, polypyrrole; PSS, poly(styrene sulfonate).

Table 2. Electrical Properties of Synthetic Conductive Composites

Composite	*Conductivity ($S \cdot cm^{-1}$)*	*Reference*
PPy in HA	$\sim 1.2–7.3 \times 10^{-3}$	10
PPy in alginate	$3.3 \times 10^{-5}–1.1 \times 10^{-4}$	161
PPy in PCL	$\sim 10^{-5}–10^{-1}$	69
PANI in PCL	$\sim 2 \times 10^{-4}$	162
Poly(glycerol sebacate)-co-aniline	$1.4 \times 10^{-6}–8.5 \times 10^{-5}$	114
PEDOT-HA nanoparticles in chitosan	$\sim 10^{-4}–10^{-2}$	109
PEDOT:PSS in PEG diglycidyl ether	5.22×10^{2}	110
CNTs in PCL + silk fibroin	$6.5–8.1 \times 10^{-7}$	163
MWCNT in PEG	$\sim 10^{-3}–10^{-2}$	116
CNTs + rGO sheets in PEG	5.75×10^{-5}	15
Graphene in collagen	6.5×10^{-3}	18
GO in polydopamine	8×10^{-2}	164
AuNPs in chitosan	1.3×10^{-3}	19
Collagen doped with iron oxide nanoparticles	3.7×10^{-5}	81

GO, graphene oxide; HA, hyaluronic acid; MWCNT, multiwall carbon nanotube; PEG, poly(ethylene glycol); rGO, reduced GO.

bonds within the polymer structure are made up of a σ bond and a π bond. Electrons are not as strongly bound to π bonds, which allows them to delocalize. To activate electron movement, the polymer chain must be disrupted by the introduction of a dopant. Oxidation, or *p*-doping, removes electrons from the system and reduction, or *n*-doping, inserts electrons into the system.[92] Charge delocalization can also occur when polymers contain aromatic rings spaced such that their π-orbitals overlap (i.e., π-π stacking). This phenomenon can result in organic materials having metallic-like conductivity.[95,96] Conjugated double-bond structures are frequently seen in synthetic materials used for tissue engineering, but can also appear in natural conductive materials. Understanding the origin of conductivity can promote purposeful design of materials and aid in understanding material synthesis.

Polypyrrole. PPy is the most studied conductive polymer for biomedical applications following its initial description by Wong et al., who tested the stability of conductive polymers in cell culture conditions.[97] When oxidized, PPy exhibits conductivity on the order of 10^3 $S \cdot cm^{-1}$, where S is siemens. Its environmental stability, capacity to support adhesion and growth of many cell types, and ease of synthesis make it an attractive additive for biomedical applications.[94]

Because PPy is mechanically rigid and brittle after synthesis, it is frequently combined with other polymers to achieve more desirable mechanical properties for tissue engineering applications.[76,98] Peripheral nerve conduits composed of electrospun poly(L-lactic acid-co-ε-caprolactone) coated with PPy were created to facilitate ES. When tested *in vivo* as a nerve conduit, the stimulated conductive scaffolds performed similarly to autograft.[54] These findings not only imply that the presence of a conductive material can influence cell response but also raise questions about how conductivity and other properties (e.g., topography) influence each other.[11,51]

PPy has also been used for musculoskeletal tissue engineering. For example, adipose-derived MSCs grown on PPy-PCL composites achieved a 100% increase in calcium deposition

Table 3. Electrical Properties of Native Tissues, Unmodified Biomaterials, and Natural Conductive Materials

Material	*Conductivity ($S \cdot cm^{-1}$)*	*Reference*
Myocardium	$\sim 10^{-5}–10^{-3}$	3,144
Nerve/spinal cord	$\sim 10^{-2}–10^{-1}$	21
Skeletal muscle (feline, porcine)	$\sim 2–8 \times 10^{-3}$	144,165
Bone	$\sim 9.1 \times 10^{-5}$	166
Cartilage (porcine)	$\sim 10^{-3}$	167
Skin	$\sim 10^{-6}–10^{-3}$	165,168
Collagen type I	2.98×10^{-10}	81
	$\sim 2.5 \times 10^{-3}$	169
	3×10^{-3}	170
Alginate	$\sim 0.1–2$	171
	8.2×10^{-6}	161
PEGDA	7.6×10^{-11}	103
Polyacrylamide	$\sim 10^{-16}$	17
Chitosan	1.91×10^{-10}	172
Wild-type *Geobacter sulfurreducens* Pil-A monomers	$5 \times 10^{-3}–188 \times 10^{-3}$	95,124
Modified *G. sulfurreducens* Pil-A monomers	$\sim 10^{-1}–10^{2}$	129
Hemin-doped serum albumin	$\sim 10^{-3}$	138
GelMA-Bio-IL	$\sim 10^{-7}–10^{-5}$	3

Bio-IL, bioionic liquids; GelMA, methacrylated gelatin.

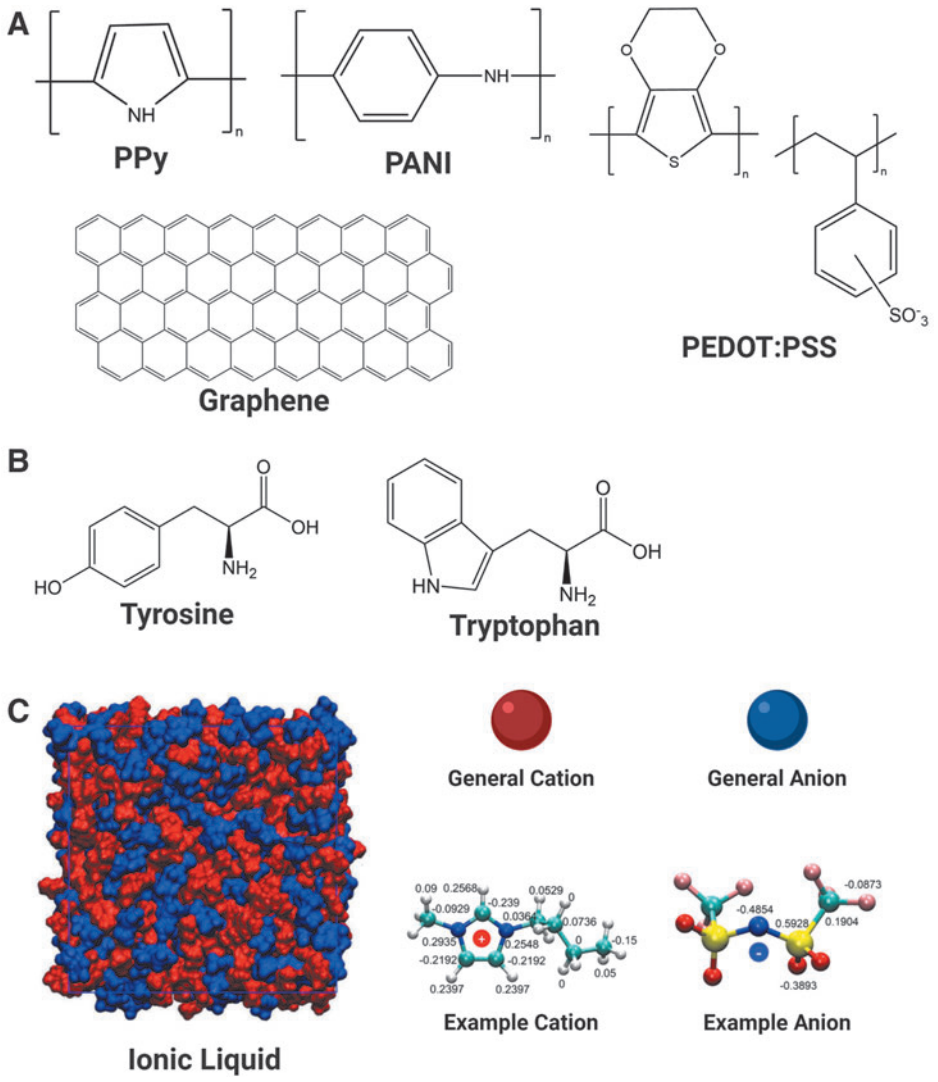

FIG. 4. Chemical structure of conductive materials used for tissue engineering applications. **(A)** Synthetic polymers and carbon-based materials have conjugated (alternating single and double bond) structures that facilitate electron movement within and between polymer chains. **(B)** Conjugated structures are present in aromatic amino acids and can give rise to metallic-like conductivity in naturally derived proteins and peptides. Chemical structures recreated with ChemDraw 19.0. **(C)** Other natural conductive materials have charge-dense regions throughout their structure, giving rise to ionic conductivity. A molecular dynamics simulation applied to a typical example of bioionic liquid is reproduced from Feng et al., in which cations and anions are represented in red and blue, respectively. There are many formulations of ionic liquids, but the molecular structures of the cations (1-butyl-3-methylimidazolium) and anions (bis(trifluorome thanesulfonyl)imide) comprising the ionic liquid used by Feng et al.[140] are provided as an example.

when electrically stimulated. The investigators interrogated the role of voltage-gated ion channels to better understand the mechanistic downstream effects of ES for bone tissue engineering.[69] Through inhibitory experiments, they determined that voltage-gated Ca^{2+} channels play a more significant role in regulating adipose-derived MSC functions than other ion channels. In addition, de Castro et al. observed increased alkaline phosphatase (ALP) activity in osteoblasts grown on electrospun scaffolds containing PPy and poly(butylene adipate-*co*-terephthalate) (PBAT) after 21 days compared to PBAT controls, indicating that substrate conductivity can enhance osteogenic potential.[99]

Polyaniline. PANI is another commonly used conjugated polymer, owing to its low cost of production, environmental stability, and greater range of conductive properties over PPy.[22,76,100] Despite these advantages, PANI is used less frequently, given multiple conflicting reports of it stimulating an elevated immune response or chronic inflammation.[76,100–102] However, when combined with natural biomaterials such as chitosan, some groups have shown promising scaffolds for cardiac,[90] nerve,[103] and musculoskeletal tissue engineering. Murine-derived C2C12 myoblasts exhibited increased proliferation and myogenic differentiation markers when grown on silk fibroin combined with a PANI-based material (poly (aniline-*co*-*N*-(4-sulfophenyl)aniline).[104] In a different study, C2C12s were cultured on aligned, PANI-coated PCL fibers and demonstrated greater capacity toward myotube formation than controls.[105] Endothelial cells better adhered to and proliferated on PANI-coated PCL fibers as well, and proliferation was further improved by ES.[106] Chen et al. combined PANI and PCL to make conductive nanofibers, and the addition of PANI caused MSCs to undergo osteogenic differentiation and deposit higher levels of calcium compared to PCL-only controls, making it a relevant additive for bone tissue engineering. It is important to note that these results were achieved with the material containing an intermediate amount of PANI, which should redirect strategies that are focused on continuously increasing the conductivity of materials they intend to use for similar applications.[107]

Poly(3,4-ethylenedioxythiophene). PEDOT is frequently used as a conductive additive for making electroactive materials, whether alone or in combination with poly(styrene sulfonate) (PSS). PEDOT alone has distinct advantages over other conductive polymers, including higher conductivity and better chemical stability.[12] Wang et al. incorporated hyaluronic acid

(HA)-doped PEDOT nanoparticles into PLLA films, which improved PC12 cell adhesion, spreading, and survival compared to PLLA control films. Electrically stimulated PC12 cells grown on the conductive films exhibited more advanced neurite extension compared to unstimulated controls.[108] When incorporated into chitosan/gelatin gels, these HA-doped PEDOT nanoparticles promoted nerve regeneration.[12,13,109]

Despite the positive effects of PEDOT alone, PEDOT:PSS has risen to the forefront in tissue engineering studies. By doping hydrophobic PEDOT with hydrophilic PSS, the conductive agent is easier to disperse and incorporate into hydrogels. Its structure also uniquely provides both electron conduction through PEDOT and ionic conduction through PSS, making it a more suitable material for bridging biological and synthetic systems. PEDOT:PSS is associated with low cytotoxicity, although like many other polymers, its stability in biological applications can be greatly influenced by choice of polymer crosslinker.[110,111] PEDOT:PSS incorporated into collagen-alginate hydrogels at low concentrations improved cardiomyocyte coupling and maturation, even without ES.[14] Multiple groups have used PEDOT:PSS to dope methacrylated gelatin (GelMA) for bioprintable, electroactive materials for tissue engineering.[112,113]

Although some novel polymer-based materials address several physical disadvantages of more ubiquitous polymers,[5,114] all synthetic conjugated polymers thus far share the limitations of being unable to be degraded or resorbed by the body and having unknown long-term toxic effects, which calls into question their use in tissue replacements.

Carbon-based materials. Carbon-based materials such as graphene and CNTs receive attention for applications in tissue engineering because of their versatility, high conductivity, and ease of synthesis.[18] Many reports also indicate carbon-based materials enhance nerve cell response.[15] These properties make carbon-based materials attractive for use in other tissue engineering applications.

CNTs are perhaps best known for their unique mechanical and thermal properties for applications in nonmedical fields, but their high conductivity has resulted in greater attention in recent years for use as electroactive substrates.[75] CNTs also have form and dimensions similar to biological structures such as neurological processes or proteins of the extracellular matrix that may aid in tissue organization.[16,115] Functionalized CNTs and reduced GO sheets were incorporated into a poly(ethylene glycol) (PEG)-based hydrogel to create composites providing both electrical conductivity and positive surface charge to serve as a nerve conduit.[15] Compared to unmodified PEG hydrogels, the conductive substrate with positive surface charge resulted in slightly less circular PC12s and an increase in the number of cells bearing neurites, both of which are indicative of neuronal-like behavior.

Multiwall carbon nanotubes (MWCNTs) embedded in PEG gels were used to investigate the synergistic effects of conductivity, mechanical properties, and ES on neuronal differentiation and extension.[116] Neuronal outputs were greatest in groups with high PEG concentration (20%), MWCNTs, and exposure to ES. With the removal of ES, this hydrogel still outperformed gels with a lower concentration of PEG, and ES further magnified those differences. These data have two major implications. First, a material's conductive properties alone can support significant improvements in cell behavior. Second, desired effects can be significantly enhanced by tuning other material properties and applying ES.

Graphene is another class of carbon-based materials, but tends to be easier and less expensive to synthesize compared to CNTs.[17] GO possesses high biocompatibility and promotes cell adhesion in many applications, but has restricted conductivity that can be mitigated by chemical reduction, resulting in reduced GO (rGO)[17,117] Pristine graphene has the highest conductivity compared to GO and rGO, but all three variants are frequently used as conductive additives.[22]

Balikov et al. used a graphene-based material to investigate how material type, ES, and physical patterns influence human MSCs toward an osteogenic or neurogenic lineage.[118] Physical cues were necessary for expression of late osteogenic markers (e.g., osteopontin), but were unable to influence neuronal markers (e.g., MAP2 and β_3-tubulin), which were only enhanced by ES. Pristine graphene and collagen were combined to stimulate cardiomyocytes, resulting in increased metabolic activity and sarcomeric structures.[18] The conductive material alone brought about significant changes, but the observations were enhanced with the addition of ES.

Overall, carbon-based materials are commonly used in tissue engineering applications to impart electroactivity and are frequently touted for surpassing conductive polymers in their ability to improve the mechanical properties of hydrogels. However, these improvements cannot overshadow the reports that carbon-based materials still face similar drawbacks as synthetic polymers, including cytotoxicity,[119,120] hydrophobicity, and being nondegradable,[121–123] which reinforces the need for natural conductive materials.

Metal-based materials. Metal-based materials, namely nanoparticles, nanorods, and nanowires, are another class of synthetic conductive materials that are under investigation for tissue engineering. Gold is most often used due to its inert behavior in the body, but iron oxide has also been used to modify hydrogels, although for applications that have not yet been tested *in vivo*.[81] The use of less common silver, platinum, and zinc nanoparticles has been summarized elsewhere.[22] There is an established history of incorporating gold nanoparticles (AuNPs) into a variety of hydrogel materials and eliciting desired changes in cardiac applications, making them a popular choice as a conductive additive.[19]

MSCs incorporated into AuNP-infused chitosan hydrogels exhibited early cardiac markers and enhanced cardiac differentiation compared to unmodified chitosan gels, even in the absence of ES.[19] Cardiomyocytes entrapped in gold-infused GelMA substrates expressed cardiac-specific markers homogeneously throughout the hydrogel, and the gold nanorod groups supported synchronous beating.[20] When ES was applied, a lower excitation threshold was observed for the groups containing higher concentrations of gold nanorods, indicating that the conductive substrates could better promote electrical integration with endogenous tissue.

Although AuNPs are considered biocompatible, have high conductivity, and are effective for stimulating cells *in vitro*, gold cannot be resorbed by the body. Long-term studies using well-characterized AuNPs confirm that both acute and chronic exposure to AuNPs can alter the expression of genes related to cell cycle regulation and oxidative stress.[24]

Summary. The use of synthetic materials for conductive substrates for tissue engineering is gaining popularity. However, substantial hurdles and disadvantages remain. Synthetic materials conduct electric signals through electrons, which does not mimic the endogenous use of ionic gradients for bioelectricity. Knowledge gaps about the mechanism by which electrically conductive materials and the body interact prevent optimal or significant improvement in material performance. Furthermore, there are conflicting reports about cellular and bodily response to synthetic materials. While most polymeric materials are reported to be biocompatible, synthetic conjugated polymers are unable to be degraded or resorbed by the body and there are numerous reports of elevated immune response when PANI is used. Other conductive polymers, such as PPy and PEDOT, have only recently begun to appear in biomedical engineering applications, which limits knowledge of the long-term toxic effects of these materials on the body.

The long-term effects of carbon-based materials and AuNPs have been explored and are linked to cytotoxicity, permanent elevation of stress response in some cell types, and particle accumulation in many organs. Although synthetic conductive materials possess many attractive properties for use in tissue engineering, there is a critical need for conductive materials that can safely interact with the body's native tissues, either in a short-term manner or for permanent implantation.

Natural conductive biomaterials

While the availability of synthetic conductive materials is expansive, natural conductive biomaterials can be categorized into two types. The first is analogous to conjugated polymers, in that charge transport originates from π-π stacking, and is most often seen in materials containing aromatic amino acids (e.g., proteins and peptides) (Fig. 4B). The other contains charge-dense regions throughout its chemical structure and mainly derives its conductive properties from the movement of ions rather than electrons (Fig. 4C).

One of the most prominent models for naturally occurring conjugated conductive ''polymers'' is the pili proteins of *Geobacter sulfurreducens*.[96,124–132] These short proteins conduct electrons over μm to cm distances with conductivity around 5×10^{-3} $S\cdot cm^{-1}$.[95,124] The mechanism of electron transfer is believed to be electron hopping, made possible by the π-π interchain stacking, which occurs when the phenyl rings of aromatic amino acids are in appropriate proximity (*d*-spacing, the distance between atomic planes, of $\sim$3.5 Å).[95]

However, not all aromatic amino acids are equally conductive. The conductivity of the wild-type PilA monomer (the precursor to the *G. sulfurreducens* pili) was increased 2000-fold by genetically substituting one tyrosine and one phenylalanine for tryptophan.[129] Kalyoncu et al. synthesized peptides and films based on *Escherichia coli* secretions with added aromatic amino acids and observed increased conductivity of those materials when compared to controls. In agreement with previous observations, the materials _containing tryptophan had higher conductivity compared to those containing phenylalanine or tyrosine. This study suggests that, in addition to conductive motifs, charged amino acids within a peptide sequence are also critical to conductivity[133,134] Using peptides to make conductive materials is a recent development in the field,[135–137] leaving much room to explore how peptides can be designed to mimic synthetic conductive polymers used for tissue engineering.

Beyond peptide structures, other natural conductive materials for tissue engineering have risen to the forefront. Amdursky and Hsu doped materials with the iron-based hemin for use in flexible bioelectronic interfaces[138] and neural tissue engineering,[139] respectively. Hemin is a type of porphyrin, a class of compounds containing pyrrole subunits, making it a natural corollary to the frequently used PPy. Other groups have completely deviated from metallic mimics and embraced the conductivity associated with ionic charges.

A new class of conductive hydrogels incorporates choline-based ''bioionic liquids'' (Bio-ILs).[3] Ionic liquids generally possess high ionic conductivity along with other desirable features for material synthesis (e.g., thermal and electrochemical stability), and biologically based ionic liquids have the preferential property of being naturally derived, noncytotoxic, and biodegradable. The conductivity of ionic liquids is believed to originate from ions hopping from one ion-dense site in the molecule to another, rather than through π-π stacking.[140]

When conjugated to GelMA, the addition of Bio-IL increased conductivity of the hydrogels and supported the adhesion, proliferation, and maturation of primary cardiomyocytes. These hydrogels also provided sufficient conductive signaling to cardiomyocytes without ES, as evidenced by the cells' synchronous beating and upregulated connexin 43 protein expression.[2] When probing *in vivo* degradation, the results indicated that cells were able to enzymatically degrade GelMA-Bio-IL hydrogels through hydrolysis.[3] While these results are promising for using natural and ionically conductive materials for tissue engineering, additional research is warranted to establish whether ionically conducting materials can be incorporated into a variety of biomaterials and have similar effects on different cell and tissue types.

Progress in Conductive Materials for Tissue Engineering

The following section briefly summarizes goals of engineering specific tissues, describes how conductive materials have improved tissue engineering, and proposes an outlook for incorporating electroactive elements into tissue engineering.

Conductive materials for nerve tissue engineering

Nervous tissue has limited ability, or in the case of the central nervous system, no ability to regenerate on its own upon injury. Therefore, the restoration of nervous tissue after injury remains a significant medical challenge. One of the major goals when using neuronal cells to regenerate tissue is directing their differentiation down the neuronal line, rather than supporting cell types such as astrocytes and oligodendrocytes. Electroactive materials have been repeatedly shown to be supportive of neuronal differentiation.[12,21,141] PPy, PEDOT:PSS, and carbon-based materials have been used frequently. Mass ratios of PPy greater than 0.2 in chitosan-alginate hydrogels led to substrates with conductivity on the order of 10^{-3} $S\cdot cm^{-1}$. When used as a nerve conduit, this concentration resulted in tissues with similar histological characteristics as autograft.[6] Adding 0.1 w/v%

MWCNTs to PEG resulted in conductivity around 10^{-2} $S \cdot cm^{-1}$ and greatest PC12 neurite outgrowth and mean length.[116] The addition of ES promotes the generation of action potentials, which improves synaptic function and is linked to increased secretion of neurotrophic factors, supporting functional recovery *in vivo*.[54] Conductive substrates have also been associated with increased expression of genes associated with Schwann cell myelination.[114] In light of their capacity to support multiple nerve cell types and functions, electroactive materials are promising tools for nerve regeneration.

Conductive substrates have been used as conduits for regeneration in both nervous systems, but the biosafety of and lack of biological mechanistic knowledge surrounding synthetic materials remain important issues to be addressed in future studies.[142] Few recent studies have investigated the action potential profile of neuronal cells grown on conductive substrates to confirm that they behave similar to uninjured cells.[143] This information is important to consider, because while conductive hydrogels can significantly improve functional recovery, they are yet unable to recapitulate uninjured or autograft tissue.

Conductive materials for cardiac tissue engineering

Because cardiac tissue is electroactive, conductive materials are frequently used for cardiac tissue engineering and have successfully recapitulated the conductivity of native myocardium.[144] Synthetic polymers, carbon-based materials, and gold-based materials are most often used as conductive additives. Conductive substrates are also supportive of induced pluripotent stem cell, endothelial stem cell, and embryoid body differentiation toward cardiomyocytes. The number of myotubes, myofibrils, and sarcomeres increases when cardiomyocytes are grown on electroconductive surfaces.[145]

When seeded with cardiomyocytes, conductive materials aid in cell maturation, alignment, communication (e.g., gap junction formation), synchronous beating, and physiological pacing.[4] Hydrogel composites containing CNTs resulted in more aligned cardiomyocyte organization, but it is unclear if this result was due to the mechanical or electrical features of CNTs.[115,146] Navaei et al. observed a similar effect using their hydrogel containing gold nanorods. Cardiomyocytes were more organized into the microgrooves of constructs containing gold nanorods than those of the nondoped construct.[147] These characteristics are critical for clinical translation, where development of arrhythmias remains a risk in cardiac tissue engineering. While conductive materials have improved synchronous beating, it remains to be explored whether the improved communication leads to phenotype changes related to cellular growth.[75]

When fabricating cardiac patches, material elasticity and durability are of critical importance for proper organ function and longevity. Synthetic conductive substrates are rarely characterized as highly elastic, nor have there been many reports of patches being cyclically tested to mimic *in vivo* performance. Elastic cardiac patches made from 10 w/v% GelMA and 66 v/v% Bio-IL exhibited conductivity around 1.5×10^{-3} $S \cdot cm^{-1}$ and upregulated connexin 43 expression.[2] Despite promising preliminary results, the long-term performance of conductive substrates after myocardial infarction and their potential for developing comorbidities such as constrictive pericarditis and arrhythmia remain to be evaluated.[2]

Conductive materials for muscle tissue engineering

Muscle tissue is efficient at regenerating small injuries, but critically sized injuries (e.g., volumetric muscle loss) require intervention. The main goals of muscle tissue engineering are to promote differentiation of satellite cells or MSCs down the myogenic lineage, create a tissue with anisotropy to allow myoblasts to fuse into myotubes, and develop vascularized, innervated constructs for functional and electrophysiological recovery. Tissue elasticity is also critical to support muscle contraction.

Conductive materials have been effective at differentiating C2C12 myoblasts, upregulating myogenic genes and proteins, and promoting cell fusion.[145] Silk fibroin and a PANI-based polymer were combined to make scaffolds with conductivity on the order of 10^{-4} $S \cdot cm^{-1}$. When C2C12 myoblasts were seeded on scaffolds with 2 w/v% polymer, myogenic genes such as myogenic differentiation 1 (*MyoD1*), myogenin, and troponin T1 (*TNNT1*) were upregulated, although the elasticity of these materials was not tested.[104] While elastic conductive materials have been developed, their material choice (e.g., polyacrylamide) does not facilitate cell attachment or encapsulation, a factor that can be addressed in future studies.[17]

In addition to supporting myogenic differentiation, the future of conductive materials can also be used to support the electroactivity of muscle tissue, at large, by encouraging innervation and neuromuscular junction (NMJ) formation.[148] Multiple studies have probed the cellular interplay between muscle and nerve and have reported spontaneous NMJ development. However, the majority of studies using conductive substrates for muscle tissue engineering do not explore co-culture systems. While many studies investigate how ES and physical exercise influence muscle repair after injury, the possible synergy when using conductive substrates as a tissue scaffold remains uninvestigated.[149]

Conductive materials for bone tissue engineering

The primary goal of bone tissue engineering is to replace critically sized defects unable to spontaneously heal, whether caused by trauma, bone-related diseases, or surgical excision. Strategies for bone tissue engineering focus on making a mechanically stable, osteoinductive, and osteoconductive material to promote bone formation. Although bone cells are not electrically excitable like neuronal and cardiac cells, they are piezoelectric, meaning they generate electric potentials as they are mechanically loaded. Piezoelectric polymers, most commonly polyvinylidene fluoride, have been used to for bone tissue engineering.[145,150]

Dynamic mechanical loading of osteoblasts on piezoelectric scaffolds improved growth and proliferation of osteoblasts[151] and osteogenic differentiation of adipose-derived MSCs.[152] Even in the absence of mechanical loading, the association of cells with conductive substrates and ES enhances osteogenic activity.[153] PLA scaffolds with 10 wt% PANI possessed conductivities around 9×10^{-3} $S \cdot cm^{-1}$ and promoted osteogenic gene expression and ALP activity of bone marrow-derived MSCs.[107] Graphene outperformed nonconductive groups in treating critically sized calvarial defects *in vivo*.[154] These findings indicate that substrate electroactivity is an important contributor to the regenerative capacity of bone cells.

Many bone tissue engineering strategies to date have recapitulated the mechanical environment of native bone and

demonstrated efficacy *in vitro* and *in vivo*. Because bone is piezoelectric, it is important to confirm electrical functional outcomes in future studies.[155] Conductive substrates have been used as scaffold materials to improve osteogenic behavior. However, few studies have evaluated critical mechanical properties (e.g., Young's modulus) as a function of substrate modification with electroactive polymers, which may lead to discrepancies in reproducibility. Possible synergies between electroactivity, mechanical cues, and chemical signals for bone tissue engineering are largely unexplored and provide great opportunity to expand foundational knowledge of bone regeneration.

Conclusion

Cells rely on mechanical, chemical, and electrical information to properly function during development and homeostasis. The field of tissue engineering has focused on the composition and mechanical properties of engineered substrates to instruct cell fate. Evidence-based advances in bioelectricity motivate the pursuit of novel strategies that cater to cells' electrical needs. Despite the promising reports that conductive synthetic substrates influence cell behavior and promote engineered tissue function, these materials have several drawbacks that may be mitigated by the design of conductive natural biomaterials.[156] In addition, the mismatch in conducting mechanism between electrically conductive substrates and bioelectric tissues has revealed gaps in understanding how to design materials for the most relevant and significant clinical outcomes. Finally, variations across conductivity studies, whether in ES parameters, methods to measure conductivity, and the lack of positive control groups prevent reproducibility within the field and hinder progress toward clinical translation.[157]

In particular, foundational experiments to understand the effects of altering the many parameters of studies using conductive materials (e.g., level of conductivity, seal resistance, type of material or mechanism of conduction, or how electrical properties interplay with other properties within cell- and material-based therapies) will be important to propel the field forward. The results of such foundational studies could then be used to design studies with more translational outputs both *in vitro* and *in vivo*. They also establish general fundamental understanding that allows for extension of using conductive materials for a variety of biomedical applications (e.g., improving *in vitro* modeling systems).

The field of tissue engineering has evolved far beyond combining cells with materials and implanting in hopes of growing neotissues or promoting repair. There are many examples of preimplantation characterization of cell adhesion, proliferation, migration, and differentiation in response to engineered materials. In contrast, the application of conductive materials in tissue engineering is only now emerging. The use of conductive materials for this purpose provides new opportunities to promote cellular organization *in vitro* before implantation, enabling the introduction of more advanced, functional tissues that possess greater therapeutic potential. Thus, there is tremendous opportunity on the horizon for developing materials that better recapitulate endogenous electrical signaling and support tissue engineering applications.

Authorship Confirmation Statement

All authors contributed to the design and concept of the article. A.C. wrote the review, and A.P. and J.K.L. critically revised the article. A.C. conceived and edited figures. All authors have reviewed and approved the contents of the article. This article has been submitted solely to this journal and is not published, in press, or submitted elsewhere.

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References

1. Funk RH, Monsees T, Ozkucur N. Electromagnetic effects—From cell biology to medicine. Prog Histochem Cytochem 2009;43:177–264.
2. Walker BW, Lara RP, Yu CH, et al. Engineering a naturally-derived adhesive and conductive cardiopatch. Biomaterials 2019;207:89–101.
3. Noshadi I, Walker BW, Portillo-Lara R, et al. Engineering biodegradable and biocompatible bio-ionic liquid conjugated hydrogels with tunable conductivity and mechanical properties. Sci Rep 2017;7:4345.
4. Cui Z, Ni NC, Wu J, et al. Polypyrrole-chitosan conductive biomaterial synchronizes cardiomyocyte contraction and improves myocardial electrical impulse propagation. Theranostics 2018;8:2752–2764.
5. Yang B, Yao F, Hao T, et al. Development of electrically conductive double-network hydrogels via one-step facile strategy for cardiac tissue engineering. Adv Healthc Mater 2016;5:474–488.
6. Bu Y, Xu HX, Li X, et al. A conductive sodium alginate and carboxymethyl chitosan hydrogel doped with polypyrrole for peripheral nerve regeneration. RSC Adv 2018; 8:10806–10817.
7. Mihic A, Cui Z, Wu J, et al. A conductive polymer hydrogel supports cell electrical signaling and improves cardiac function after implantation into myocardial infarct. Circulation 2015;132:772–784.
8. Shastri VR, Schmidt CE, Kim TH, et al. Polypyrrole—A potential candidate for stimulated nerve regeneration. MRS Proc 1996;414:113–118.
9. Xu H, Holzwarth JM, Yan Y, et al. Conductive PPY/PDLLA conduit for peripheral nerve regeneration. Biomaterials 2014;35:225–235.
10. Yang J, Choe G, Yang S, et al. Polypyrrole-incorporated conductive hyaluronic acid hydrogels. Biomater Res 2016;20:31.
11. Zhou X, Yang A, Huang Z, et al. Enhancement of neurite adhesion, alignment and elongation on conductive polypyrrole-poly(lactide acid) fibers with cell-derived extracellular matrix. Colloids Surf B Biointerfaces 2017; 149:217–225.

12. Wang S, Guan S, Li W, et al. 3D culture of neural stem cells within conductive PEDOT layer-assembled chitosan/gelatin scaffolds for neural tissue engineering. Mater Sci Eng C Mater Biol Appl 2018;93:890–901.
13. Wang SP, Sun CK, Guan S, et al. Chitosan/gelatin porous scaffolds assembled with conductive poly(3,4-ethylenedioxythiophene) nanoparticles for neural tissue engineering. J Mater Chem B 2017;5:4774–4788.
14. Roshanbinfar K, Vogt L, Greber B, et al. Electroconductive biohybrid hydrogel for enhanced maturation and beating properties of engineered cardiac tissues. Adv Funct Mater 2018;28:1803951.
15. Liu X, Miller AL, Park S, et al. Functionalized carbon nanotube and graphene oxide embedded electrically conductive hydrogel synergistically stimulates nerve cell differentiation. ACS Appl Mater Interfaces 2017;9:14677–14690.
16. Scapin G, Salice P, Tescari S, et al. Enhanced neuronal cell differentiation combining biomimetic peptides and a carbon nanotube-polymer scaffold. Nanomedicine 2015; 11:621–632.
17. Alam A, Kuan HC, Zhao ZH, et al. Novel polyacrylamide hydrogels by highly conductive, water-processable graphene. Compos Part A Appl Sci Manuf 2017;93:1–9.
18. Ryan AJ, Kearney CJ, Shen N, et al. Electroconductive biohybrid collagen/pristine graphene composite biomaterials with enhanced biological activity. Adv Mater 2018; 30:e1706442.
19. Baei P, Jalili-Firoozinezhad S, Rajabi-Zeleti S, et al. Electrically conductive gold nanoparticle-chitosan thermosensitive hydrogels for cardiac tissue engineering. Mater Sci Eng C Mater Biol Appl 2016;63:131–141.
20. Navaei A, Saini H, Christenson W, et al. Gold nanorod-incorporated gelatin-based conductive hydrogels for engineering cardiac tissue constructs. Acta Biomater 2016; 41:133–146.
21. Zhou L, Fan L, Yi X, et al. Soft conducting polymer hydrogels cross-linked and doped by tannic acid for spinal cord injury repair. ACS Nano 2018;12:10957–10967.
22. Min JH, Patel M, Koh WG. Incorporation of conductive materials into hydrogels for tissue engineering applications. Polymers 2018;10:1078.
23. Guiseppi-Elie A. Electroconductive hydrogels: Synthesis, characterization and biomedical applications. Biomaterials 2010;31:2701–2716.
24. Falagan-Lotsch P, Grzincic EM, Murphy CJ. One low-dose exposure of gold nanoparticles induces long-term changes in human cells. Proc Natl Acad Sci U S A 2016; 113:13318–13323.
25. Piccolino M. Luigi Galvani and animal electricity: Two centuries after the foundation of electrophysiology. Trends Neurosci 1997;20:443–448.
26. Levin M, Pezzulo G, Finkelstein JM. Endogenous bioelectric signaling networks: Exploiting voltage gradients for control of growth and form. Annu Rev Biomed Eng 2017;19:353–387.
27. McLaughlin KA, Levin M. Bioelectric signaling in regeneration: Mechanisms of ionic controls of growth and form. Dev Biol 2018;433:177–189.
28. Funk RH. Endogenous electric fields as guiding cue for cell migration. Front Physiol 2015;6:143.
29. Snyder S, DeJulius C, Willits RK. Electrical stimulation increases random migration of human dermal fibroblasts. Ann Biomed Eng 2017;45:2049–2060.
30. Kojima J, Shinohara H, Ikariyama Y, et al. Electrically promoted protein production by mammalian cells cultured on the electrode surface. Biotechnol Bioeng 1992;39:27–32.
31. Mathews J, Levin M. The body electric 2.0: Recent advances in developmental bioelectricity for regenerative and synthetic bioengineering. Curr Opin Biotechnol 2018; 52:134–144.
32. Reid B, Song B, Zhao M. Electric currents in Xenopus tadpole tail regeneration. Dev Biol 2009;335:198–207.
33. Thrivikraman G, Boda SK, Basu B. Unraveling the mechanistic effects of electric field stimulation towards directing stem cell fate and function: A tissue engineering perspective. Biomaterials 2018;150:60–86.
34. Balint R, Cassidy NJ, Cartmell SH. Electrical stimulation: A novel tool for tissue engineering. Tissue Eng Part B Rev 2013;19:48–57.
35. Cao L, Wei D, Reid B, et al. Endogenous electric currents might guide rostral migration of neuroblasts. EMBO Rep 2013;14:184–190.
36. Yao L, Shanley L, McCaig C, et al. Small applied electric fields guide migration of hippocampal neurons. J Cell Physiol 2008;216:527–535.
37. Nie K, Henderson A. MAP kinase activation in cells exposed to a 60 Hz electromagnetic field. J Cell Biochem 2003;90:1197–1206.
38. Wolf-Goldberg T, Barbul A, Ben-Dov N, et al. Low electric fields induce ligand-independent activation of EGF receptor and ERK via electrochemical elevation of H(+) and ROS concentrations. Biochim Biophys Acta 2013;1833:1396–1408.
39. Hart FX. Integrins may serve as mechanical transducers for low-frequency electric fields. Bioelectromagnetics 2006;27:505–508.
40. Delle Monache S, Angelucci A, Sanita P, et al. Inhibition of angiogenesis mediated by extremely low-frequency magnetic fields (ELF-MFs). PLoS One 2013;8:e79309.
41. Zrimec A, Jerman I, Lahajnar G. Alternating electric fields stimulate ATP synthesis in Escherichia coli. Cell Mol Biol Lett 2002;7:172–174.
42. Tarasov AI, Semplici F, Li D, et al. Frequency-dependent mitochondrial Ca(2+) accumulation regulates ATP synthesis in pancreatic beta cells. Pflugers Arch 2013;465: 543–554.
43. Sheikh AQ, Taghian T, Hemingway B, et al. Regulation of endothelial MAPK/ERK signalling and capillary morphogenesis by low-amplitude electric field. J R Soc Interface 2013;10:20120548.
44. Lozano MM, Hovis JS, Moss FR, 3rd, et al. Dynamic reorganization and correlation among lipid raft components. J Am Chem Soc 2016;138:9996–10001.
45. Lin BJ, Tsao SH, Chen A, et al. Lipid rafts sense and direct electric field-induced migration. Proc Natl Acad Sci U S A 2017;114:8568–8573.
46. Feng JF, Liu J, Zhang XZ, et al. Guided migration of neural stem cells derived from human embryonic stem cells by an electric field. Stem Cells 2012;30:349–355.
47. Guo A, Song B, Reid B, et al. Effects of physiological electric fields on migration of human dermal fibroblasts. J Invest Dermatol 2010;130:2320–2327.
48. Zimolag E, Borowczyk-Michalowska J, Kedracka-Krok S, et al. Electric field as a potential directional cue in homing of bone marrow-derived mesenchymal stem cells to cutaneous wounds. Biochim Biophys Acta Mol Cell Res 2017;1864:267–279.

49. Long Y, Wei H, Li J, et al. Effective wound healing enabled by discrete alternative electric fields from wearable nanogenerators. ACS Nano 2018;12:12533–12540.
50. Gao J, Raghunathan VK, Reid B, et al. Biomimetic stochastic topography and electric fields synergistically enhance directional migration of corneal epithelial cells in a MMP-3-dependent manner. Acta Biomater 2015;12:102–112.
51. Tian L, Prabhakaran MP, Hu J, et al. Synergistic effect of topography, surface chemistry and conductivity of the electrospun nanofibrous scaffold on cellular response of PC12 cells. Colloids Surf B Biointerfaces 2016;145:420–429.
52. Cho MR, Thatte HS, Lee RC, et al. Reorganization of microfilament structure induced by ac electric fields. FASEB J 1996;10:1552–1558.
53. Tsai CH, Lin BJ, Chao PH. alpha2beta1 integrin and RhoA mediates electric field-induced ligament fibroblast migration directionality. J Orthop Res 2013;31:322–327.
54. Song J, Sun B, Liu S, et al. Polymerizing pyrrole coated poly (l-lactic acid-co-epsilon-caprolactone) (PLCL) conductive nanofibrous conduit combined with electric stimulation for long-range peripheral nerve regeneration. Front Mol Neurosci 2016;9:117.
55. Choi WJ, Jung J, Lee S, et al. Effects of substrate conductivity on cell morphogenesis and proliferation using tailored, atomic layer deposition-grown ZnO thin films. Sci Rep 2015;5:9974.
56. Wu Y, Guo L. Enhancement of intercellular electrical synchronization by conductive materials in cardiac tissue engineering. IEEE Trans Biomed Eng 2018;65:264–272.
57. Kotwal A, Schmidt CE. Electrical stimulation alters protein adsorption and nerve cell interactions with electrically conducting biomaterials. Biomaterials 2001;22: 1055–1064.
58. Lei KF, Hsieh SC, Goh A, et al. Proliferation arrest, selectivity, and chemosensitivity enhancement of cancer cells treated by a low-intensity alternating electric field. Biomed Microdevices 2018;20:90.
59. Martinelli V, Cellot G, Toma FM, et al. Carbon nanotubes promote growth and spontaneous electrical activity in cultured cardiac myocytes. Nano Lett 2012;12:1831–1838.
60. Cao L, Liu J, Pu J, et al. Endogenous bioelectric currents promote differentiation of the mammalian lens. J Cell Physiol 2018;233:2202–2212.
61. Diaz-Vegas A, Campos CA, Contreras-Ferrat A, et al. ROS production via P2Y1-PKC-NOX2 is triggered by extracellular ATP after electrical stimulation of skeletal muscle cells. PLoS One 2015;10:e0129882.
62. Yamada M, Tanemura K, Okada S, et al. Electrical stimulation modulates fate determination of differentiating embryonic stem cells. Stem Cells 2007;25:562–570.
63. Tonelli FMP, Santos AK, Gomes DA, et al. Stem cells and calcium signaling. In: Islam M, ed. Calcium Signaling. Dordrecht, Netherlands: Springer, 2012: 891–916.
64. Sauer H, Wartenberg M, Hescheler J. Reactive oxygen species as intracellular messengers during cell growth and differentiation. Cell Physiol Biochem 2001;11:173–186.
65. Manthey AL, Liu W, Jiang ZX, et al. Using electrical stimulation to enhance the efficacy of cell transplantation therapies for neurodegenerative retinal diseases: Concepts, challenges, and future perspectives. Cell Transplant 2017;26:949–965.
66. Matsumoto M, Imura T, Fukazawa T, et al. Electrical stimulation enhances neurogenin2 expression through beta-catenin signaling pathway of mouse bone marrow stromal cells and intensifies the effect of cell transplantation on brain injury. Neurosci Lett 2013;533:71–76.
67. George PM, Bliss TM, Hua T, et al. Electrical preconditioning of stem cells with a conductive polymer scaffold enhances stroke recovery. Biomaterials 2017; 142:31–40.
68. Kwon HJ, Lee GS, Chun H. Electrical stimulation drives chondrogenesis of mesenchymal stem cells in the absence of exogenous growth factors. Sci Rep 2016;6:39302.
69. Zhang J, Li M, Kang ET, et al. Electrical stimulation of adipose-derived mesenchymal stem cells in conductive scaffolds and the roles of voltage-gated ion channels. Acta Biomater 2016;32:46–56.
70. Pitcairn E, Harris H, Epiney J, et al. Coordinating heart morphogenesis: A novel role for hyperpolarization-activated cyclic nucleotide-gated (HCN) channels during cardiogenesis in *Xenopus laevis*. Commun Integr Biol 2017;10:e1309488.
71. Grossemy S, Chan PPY, Doran PM. Electrical stimulation of cell growth and neurogenesis using conductive and nonconductive microfibrous scaffolds. Integr Biol (Camb) 2019;11:264–279.
72. Hardy JG, Lee JY, Schmidt CE. Biomimetic conducting polymer-based tissue scaffolds. Curr Opin Biotechnol 2013;24:847–854.
73. Balint R, Cassidy NJ, Cartmell SH. Conductive polymers: Towards a smart biomaterial for tissue engineering. Acta Biomater 2014;10:2341–2353.
74. Yuk H, Lu B, Zhao X. Hydrogel bioelectronics. Chem Soc Rev 2019;48:1642–1667.
75. Burnstine-Townley A, Eshel Y, Amdursky N. Conductive scaffolds for cardiac and neuronal tissue engineering: Governing factors and mechanisms. Adv Funct Mater 2019;30:1901369.
76. Ning C, Zhou Z, Tan G, et al. Electroactive polymers for tissue regeneration: Developments and perspectives. Prog Polym Sci 2018;81:144–162.
77. Anderson M, Shelke NB, Manoukian OS, et al. Peripheral nerve regeneration strategies: Electrically stimulating polymer based nerve growth conduits. Crit Rev Biomed Eng 2015;43:131–159.
78. Abidian MR, Daneshvar ED, Egeland BM, et al. Hybrid conducting polymer-hydrogel conduits for axonal growth and neural tissue engineering. Adv Healthc Mater 2012;1: 762–767.
79. Green RA, Baek S, Poole-Warren LA, et al. Conducting polymer-hydrogels for medical electrode applications. Sci Technol Adv Mater 2010;11:014107.
80. Guimard NK, Gomez N, Schmidt CE. Conducting polymers in biomedical engineering. Prog Polym Sci 2007;32: 876–921.
81. Bonfrate V, Manno D, Serra A, et al. Enhanced electrical conductivity of collagen films through long-range aligned iron oxide nanoparticles. J Colloid Interface Sci 2017;501: 185–191.
82. Broda CR, Lee JY, Sirivisoot S, et al. A chemically polymerized electrically conducting composite of polypyrrole nanoparticles and polyurethane for tissue engineering. J Biomed Mater Res A 2011;98:509–516.
83. Gan D, Han L, Wang M, et al. Conductive and tough hydrogels based on biopolymer molecular templates for controlling in situ formation of polypyrrole nanorods. ACS Appl Mater Interfaces 2018;10:36218–36228.

84. Liu X, Miller AL, 2nd, Park S, et al. Covalent crosslinking of graphene oxide and carbon nanotube into hydrogels enhances nerve cell responses. J Mater Chem B 2016;4: 6930–6941.
85. Feiner R, Fleischer S, Shapira A, et al. Multifunctional degradable electronic scaffolds for cardiac tissue engineering. J Control Release 2018;281:189–195.
86. Pena B, Maldonado M, Bonham AJ, et al. Gold nanoparticle-functionalized reverse thermal gel for tissue engineering applications. ACS Appl Mater Interfaces 2019;11:18671–18680.
87. Mobini S, Spearman BS, Lacko CS, et al. Recent advances in strategies for peripheral nerve tissue engineering. Curr Opin Biomed Eng 2017;4:134–142.
88. Song S, George PM. Conductive polymer scaffolds to improve neural recovery. Neural Regen Res 2017;12: 1976–1978.
89. Spearman BS, Desai VH, Mobini S, et al. Tissue-engineered peripheral nerve interfaces. Adv Funct Mater 2018;28:1701713.
90. Mawad D, Mansfield C, Lauto A, et al. A conducting polymer with enhanced electronic stability applied in cardiac models. Sci Adv 2016;2:e1601007.
91. MacDiarmid AG. ''Synthetic metals'': A novel role for oganic polymers (Nobel lecture). Angew Chem Int Ed 2001;40:2581–2590.
92. The Nobel Prize in Chemistry 2000. NobelPrize.org. Nobel Media AB 2020. https://www.nobelprize.org/prizes/chemistry/2000/summary/. Last accessed October 8, 2020.
93. Tandon B, Magaz A, Balint R, et al. Electroactive biomaterials: Vehicles for controlled delivery of therapeutic agents for drug delivery and tissue regeneration. Adv Drug Deliv Rev 2018;129:148–168.
94. Kaur G, Adhikari R, Cass P, et al. Electrically conductive polymers and composites for biomedical applications. RSC Adv 2015;5:37553–37567.
95. Malvankar NS, Vargas M, Nevin KP, et al. Tunable metallic-like conductivity in microbial nanowire networks. Nat Nanotechnol 2011;6:573–579.
96. Malvankar NS, Vargas M, Nevin K, et al. Structural basis for metallic-like conductivity in microbial nanowires. mBio 2015;6:e00084.
97. Wong JY, Langer R, Ingber DE. Electrically conducting polymers can noninvasively control the shape and growth of mammalian cells. Proc Natl Acad Sci U S A 1994;91: 3201–3204.
98. Brahim S, Guiseppi-Elie A. Electroconductive hydrogels: Electrical properties of polypyrrole-poly(HEMA) and electrochemical composites. Electroanalysis 2005;17:556–570.
99. de Castro JG, Rodrigues BVM, Ricci R, et al. Designing a novel nanocomposite for bone tissue engineering using electrospun conductive PBAT/polypyrrole as a scaffold to direct nanohydroxyapatite electrodeposition. RSC Adv 2016;6:32615–32623.
100. Solazzo M, O'Brien FJ, Nicolosi V, et al. The rationale and emergence of electroconductive biomaterial scaffolds in cardiac tissue engineering. APL Bioeng 2019;3:041501.
101. Humpolicek P, Kasparkova V, Pachernik J, et al. The biocompatibility of polyaniline and polypyrrole: A comparative study of their cytotoxicity, embryotoxicity and impurity profile. Mater Sci Eng C Mater Biol Appl 2018;91:303–310.
102. Qazi TH, Rai R, Boccaccini AR. Tissue engineering of electrically responsive tissues using polyaniline based polymers: A review. Biomaterials 2014;35:9068–9086.
103. Guarino V, Alvarez-Perez MA, Borriello A, et al. Conductive PANi/PEGDA macroporous hydrogels for nerve regeneration. Adv Healthc Mater 2013;2:218–227.
104. Zhang M, Guo B. Electroactive 3D scaffolds based on silk fibroin and water-bornepolyaniline for skeletal muscle tissue engineering. Macromol Biosci 2017;17:1700147.
105. Chen M-C, Sun Y-C, Chen Y-H. Electrically conductive nanofibers with highly oriented structures and their potential application in skeletal muscle tissue engineering. Acta Biomater 2013;9:5562–5572.
106. Li Y, Li X, Zhao R, et al. Enhanced adhesion and proliferation of human umbilical vein endothelial cells on conductive PANI-PCL fiber scaffold by electrical stimulation. Mater Sci Eng C Mater Biol Appl 2017;72:106–112.
107. Chen J, Yu M, Guo B, et al. Conductive nanofibrous composite scaffolds based on in-situ formed polyaniline nanoparticle and polylactide for bone regeneration. J Colloid Interface Sci 2018;514:517–527.
108. Wang S, Guan S, Wang J, et al. Fabrication and characterization of conductive poly (3,4-ethylenedioxythiophene) doped with hyaluronic acid/poly (l-lactic acid) composite film for biomedical application. J Biosci Bioeng 2017;123: 116–125.
109. Wang S, Guan S, Zhu Z, et al. Hyaluronic acid doped-poly(3,4-ethylenedioxythiophene)/chitosan/gelatin (PEDOT-HA/Cs/Gel) porous conductive scaffold for nerve regeneration. Mater Sci Eng C Mater Biol Appl 2017;71:308–316.
110. Solazzo M, Krukiewicz K, Zhussupbekova A, et al. PEDOT:PSS interfaces stabilised using a PEGylated crosslinker yield improved conductivity and biocompatibility. J Mater Chem B 2019;7:4811–4820.
111. Lu B, Yuk H, Lin S, et al. Pure PEDOT:PSS hydrogels. Nat Commun 2019;10:1043.
112. Spencer AR, Shirzaei Sani E, Soucy JR, et al. Bioprinting of a cell-laden conductive hydrogel composite. ACS Appl Mater Interfaces 2019;11:30518–30533.
113. Heo DN, Lee SJ, Timsina R, et al. Development of 3D printable conductive hydrogel with crystallized PEDOT: PSS for neural tissue engineering. Mater Sci Eng C Mater Biol Appl 2019;99:582–590.
114. Wu Y, Wang L, Guo B, et al. Electroactive biodegradable polyurethane significantly enhanced Schwann cells myelin gene expression and neurotrophin secretion for peripheral nerve tissue engineering. Biomaterials 2016;87:18–31.
115. Shin SR, Jung SM, Zalabany M, et al. Carbon-nanotube-embedded hydrogel sheets for engineering cardiac constructs and bioactuators. ACS Nano 2013;7:2369–2380.
116. Imaninezhad M, Pemberton K, Xu F, et al. Directed and enhanced neurite outgrowth following exogenous electrical stimulation on carbon nanotube-hydrogel composites. J Neural Eng 2018;15:056034.
117. Li D, Muller MB, Gilje S, et al. Processable aqueous dispersions of graphene nanosheets. Nat Nanotechnol 2008;3: 101–105.
118. Balikov DA, Fang B, Chun YW, et al. Directing lineage specification of human mesenchymal stem cells by decoupling electrical stimulation and physical patterning on unmodified graphene. Nanoscale 2016;8:13730–13739.
119. Jia G, Wang H, Yan L, et al. Cytotoxicity of carbon nanomaterials: Single-wall nanotube, multi-wall nanotube, and fullerene. Environ Sci Technol 2005;39:1378–1383.
120. Lam CW, James JT, McCluskey R, et al. Pulmonary toxicity of single-wall carbon nanotubes in mice 7 and 90

days after intratracheal instillation. Toxicol Sci 2004;77: 126–134.
121. Di Giorgio ML, Di Bucchianico S, Ragnelli AM, et al. Effects of single and multi walled carbon nanotubes on macrophages: Cyto and genotoxicity and electron microscopy. Mutat Res 2011;722:20–31.
122. Principi E, Girardello R, Bruno A, et al. Systemic distribution of single-walled carbon nanotubes in a novel model: Alteration of biochemical parameters, metabolic functions, liver accumulation, and inflammation in vivo. Int J Nanomedicine 2016;11:4299–4316.
123. Prajapati SK, Malaiya A, Kesharwani P, et al. Biomedical applications and toxicities of carbon nanotubes. Drug Chem Toxicol 2020 [Epub ahead of print]; DOI: 10.1080/01480545.2019.1709492.
124. Adhikari RY, Malvankar NS, Tuominen MT, et al. Conductivity of individual Geobacter pili. RSC Adv 2016;6: 8363–8366.
125. Bond DR, Lovley DR. Electricity production by *Geobacter sulfurreducens* attached to electrodes. Appl Environ Microbiol 2003;69:1548–1555.
126. Ing NL, Nusca TD, Hochbaum AI. *Geobacter sulfurreducens* pili support ohmic electronic conduction in aqueous solution. Phys Chem Chem Phys 2017;19:21791–21799.
127. Malvankar NS, Lovley DR. Microbial nanowires: A new paradigm for biological electron transfer and bioelectronics. ChemSusChem 2012;5:1039–1046.
128. Reardon PN, Mueller KT. Structure of the type IVa major pilin from the electrically conductive bacterial nanowires of *Geobacter sulfurreducens*. J Biol Chem 2013;288:29260–29266.
129. Tan Y, Adhikari RY, Malvankar NS, et al. Synthetic biological protein nanowires with high conductivity. Small 2016;12:4481–4485.
130. Vargas M, Malvankar NS, Tremblay PL, et al. Aromatic amino acids required for pili conductivity and long-range extracellular electron transport in *Geobacter sulfurreducens*. mBio 2013;4:e00105–e00113.
131. Xiao K, Malvankar NS, Shu C, et al. Low energy atomic models suggesting a pilus structure that could account for electrical conductivity of *Geobacter sulfurreducens* pili. Sci Rep 2016;6:23385.
132. Yates MD, Strycharz-Glaven SM, Golden JP, et al. Measuring conductivity of living *Geobacter sulfurreducens* biofilms. Nat Nanotechnol 2016;11:910–913.
133. Kalyoncu E, Ahan RE, Olmez TT, et al. Genetically encoded conductive protein nanofibers secreted by engineered cells. RSC Adv 2017;7:32543–32551.
134. Sepunaru L, Refaely-Abramson S, Lovrincic R, et al. Electronic transport via homopeptides: The role of side chains and secondary structure. J Am Chem Soc 2015;137:9617–9626.
135. Ing NL, Spencer RK, Luong SH, et al. Electronic conductivity in biomimetic α-helical peptide nanofibers and gels. ACS Nano 2018;12:2652–2661.
136. Hayamizu Y, So CR, Dag S, et al. Bioelectronic interfaces by spontaneously organized peptides on 2D atomic single layer materials. Sci Rep 2016;6:33778.
137. Hauser CAE, Zhang S. Peptides as biological semiconductors. Nature 2010;468:516–517.
138. Amdursky N, Wang X, Meredith P, et al. Electron hopping across hemin-doped serum albumin mats on centimeter-length scales. Adv Mater 2017;29:1700810.
139. Hsu CC, Serio A, Amdursky N, et al. Fabrication of hemin-doped serum albumin-based fibrous scaffolds for neural tissue engineering applications. ACS Appl Mater Interfaces 2018;10:5305–5317.
140. Feng G, Chen M, Bi S, et al. Free and bound states of ions in ionic liquids, conductivity, and underscreening paradox. Phys Rev X 2019;9:021024.
141. Niu Y, Chen X, Yao D, et al. Enhancing neural differentiation of induced pluripotent stem cells by conductive graphene/silk fibroin films. J Biomed Mater Res A 2018; 106:2973–2983.
142. Yang M, Liang Y, Gui Q, et al. Electroactive biocompatible materials for nerve cell stimulation. Mater Res Express 2015;2:042001.
143. Acosta-Garcia MC, Morales-Reyes I, Jimenez-Anguiano A, et al. Simultaneous recording of electrical activity and the underlying ionic currents in NG108–NG115 cells cultured on gold substrate. Heliyon 2018;4:e00550.
144. Surowiec A, Stuchly SS, Eidus L, et al. In vitro dielectric properties of human tissues at radiofrequencies. Phys Med Biol 1987;32:615–621.
145. da Silva LP, Kundu SC, Reis RL, et al. Electric phenomenon: A disregarded tool in tissue engineering and regenerative medicine. Trends Biotechnol 2020;38:24–49.
146. Mostafavi E, Medina-Cruz D, Kalantari K, et al. Electroconductive nanobiomaterials for tissue engineering and regenerative medicine. Bioelectricity 2020;2:120–149.
147. Navaei A, Moore N, Sullivan RT, et al. Electrically conductive hydrogel-based micro-topographies for the development of organized cardiac tissues. RSC Adv 2017;7:3302–3312.
148. Freeman JW, Browe DP. Bio-instructive scaffolds for skeletal muscle regeneration. In: Brown JL, Banik BL, Kumbar SG, eds. Bio-Instructive Scaffolds for Musculoskeletal Tissue Engineering and Regenerative Medicine. London, UK: Elsevier, 2017:187–199.
149. Gilbert-Honick J, Grayson W. Vascularized and innervated skeletal muscle tissue engineering. Adv Healthc Mater 2020;9:e1900626.
150. Ribeiro C, Sencadas V, Correia DM, et al. Piezoelectric polymers as biomaterials for tissue engineering applications. Colloids Surf B Biointerfaces 2015;136:46–55.
151. Ribeiro C, Moreira S, Correia V, et al. Enhanced proliferation of pre-osteoblastic cells by dynamic piezoelectric stimulation. RSC Adv 2012;2:11504–11509.
152. Ribeiro C, Parssinen J, Sencadas V, et al. Dynamic piezoelectric stimulation enhances osteogenic differentiation of human adipose stem cells. J Biomed Mater Res A 2015; 103:2172–2175.
153. Guex AG, Puetzer JL, Armgarth A, et al. Highly porous scaffolds of PEDOT:PSS for bone tissue engineering. Acta Biomater 2017;62:91–101.
154. Wang W, Junior JRP, Nalesso PRL, et al. Engineered 3D printed poly(varepsilon-caprolactone)/graphene scaffolds for bone tissue engineering. Mater Sci Eng C Mater Biol Appl 2019;100:759–770.
155. Saberi A, Jabbari F, Zarrintaj P, et al. Electrically conductive materials: Opportunities and challenges in tissue engineering. Biomolecules 2019;9:448.
156. Jin G, Li K. The electrically conductive scaffold as the skeleton of stem cell niche in regenerative medicine. Mater Sci Eng C Mater Biol Appl 2014;45:671–681.
157. Shi Z, Gao X, Ullah MW, et al. Electroconductive natural polymer-based hydrogels. Biomaterials 2016;111:40–54.
158. Pan L, Yu G, Zhai D, et al. Hierarchical nanostructured conducting polymer hydrogel with high electrochemical activity. Proc Natl Acad Sci U S A 2012;109:9287–9292.

159. Yu Z, Xia Y, Du D, et al. PEDOT:PSS films with metallic conductivity through a treatment with common organic solutions of organic salts and their application as a transparent electrode of polymer solar cells. ACS Appl Mater Interfaces 2016;8:11629–11638.
160. Wang Y, Weng GJ. Electrical conductivity of carbon nanotube- and graphene-based nanocomposites. In: Meguid S, Weng G, eds. Micromechanics and Nanomechanics of Composite Solids. Cham, Switzerland: Springer, 2018: 123–156.
161. Yang S, Jang L, Kim S, et al. Polypyrrole/Alginate hybrid hydrogels: Electrically conductive and soft biomaterials for human mesenchymal stem cell culture and potential neural tissue engineering applications. Macromol Biosci 2016;16:1653–1661.
162. Wibowo A, Vyas C, Cooper G, et al. 3D printing of polycaprolactone-polyaniline electroactive scaffolds for bone tissue engineering. Materials (Basel) 2020;13:512.
163. Wu Y, Wang L, Guo B, et al. Interwoven aligned conductive nanofiber yarn/hydrogel composite scaffolds for engineered 3D cardiac anisotropy. ACS Nano 2017;11:5646–5659.
164. Han L, Lu X, Wang M, et al. A mussel-inspired conductive, self-adhesive, and self-healable tough hydrogel as cell stimulators and implantable bioelectronics. Small 2017;13:1601916.
165. Gabriel C, Peyman A, Grant EH. Electrical conductivity of tissue at frequencies below 1 MHz. Phys Med Biol 2009;54:4863–4878.
166. Balmer TW, Vesztergom S, Broekmann P, et al. Characterization of the electrical conductivity of bone and its correlation to osseous structure. Sci Rep 2018;8:8601.
167. Binette JS, Garon M, Savard P, et al. Tetrapolar measurement of electrical conductivity and thickness of articular cartilage. J Biomech Eng 2004;126:475–484.
168. Zarrintaj P, Manouchehri S, Ahmadi Z, et al. Agarose-based biomaterials for tissue engineering. Carbohydr Polym 2018;187:66–84.
169. Sun H, Zhou J, Huang Z, et al. Carbon nanotube-incorporated collagen hydrogels improve cell alignment and the performance of cardiac constructs. Int J Nanomedicine 2017;12:3109–3120.
170. MacDonald RA, Voge CM, Kariolis M, et al. Carbon nanotubes increase the electrical conductivity of fibroblast-seeded collagen hydrogels. Acta Biomater 2008;4:1583–1592.
171. Kaklamani G, Kazaryan D, Bowen J, et al. On the electrical conductivity of alginate hydrogels. Regen Biomater 2018;5:293–301.
172. Marroquin JB, Rhee KY, Park SJ. Chitosan nanocomposite films: Enhanced electrical conductivity, thermal stability, and mechanical properties. Carbohydr Polym 2013;92:1783–1791.

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