1

Electroactive Materials for Tissue Engineering

Teresa Marques-Almeida[1], Estela O. Carvalho[1], Unai Silvan[2,3], Senentxu Lanceros-Méndez[1,2,3], and Clarisse Ribeiro[1]

[1] *University of Minho, CF-UM-UP–Physics Centre of Minho and Porto Universities and LaPMET–Laboratory of Physics for Materials and Emergent Technologies, Campus de Gualtar, 4710-057 Braga, Portugal*
[2] *BCMaterials, Basque Center for Materials, Applications and Nanostructures, UPV/EHU Science Park, 48940 Leioa, Spain*
[3] *Basque Foundation for Science, Ikerbasque, 48009 Bilbao, Spain*

1.1 Introduction

Tissue engineering (TE) is a branch of regenerative medicine that aims to repair and restore damaged or lost tissues using biological substitutes [1]. TE combines three key factors to seek this ultimate goal: cells, scaffolds, and biochemical and/or biophysical cues [2]. Scaffolds are used to provide a supportive setting that encourages cell growth and matrix synthesis, thereby promoting the formation of new tissue. In turn, biochemical and biophysical cues are employed to create the optimal microenvironment for long-term communications between cells and surrounding tissues/organs. The ultimate objective is to closely mimic the native microenvironment of the impaired tissue [2, 3].

Besides the well-established biochemical cues provided by bioactive factors, electrical signals play a crucial role in cell activity as a biophysical cue. Electrical signals regulate a variety of typical physiological processes, from brain activity to heartbeat [2, 4]. In this context, electroactive biomaterials have emerged as one of the most promising scaffolds for TE application in recent decades. These materials promote conduction of electrical charges and exchange of ions with the surrounding environment. Therefore, the incorporation of electroactive materials into scaffolds for TE can provide a platform for delivering electrical signals to cells, promoting cell proliferation or differentiation, and consequently tissue regeneration [5]. The characteristics of these materials can be modified by adjusting their composition, morphology, and processing conditions to optimize their performance for specific TE applications.

An in-depth understanding of how the cellular microenvironment evolves over time and the pathways that might be used to impose electricity on cells/tissues via electroactive materials is critical for the development of active scaffolds. Additional

Stimuli-Responsive Materials for Tissue Engineering, First Edition.
Edited by Clarisse Ribeiro, Unai Silván, and Senentxu Lanceros-Méndez.

investigations in this field aim to develop tunable electroactive materials with enhanced biocompatibility and improved electrical properties, opening up new possibilities for TE and regenerative medicine [5, 6].

1.2 Relevance of the Electrical Signals in the Human Body

Human body functions and homeostasis are influenced by physical stimuli [7]. Electromagnetic radiation, temperature, and mechanical forces are external physical stimuli known to present a significant impact on biological events. On the other hand, intrinsic electrical signals and mechanical forces (compressive loading, hydraulic pressure, shear stress, and tensile forces) are the internal physical stimuli that also have been demonstrated to naturally control cell fate by enhancing cell target functions such as adhesion, migration, proliferation, and differentiation [8]. Although all physical stimuli are important and have relevance on the cellular level, it is widely accepted that electrical signals are the most prominent physical stimuli for controlling many physiological processes and may even outperform other physical cues [2, 4, 9].

In the eighteenth century, Luigi Galvani and coworkers demonstrated the presence of an intrinsic form of electricity responsible for nerve conduction and muscle contraction. For the first time, they reported the possibility of causing muscle twitches in freshly killed animals using electric signals and then demonstrated the existence of the injury potential [10]. Since then, many researchers corroborated those findings and clearly defined phenomena such as membrane potentials (Vm) and later action and resting potentials. Currently, it is stablished that Vm are fundamental features of all cells and are caused by the movement of ions across the cell membrane, which results in charge separation and the generation of an electrical potential difference. This occurrence is both a by-product and a regulator of a wide range of essential properties at multiple biological organization levels, intrinsic to the normal function of all cells, organelles, and molecules [9, 11]. For instance, these signals drive processes such as respiration, influence pH levels, and modulate the redox state. Additionally, they facilitate cell-to-cell communication, guide cell migration (in a process known as galvanotaxis), and contribute to tissue repair [12].

Electrical signals enable cells to communicate with each other at the tissue and organ levels, which in turn plays a crucial role in the organ's performance [13]. Various types of tissues exhibit specific electrically conductive and/or electrically responsive properties, such as piezoelectricity and ferroelectricity, and thus several functions are controlled by electrical signals. Figure 1.1 depicts tissues that inherently make use of these signals to regulate different physiological processes [2, 4], such as neural communication [14, 15], bone regeneration [16, 17], heartbeat activities [18, 19], muscle contraction [20, 21], and wound healing [22, 23]. Moreover, compelling evidence indicates that alterations in cellular and tissue excitability, along with more extensive electrical fields across tissues, may have implications for embryo development and tumorigenesis [12, 24].

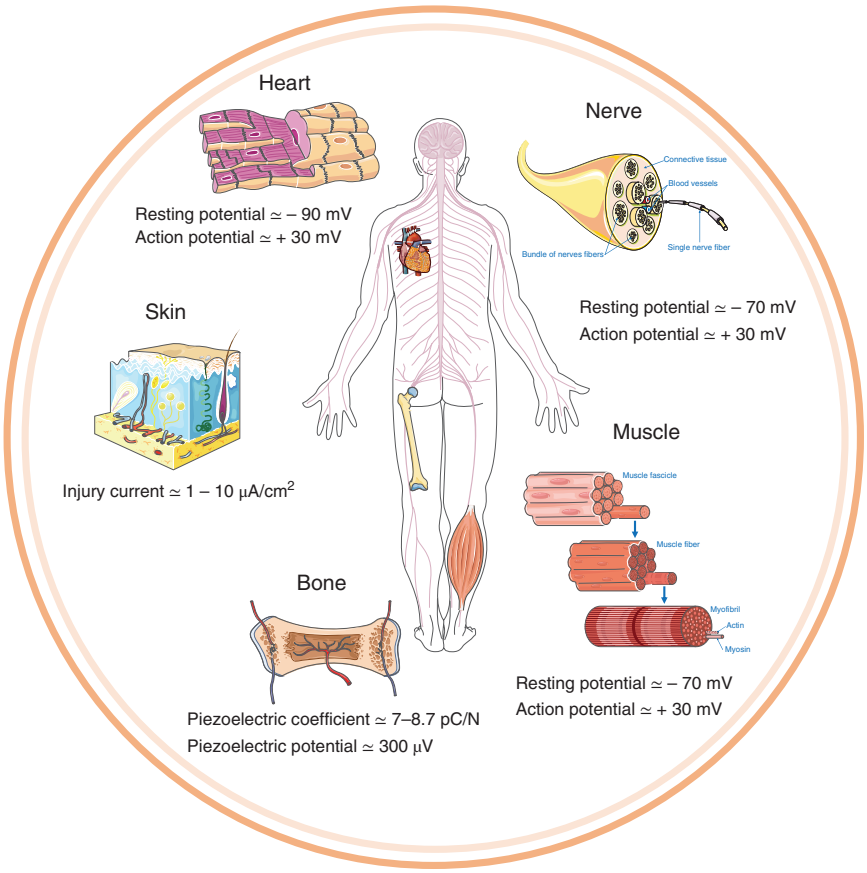

Figure 1.1 Electric activity in the human body.

All these organs and tissues are known as electroactive tissues given their ability to generate and transmit electrical signals [13]. The nervous system exhibits the highest electrical activity, facilitating the transmission of signals from neurons through synapses to their respective destinations. The pacemaker cells in the heart, a specialized subpopulation of cardiomyocytes, generate rhythmic impulses that propagate through the entire heart and trigger mechanical activity, i.e., heart beating, in ventricular myocytes [6]. Bone exhibits piezoelectric properties, and it generates electrical charges in response to mechanical stress, which ultimately triggers cell growth and repair. Regarding skin, the normal electrical fields are disrupted after an injury, resulting in abnormalities. The Vm is severely disrupted, and a wound electrical field forms, driving cells to the wound for healing purposes, such as epithelial cells in skin injuries [25].

Given the importance of electrical cues in physiological tissue function, disease manifestation and progression, and regeneration, extensive research has been conducted to identify the ideal conditions for electrically stimulating tissues and modulating cell response in vitro, in order to understand the action mechanisms

Table 1.1 Overview of *in vitro* and *in vivo* electrical stimulation effects on different mammalian cells.

Cell type	*In vitro* effect of electrical stimulation	*In vivo* effect of electric stimulation	Refs.
Neurons	Enhanced neurite outgrowth, improved myelinization, and increased synaptic activity	Enhanced axonal sprouting, and improved functional recovery after spinal cord injury	[14, 15, 26, 27]
Skeletal muscle cells	Enhanced myogenic differentiation and myotubes contraction	Improved muscle function in mice with muscular atrophy	[20, 21, 28, 29]
Bone cells	Increased osteoblast cell proliferation, suppressed osteoclast recruitment, and enhanced calcification	Improved bone regeneration, and increased matrix formation around orthopedic implants	[16, 30, 31]
Skin cells	Increased collagen production, stimulation of proliferation, and differentiation of keratinocytes, fibroblasts, and endothelial cells	Enhanced epithelialization and improved wound healing	[32]
Cardiac muscle cells	Enhanced stem cell differentiation, improved contractile function and maturation, and promoted cardiomyocyte alignment and synchronization	Promoted angiogenesis, reduced apoptosis, and inflammation in ischemic myocardium	[33–35]
Cancer cells	Reduced proliferation, apoptosis induction, and metastasis suppression	Enhanced sensitivity to usual therapies, and decreased tumor growth in mice with breast cancer	[36, 37]

and develop functional therapeutic interventions, especially in the scope of TE. Table 1.1 provides a few examples of the influence of in vitro and in vivo electrical stimulation on different mammalian cell types. Notwithstanding, the given stimulation may vary depending on several factors, such as the strength and frequency of the electric field, its duration, and specific characteristics of the cells being stimulated.

The evaluation of electric fields in a biological context has led to a better understanding of several aspects of living organisms, including their pivotal role in tissue development, regeneration, and repair processes. Furthermore, it has shed light on the mechanisms underlying cellular detection of electric fields and subsequent modulation of downstream signaling pathways, responsible for orchestrating cellular responses. These discoveries have not only opened up new avenues for scientific inquiry but also hold great promise for the development of innovative therapeutic interventions [38].

1.3 Relevance of the Electrical Signals in Cell Processes

The delivery of electrical signals to cells is closely related to changes in their Vm. Every cell presents a Vm that reflects the electrical potential difference across its plasma membrane. This potential is determined by the balance of ionic concentration on both sides of the membrane, and this balance is tightly regulated by ion channels and pumps [39].

Almost all cells present a Vm in the range of −10 to −90 mV at a steady state [40]. However, the specific value varies among different cell types, for instance, neurons typically exhibit a Vm around −70 mV [41], while cardiac cells tend to be closer to −90 mV [42]. The maintenance of this long-term steady state is critical for regulating cell proliferation and establishing tissue-level behaviors and patterns. Nevertheless, this state is influenced by several factors, including a diverse array of ion channels, variations in the expression of channels and isoforms with distinct response characteristics and ion affinities, and posttranslational channel alteration [40].

The difference in the electrical potential across the cell membrane is dynamic in the vast majority of cells. The mechanism through which each cell manages electrical signals determines whether the cell is excitable or non-excitable [43]. In excitable cells, such as muscle and neuron cells, Vm is also named resting potential and defines the non-excited state of the cell responsible for internal processes such as basal metabolic activities that sustain their homeostasis and prepare for forthcoming signaling events. These cells can change to non-resting states, known as action potentials, which correspond to the moment when the cell is actively involved in signaling, communication, or other forms of cellular activity [44]. Action potentials involve a rapid change in Vm when excitable cells receive a stimulus that exceeds a certain threshold. The stimulus triggers a chain of events that includes the opening and closure of voltage-gated ion channels, resulting in a distinct shift in Vm, as represented in Figure 1.2. In the nervous system, it serves the purpose of transmitting signals throughout the body. In skeletal and

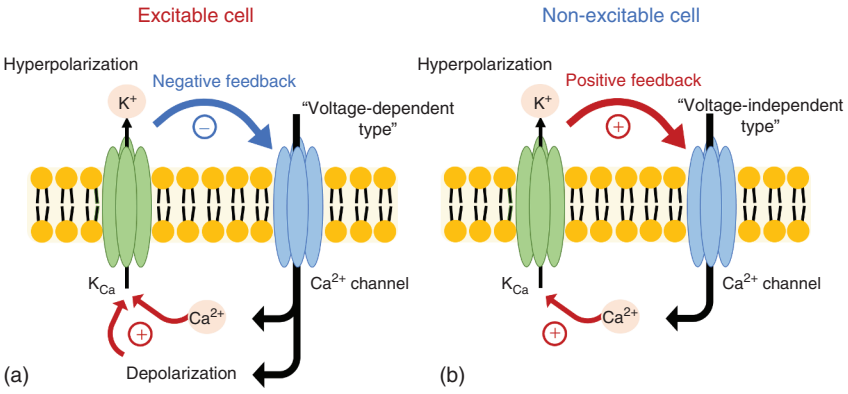

Figure 1.2 Correlation between changes in Vm and intracellular calcium concentration ($[Ca^{2+}]$) in both (a) excitable and (b) non-excitable cells.

cardiac muscle, action potentials are responsible for coordinating and regulating the excitation–contraction coupling. Despite not having an action potential, non-excitable cells frequently have a dynamic Vm (Figure 1.2) [44], which serves a wide range of essential biological functions [14, 16, 45, 46]. Additionally, in excitable cells, depolarization triggers the opening of voltage-dependent Ca^{2+} channels (VDCC), resulting in calcium influx. Ca^{2+}-activated K^+ (KCa) channels, particularly large-conductance Ca^{2+}-activated K^+ channels (BKCa – Big potassium calcium-activated channels), are activated and subsequently induce hyperpolarization, thereby reducing Ca^{2+} influx through VDCC. Thus, BKCa channels act as a negative feedback mechanism to VDCC activity. In non-excitable cells, Ca^{2+} channels mainly consist of voltage-independent calcium channels (VICC) instead of VDCC. Ca^{2+} release-activated Ca^{2+} (CRAC) channels (VICC type) activate KCa channels, leading to hyperpolarization [47]. Unlike VDCC, CRAC channels are not closed by hyperpolarization since they lack a voltage-sensing domain. Stromal interaction molecules open the pores of CRAC channels, enabling them to conduct Ca^{2+} even at hyperpolarized potentials. The hyperpolarization induced by the KCa channel increases the driving force for Ca^{2+}, promoting its influx through the CRAC channel. Consequently, BKCa channels contribute to positive feedback for Ca^{2+} influx through CRAC channels. Activation of CRAC channels results in minimal membrane depolarization due to their lower single-channel conductance compared to VDCC. The slight depolarization resulting from Ca^{2+} influx through CRAC channels is counteracted by the significant membrane hyperpolarization caused by K^+ conductance through the BKCa channels [48, 49].

Calcium influx is a fundamental requirement for normal matrix metabolic and secretory functions [50, 51], the change in Vm being closely related to calcium influx, independently of the cell type [37]. It can boost cell metabolism and promote adenosine triphosphate (ATP) depletion, resulting in cytoskeleton reorganization and alterations in membrane-related cellular activities like endocytosis, exocytosis, adhesion, migration, and proliferation [52].

Although the Ca^{2+} release-activated Ca^{2+} (CRAC) channel is primarily responsible for calcium influx in non-excitable cells (Figure 1.2b), voltage-gated ion channels are also expressed in these cells and are also responsible for cell membrane depolarization, similar to what happens in excitable cells [53–55]. The precise physiological functions assigned to these channels in non-excitable cells, as well as their regulation mechanisms, are still debated and being researched [56, 57]. However, it is well established that electrocoupling is the response mechanism to electrical stimulation in the widest range of cells, both excitable and non-excitable cells, such as neurons [58], pancreatic and bone cells, and even tumor cells [54]. Exposing cells to electric fields can directly activate L-type voltage-gated calcium channels, a type of VDCC. This activation triggers several regulatory responses through enzymatic activities, enhancing the expression of differentiation-related genes [59]. An example of a molecular signaling pathway that triggers cell differentiation processes in the presence of electrical fields is the cyclic adenosine monophosphate (cAMP)-dependent pathway [58, 60, 61]. This pathway is activated by the elevated intracellular concentration of Ca^{2+}, which leads to the activation

of adenyl cyclase (AC). The AC catalyzes the conversion of ATP to cyclic AMP (cAMP), a secondary messenger molecule involved in numerous regulatory signaling pathways [62]. Thus, the activation of VDCC by electrical pulses increases cellular Ca^{2+} influx and consequently starts the AC signaling cascade. In addition to this pathway, the pERK and Wnt/β-catenin pathways are also activated by the opening of the VDCC and play an important role in stem cell differentiation [63, 64]. Note that calcium oscillations were always the trigger [54, 59, 62, 65].

Besides calcium ions, additional electrical-signal-related pathways regulate proliferation, differentiation, and apoptosis [37, 55]. Figure 1.3 summarizes possible pathways involved in biological response to electrical stimulation.

Reactive oxygen species (ROS) are thought to be important mechanisms involved in cell response to electric signals. Controlled induction of ROS at physiological levels can improve interactions with signaling molecules [66]. Several studies have demonstrated that moderate ROS levels activate mitogen-activated protein kinase (MAPK) cascades, which are central signaling pathways that regulate cellular processes, including proliferation, differentiation, and apoptosis. Thus, a mild increase in hypoxia-induced ROS has been revealed to mediate cell proliferation and differentiation [67–69].

Regarding transmembrane proteins, it has been reported that electrical signals can reorganize the membrane proteins and lipids on the cell's external site due to the

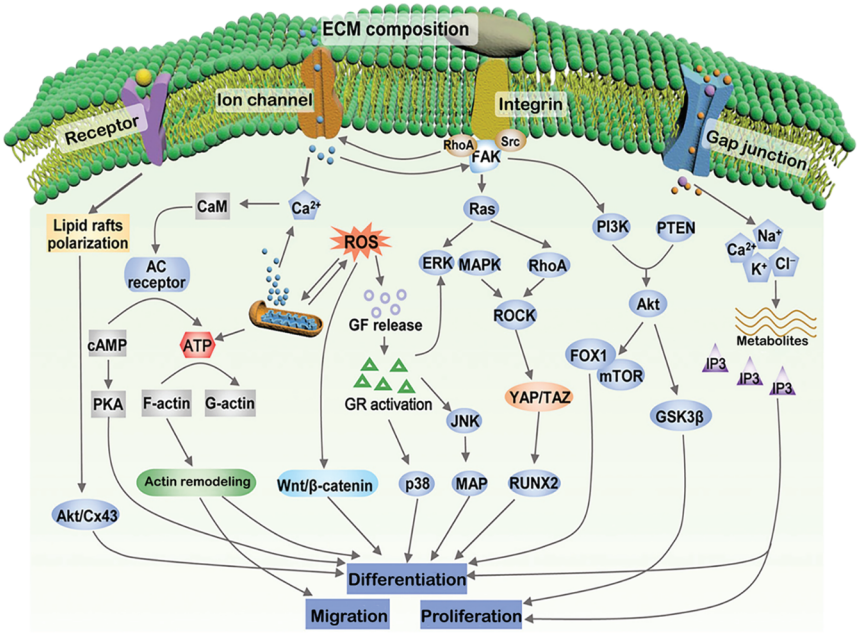

Figure 1.3 Possible intracellular responses to electrical stimulation. Membrane receptors, ion channels, integrins, and gap junctions mediate electrical stimulation, triggering responses such as cell proliferation, migration, and differentiation. Source: Liu et al. [40]/ John Wiley & Sons.

induction of a relative electrophoretic movement of these components on the cell's exterior [70]. Electrical fields can up-regulate the expression of epidermal growth factor receptor (EGFR) on fibroblasts, corneal epithelial cells, and keratinocytes [69], inducing its redistribution and accumulation primarily on the cathode side of the cell. Besides this asymmetric distribution, it can cause the colocalization of membrane lipids, resulting again in the triggering of MAPK signaling cascades [69, 71].

Considering the previous remarks, it appears that the application of electrical stimuli in diverse tissues, whether excitable or non-excitable, might be an efficient strategy for repairing injured tissues due to their ability to promote tissue proliferation and differentiation. Even though each tissue has a distinct response to electrical signals with varying degrees of sensitivity, electrical signals appear to trigger biochemical and physiological processes, leading to effective and specific tissue regeneration responses. By applying controlled electrical stimulation, electroactive materials can harness this potential and actively influence tissue repair. They offer a unique and powerful approach to tissue regeneration by delivering electrical stimulation that mimics natural cellular microenvironments.

1.4 Types of Electroactive Materials

Electroactive materials are a subset of smart materials that can translate a physical or chemical stimulus into an electrical signal, and vice versa, in a reproducible and predictable manner. They are used in a wide range of applications, including sensors, actuators, energy-harvesting, and biomedical devices [72]. Further, electrically conductive materials also play a relevant role in the area of tissue engineering and will be considered in this section. As the basic principle of tissue engineering is to mimic the extracellular microenvironment and, as described, electrical signals are one of the human body's main physical stimuli, electroactive materials and electrically conductive materials are being highly pursued for tissue engineering purposes. Among the different electroactive materials, in the following, conductive, piezoelectric, magnetoelectric, and thermoelectric materials will be considered based on their relevance in the tissue regeneration area.

1.4.1 Conductive Materials

Conductive materials can conduct electricity through them, presenting low resistance to the movement of electric current, whether the charge carriers are ions or electrons [73]. The conductivity (σ, S/m) of a material represents its ability to conduct electric current and can be quantified by Eq. (1.1), where R is the material's electrical resistance, A is its cross-sectional area, and l is its length.

$$\sigma = \frac{l}{RA} \tag{1.1}$$

The electronic structure of a material defines whether a material is an insulator, a semiconductor, or a conductor. The band gap theory is commonly used to distinguish these three types of materials (Figure 1.4a). Conductors are characterized

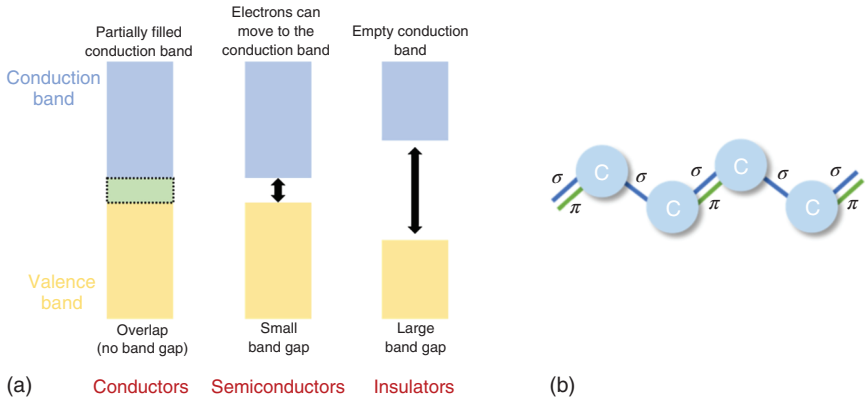

Figure 1.4 (a) Band gap theory of conductors, semiconductors, and insulators; (b) Illustration of a conductive polymer-conjugated backbone with alternating patterns of double and single bonds.

by valence bands overlapping the conduction bands, allowing the valence electrons to move freely and propagate in the conduction band. Semiconductors have small energy gaps and, upon excitation, electrons can cross to reach the valence band, allowing the conduction of current. On the other hand, insulators have larger band gaps that cannot be crossed by electrons and thus disabled them from conducting current [74]. As electrons in polymers are not delocalized, they cannot flow easily from atom to atom to conduct currents. Hence, conductive polymers' conductivity cannot be explained by the gap band theory; rather, it is explained by the existence of a conjugated backbone, whereby carbon atoms are alternately connected by single and double bonds (Figure 1.4b). The single bond detains a strong localized s-bond, and the double bond has a weaker localized p-bond and a stronger s-bond. This creates a continuous overlapping of p_z-orbitals in the chain of p-bonds allowing for p-bonds electrons to be easily delocalized and transferred along the polymer chain, consenting electrical flow [46, 74].

Electronic conductors include metals (e.g., gold, silver, iron), members of the carbon family (e.g., carbon nanotubes (CNTs) and graphene), and conductive polymers (CPs), while among ionic conductors are polymer electrolytes (e.g., poly(vinyl alcohol) (PVA) and chitosan), solid-state ionic conductors (e.g., polyethylene oxide (PEO)), and ionic liquids (ILs). Due to their versatility, conductive materials can be designed to meet specific requirements concerning the target tissue. Metal-based nanoparticles, CNTs, graphene-based materials, and ILs are being used for TE applications [3]. They can be used purely or in the form of composites with other materials and have shown suitable results in promoting cellular activities, ranging from facilitating electrical signaling in neurons to influencing cell migration, adhesion, proliferation, and differentiation in different cell types [75–78]. However, the biological application of inorganic materials is limited, either by the cost or by cytotoxicity problems [46]. Organic CPs allow the combination of good biocompatibility, and chemical and mechanical properties of polymers, with the electrical and optical properties of metals, which makes them highly pursued to overcome the drawbacks

of inorganic materials [79]. The first CP discovered was polyacetylene (PA) in 1977, leading to the Nobel Prize in Chemistry for Alan MacDiarmid, Alan Heeger, and Hideki Shirakawa in 2000. Since then, a variety of CPs has been investigated, including polypyrrole (PPy), polyaniline (PANI), poly(3,4-ethylenedioxythiophene) (PEDOT), poly(*p*-phenylene vinylene) (PPV), and polyacetylene (PAc) [80]. CPs have lower conductivity compared to most metals; thus, in order to increase their conductivity or improve performance, CPs are often doped or blended with other materials, and can reach electrical conductivities as high as 10^2–10^6 S/m [79, 81, 82].

CPs' electrical conductivities range meets the conductivities present in many biological tissues, making them valuable materials for creating biomimetic environments that can influence and support cellular functions [83]. PPy, PANI, and PEDOT are the most used for TE and are particularly interesting for neural and cardiac excitable cells, and also for non-excitable bone cells [84–86]. Non-excitable cells/tissues can generate endogenous charges that stimulate regenerative responses through VDCCs activation, as it is the case of bone cells [85]. The frequent application of CPs in this field arises from the suggestion that bone healing can be promoted by restoring the bioelectrical microenvironment [87]. An overview of the most relevant conductive materials for TE applications is presented in Table 1.2.

The main drawback of conductive materials is the need for invasive electrodes, which can be overcome by piezoelectric or magnetoelectric materials, that can be remotely stimulated.

1.4.2 Piezoelectric Materials

Piezoelectric materials are the class of electroactive materials that can translate mechanical inputs into an electric output (direct piezoelectric effect), or, alternately, generate a mechanical output when exposed to an electric field (inverse piezoelectric effect).

The direct piezoelectric effect was first discovered by the Curie brothers, Jacques and Pierre Curie, in 1880 [106], and the inverse piezoelectric effect was mathematically deduced by Lippman in 1881 [107] and later confirmed by the Curie brothers. The piezoelectric effect, or piezoelectricity, occurs because of the crystal structure of

Table 1.2 Overview of relevant conductive materials for TE applications.

		Conductivity (S/m)	TE applications
Inorganic conductive materials	Gold	4.9×10^7 [88]	Bone [89–91], muscle [92, 93], skin [94, 95], neuro [96–99], cardiac [100–102]
	CNTs	10^6–10^7 [103]	
	Graphene	10^8 [103]	
Organic conductive materials	PPy	10^2–10^5 [104]	Bone [84, 90, 105], muscle [84, 92], skin [45, 84, 105], neuro [84, 96, 97, 105], cardiac [84, 101, 105]
	PANI	10^4 [82]	
	PEDOT	10^4–10^5 [104]	

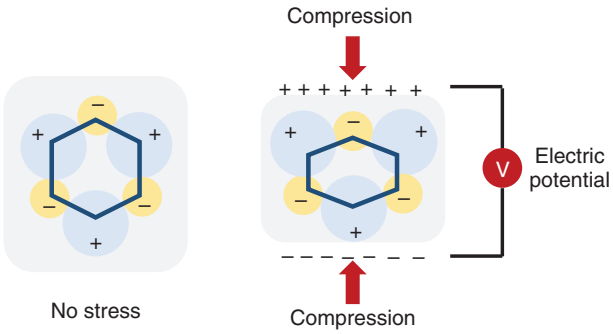

Figure 1.5 Piezoelectric effect in quartz.

these materials that lacks a center of symmetry (illustrated by the quartz piezoelectric effect in Figure 1.5).

This non-centrosymmetric structure gives rise to a non-centrosymmetric arrangement of positive and negative ions, resulting in dipolar moments. When a mechanical stress is applied, it disrupts the dipole moments, causing a charge redistribution across the two faces of the crystal lattice, resulting in the generation of an electrical potential proportional to the applied force. Conversely, when an electric field is applied, the dipolar moments within the crystal structure suffer variations along the electric field direction, and the material suffers a mechanical deformation proportional to the applied electrical field. The piezoelectric effect can be quantified using Eq. (1.2) for direct piezoelectric coefficient (d_{ij}, pC/N), or Eq. (1.3) for inverse piezoelectric coefficient (e_{ij}, C/m^2), where D is the electric displacement field, E corresponds to the electric field strength, and X and x are the mechanical stress and strain, respectively [108].

$$d_{ij} = \left(\frac{\partial D_i}{\partial X_j}\right)^E = \left(\frac{\partial x_i}{\partial E_j}\right)^X \tag{1.2}$$

$$e_{ij} = \left(\frac{\partial D_i}{\partial x_j}\right)^E = -\left(\frac{\partial X_i}{\partial E_j}\right)^x \tag{1.3}$$

Piezoelectricity is found in many biological tissues, including bone, skin, tendon, and cartilage, and has been referred to as an extensive and fundamental feature of biological tissues since it is implicated in essential physiological events [109]. As TE is mainly based on biomimetic approaches, the interest and research on these electroactive materials have been strongly increasing [110]. Piezoelectric materials can generate local electrical potentials with non-invasive methods, without the need to use electrodes and/or wires that could lead to tissue inflammation when implanted, and instead using vibration plates, sound, or ultrasound (US) stimulation, among others [41].

Piezoelectric materials can be inorganic, organic, or combination of them in composites. Among inorganic are quartz, lead zirconate titanate (PZT), barium titanate ($BaTiO_3$), and zinc oxide (ZO); and among organic are synthetic polymers such as

Table 1.3 Overview of relevant piezoelectric materials for TE applications.

		Piezoelectric coefficients (d_{ij}, pC/N)	TE applications
Inorganic piezoelectric materials	PZT	225–590 [115]	Bone [116, 117], muscle [118], skin [119], neuro [116], cardiac [19, 116, 117], muscle [118], skin [119], neuro [116], cardiac [19]
	$BaTiO_3$	191 [115]	
	ZnO	13 [115]	
Organic piezoelectric materials	PVDF	24–34 [109]	Bone [120, 121], muscle [121], skin [122], neuro [41], cardiac [19, 120, 121], muscle [121], skin [122], neuro [41], cardiac [19]
	P(VDF-TrFE)	38 [109]	
	PLLA	9.82 [109]	
	PHBV	1.3 [123]	
	Silk	1.5 [124]	

nylon, poly(vinylidene fluoride) (PVDF), PVA, poly(urethane) (PU), poly(L-lactic acid) (PLLA), poly(lactic-*co*-glycolic acid) (PLGA), and poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV)), and natural polymers including chitosan, silk, collagen, and cellulose. Although inorganic piezoelectric materials have higher piezoelectric response, they can be fragile and show limited or no biocompatibility. This makes organic piezoelectric polymers the most researched for TE applications, due to their biocompatibility and tunable mechanical characteristics [109, 111]. Further, they are easier to process in a wide variety of morphologies [110].

PVDF and its copolymers, as poly(vinylidene fluoride-trifluoroethylene) (P(VDF-TrFE)), are the most explored for TE applications due to their high dielectric constants (6–12) and piezoelectric coefficients ($|d_{33}| = 24–34$ pC/N) [109]. In the last decade, electrical stimulation delivered by PVDF-based scaffolds has been strongly focused on bone TE applications [17, 31, 112] and expanded to other tissues, such as muscle [21] and nerve [113, 114]. PLLA, PHBV, silk, cellulose, and collagen are also being investigated because of their biodegradability, although they present lower piezoelectric coefficients. An overview of the most relevant piezoelectric materials for TE applications is presented in Table 1.3.

1.4.3 Magnetoelectric Materials

Most magnetoelectric materials applied in the area of tissue regeneration are composite materials that integrate magnetic and piezoelectric components. The coupling of these materials properties results in a magnetoelectric coupling, which allows a magnetic field to switch/tune the polarization state of the material (direct magnetoelectric effect), and, conversely, its magnetization can be switched/tuned by an electric field (inverse magnetoelectric effect) [125, 126]. The magnetic component of the magnetoelectric system, which can have magnetic and magnetostrictive properties, can sense an applied magnetic field and translate it into a mechanical deformation, that is then converted by the piezoelectric component into an electrical potential (Figure 1.6) [127].

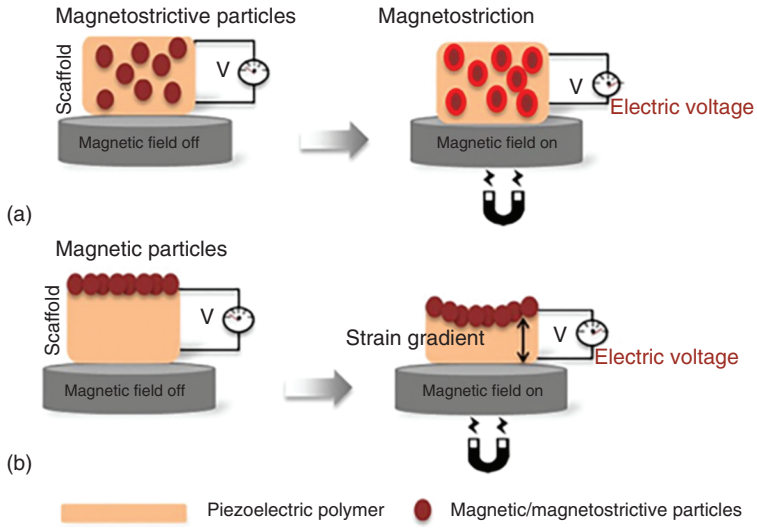

Figure 1.6 Magnetoelectric effect using (a) magnetostrictive particles or (b) magnetic particles. Source: Ribeiro et al. [127]/with permission of Elsevier.

Magnetoelectricity was first described by Pierre Curie in 1894 [128] and experimentally observed by Astrov in 1960 [129]. Magnetoelectric scaffolds are a relatively new TE paradigm. Magnetic nanoparticles such as iron oxides (Fe_3O_4, Fe_2O_4), iron-based metal oxides ($CoFe_2O_4$), and Terfenol-D are frequently combined with piezoelectric polymers such as PVDF, P(VDF-TrFE), PLLA, PLGA, and PHBV [111, 130–135]. Magnetic fields have been implemented for a wide range of biomedical applications, from magnetic resonance, and target drug delivery to hyperthermia, due to their deep tissue penetration and highly controllable motion of magnetic materials [40]. Thus, the use of magnetoelectric scaffolds may be effective in cases where the patient is immobilized and natural mechanical stimulation is not fully guaranteed, allowing the deployment of an external magnetic field to remotely promote tissue recovery [130]. These types of electroactive scaffolds are being highly investigated for bone and nerve regeneration [111, 135–138]. Since magnetic fields can penetrate brain tissue with limited signal attenuation and side effects, they are pursued from deep brain stimulation in neural tissue engineering applications [136].

1.4.4 Thermoelectric Materials

Thermoelectric materials are another class of smart electroactive materials, characterized by the ability to convert heat into electrical energy, or vice versa [139]. The thermoelectric effect applied in biomedicine is mainly based on the Seebeck effect, which was discovered in 1821 by Thomas Seebeck and consists in the generation of an electrical voltage by a temperature difference between two materials or regions [140]. When a temperature difference is applied across a material made of two metals or semiconductors – one n-type and one p-type – induces the movement of charges leading to the generation of a voltage difference (Figure 1.7a). Later in 1834, Jean-Charles-Athanase Peltier discovered the Peltier effect, which is

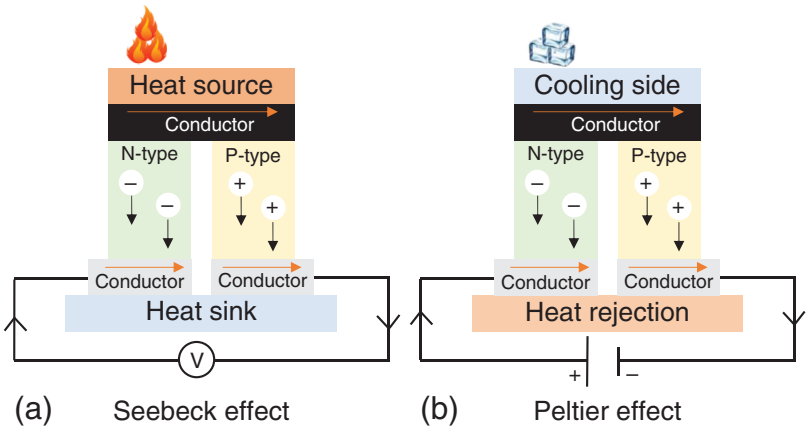

Figure 1.7 Thermoelectric materials: (a) Seebeck and (b) Peltier effects.

essentially the reverse of the Seebeck effect as it generates a temperature variation from the application of an electrical current. When an electrical current is applied across two metals or semiconductors, heat is either absorbed or released, depending on the direction of the current (Figure 1.7b). The thermoelectric effect comprises a third less-applied effect, called the Thomson effect. Discovered by William Thomson (later known as Lord Kelvin) in 1854, it establishes a mathematical relationship between the Seebeck and Peltier coefficients. It is distinguished by the generation of a temperature difference within a single conductor material when an electric current flows through it [141].

The performance of thermoelectric materials is measured by the dimensionless figure of merit (ZT), quantified by the Eq. (1.4), where S is the Seebeck coefficient, σ *is* the electrical conductivity, and K is the thermal conductivity. Typically, thermoelectric materials have low thermal conductivity and high electrical conductivity [142].

$$\mathrm{ZT} = \frac{S^2\sigma}{K} \tag{1.4}$$

Thermoelectric materials can be inorganic, including bismuth telluride (Bi_2Te_3), lead telluride (PbTe), silicon-germanium (SiGe), and skutterudite ($CoSb_3$); carbonaceous such as CNTs, and graphene; or organic, among them poly(3-hexylthiophene) (P3HT), PPy, PANI, PEDOT, and polythiophene (PTh) [139]. Inorganic thermoelectric materials are typically characterized by low mechanical performance, as they are brittle, are often toxic, and demand expensive microfabrication techniques for processing. Thus, organic polymeric thermoelectric materials are the most interesting alternatives for applications in the area of biomedicine, due to their low thermal conductivity, good flexibility, low cost, and non-toxicity [139, 143].

Thermoelectric materials and devices are being used in power generation, solid-state cooling, and heating. Regarding the biomedical field, these materials are mostly used to integrate biomedical sensor systems such as thermoelectric power generators (TEGs) or thermoelectrical coolers (TECs) [144]. Much research is being

devoted for developing implantable and wearable TEGs, to assist specific human tissues or organs as implantable medical devices [145, 146].

1.5 Relevance of the Material's Architecture

In addition to material and active response selection, it is crucial that the scaffold provides morphological cues resembling the tissue-specific microenvironment. The extracellular matrix of a defective site must be mimicked, with the scaffold providing cell support to fine tuning specific functions, as well as spatial and temporal guidance for multicellular processes of formation and regeneration of damaged or lost tissues. Therefore, the morphological features of the scaffold should meet a biomimetic approach, and thus be appropriate for the specific microenvironment in which it will be inserted.

Scaffolds with electroactive properties, particularly polymers or polymer-based materials, can be obtained in the most varied morphologies (Figure 1.8), including films, fibers, membranes, hydrogels, 3D structures, microspheres, or patterned surfaces, among others [147]. For their processing, and depending on the morphology requirements, there are several methods and techniques that can be used including doctor blade, spin-coating, screen printing, electrospinning, melt electrowriting, electrospray, phase separation techniques (e.g., thermally induced phase separation (TIPS), nonsolvent-induced phase separation (NIPS), and vapor-induced phase separation (VIPS)), salt leaching, soft lithography techniques (e.g., replica molding, microcontact printing, microtransfer printing, and micromolding in capillaries), and 3D printing, among others.

1.5.1 Films

For TE applications, films are often chosen for initial studies on the influence of material properties other than their morphology, such as the electroactive response, as they provide a simple flat surface that resembles conventional 2D culture conditions. Films can be processed by several techniques that include doctor blade and spin-coating, and printing technologies such as screen printing, and spray printing. Doctor blade, also known as blade or knife coating, is a widely used technique to obtain thin films on large area surfaces and is the most used for TE applications. The gap size between the blade and the substrate, and the polymer mass fraction define the thickness of the film. The blade is moved across a flat substrate in lab-scale production, whereas the substrate is moved, or the blade can be fixed for large-scale processes [147, 148]. After solvent evaporation, the final thickness of the film (d) decreases and is dependent on several parameters, such as the flow behavior, speed of coating, substrate surface energy, surface temperature, fluid surface tension, and viscosity, being determined by Eq. (1.5), where w is the gap width, c the concentration (g/cm^3), and ρ the density of the material in the film (g/cm^3) [147].

$$d = \frac{1}{2} w \frac{c}{\rho} \tag{1.5}$$

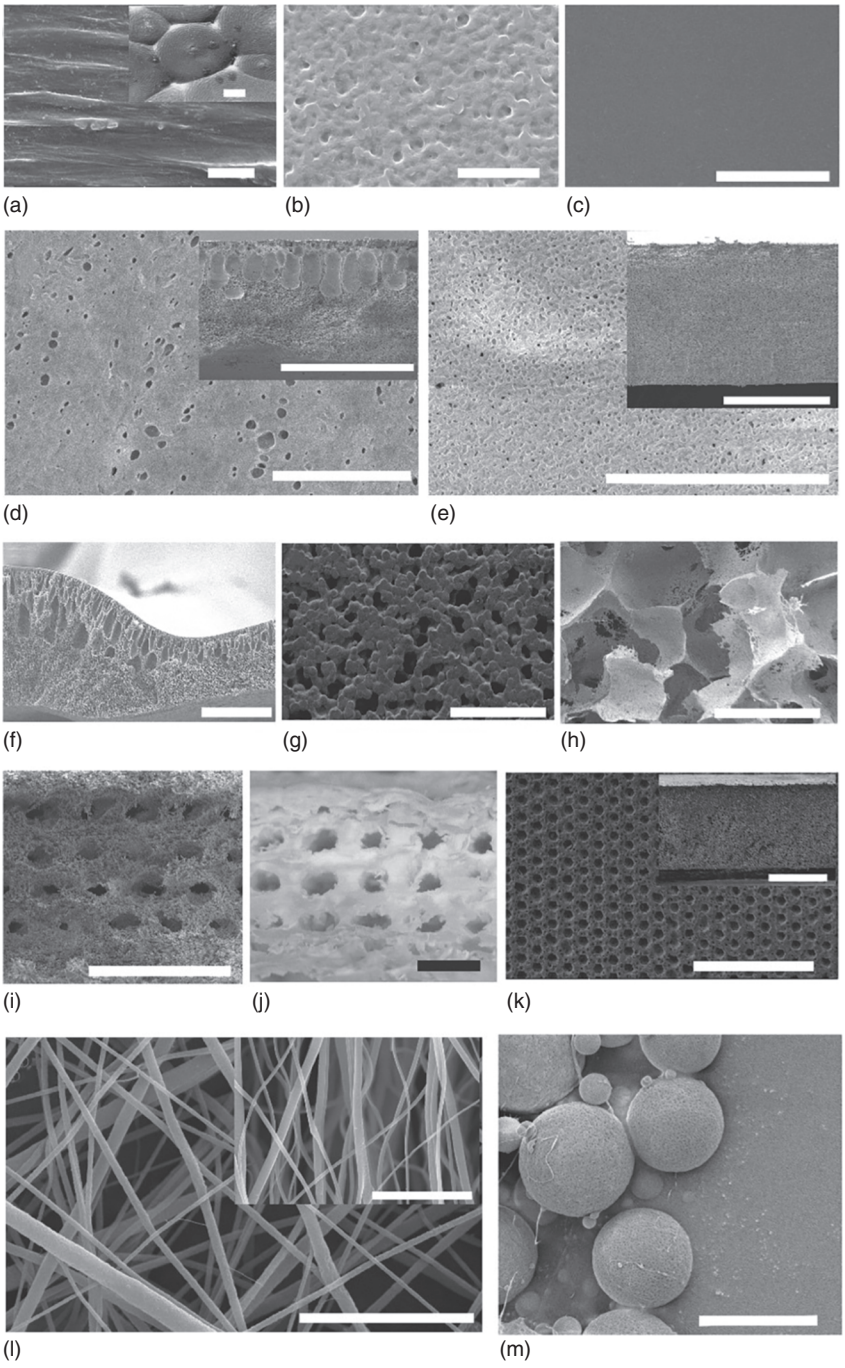
(a)
(b)
(c)
(d)
(e)
(f)
(g)
(h)
(i)
(j)
(k)
(l)
(m)

Figure 1.8 Representative SEM images of the distinct structures/morphologies: (a) β-PVDF film obtained by the doctor blade technique after mechanical stretching. Inset: The α-PVDF film before mechanical stretching. Scale bars: 5 μm (main) and 10 μm (inset). (b) β-PVDF film obtained by spin coating. Scale bar: 5 μm. (c) P(VDF-TrFE) film obtained by screen printing. Scale bar: 10 μm. (d) Porous P(VDF-HFP) film obtained by NIPS. Inset: Cross-section. Scale bars: 10 μm (main) and 100 μm (inset). (e) Porous P(VDF-CTFE) film obtained by NIPS. Inset: Cross-section. Scale bars: 30 μm (main) and 100 μm (inset). (f) Porous β-PVDF film obtained by NIPS (cross-section). Scale bar: 100 μm. (g) Porous β-PVDF film obtained by TIPS. Scale bar: 100 μm. (h) β-PVDF scaffolds obtained by solvent-casting particulate leaching. Scale bar: 500 μm. (i) β-PVDF scaffolds obtained by solvent casting and 3D nylon template. Scale bar: 400 μm. (j) β-PVDF scaffolds obtained by freeze extraction with a 3D PVA template (optical microscope image). Scale bar: 1 mm. (k) Patterned porous P(VDF-TrFE) structure obtained by replica molding. Inset: Cross-section. Scale bars: 500 μm. (l) Randomly electrospun β-PVDF fibers obtained by electrospinning. Inset: The oriented electrospun PVDF fibers. Scale bars: 10 μm. (m) β-PVDF spheres obtained by electrospraying. Scale bar: 5 μm. Source: Ribeiro et al. [147]/with permission of Springer Nature.

Surface charge and charge variation of piezoelectric and magnetoelectric biomaterials in bone, muscle, and nerve regeneration have been studied using scaffolds in film morphology [21, 112, 113, 132, 149, 150]. Besides, films with electroactive properties are designed for wound dressings due to their ability to adhere to the skin and protect the wound, as well as to be combined with different pharmaceutical compounds, including antibiotics, to promote wound regeneration and prevent bacterial infections [151–153].

1.5.2 Electrospun fibers

The fibrillar architecture of some extracellular matrix components, such as laminin, fibronectin, collagen, and elastin, has inspired the design of scaffolds with similar structures. Fiber scaffolds can be obtained by different methods and techniques, including phase separation, self-assembly, electrospinning, and melt electrowriting [154]. Electrospinning is the most used technique, due to its easy handling, low cost, and versatility. Through this technique, natural, synthetic, or composite polymer-based fibers can be obtained in random and oriented morphologies, with or without internal porosity, and with the most varied range of diameters, from micro- to nanofibers [155]. Because of fibers' easy processing and tailoring and large surface area-to-volume ratio, electrospun fibers are one of the most required and studied morphologies for TE applications. The electrospinning setup is composed of a syringe pump, a syringe filled with a polymeric solution and connected to a metallic needle, a metallic collector, and a power supply with high-voltage directly connected to the needle and the collector. Its functioning is based on an applied voltage to the polymeric solution, which is expelled as an electrically charged viscoelastic jet, with a controlled flow rate given by the syringe pump, toward the metallic collector. During this trajectory, the solvent evaporates from the jet, and the polymeric fibers are deposited onto the collector. Besides solution formulation, electrospinning

parameters can be optimized to tailor the obtained fibers, such as the flow rate, applied voltage, needle diameter, distance between the needle and the collector, and the type of collector used [147]. The use of a static collector allows to obtain fibers mats with random fiber distribution, and a rotative collector allows for obtaining aligned fiber morphologies.

Fibers not only mimic fibrous components of ECM but also enable the control of cell directionality by contact orientation, which is essential for specific applications. For instance, oriented fibers can be used to promote the directional growth of different cell types, such as neural [156, 157] and muscle cells [149, 158], to develop nerve guidance conduits to repair nerve defects [159, 160], and for the guidance of new bone formation [161, 162]. Electroactive fibers, either conductive, piezoelectric, or magnetoelectric composites, can deliver morphological and biophysical cues that are particularly interesting for TE [6, 163]. For instance, electrical stimuli given by fibrous scaffolds have proven to enhance cardiogenesis [164], myogenesis [165], neurite extension [166], and peripheral nerve repair [167].

1.5.3 3D Porous Scaffolds

The scaffold's internal structure, such as porosity, pore size, and interconnectivity, is highly influential in cell development. These features enable cell infiltration, migration, and interconnection, nutrients and waste diffusion, and help with tissue vascularization [168]. Porous architectures can be reproduced into films, fibers, membranes, or more complex 3D structures, using a variety of techniques, including phase separation, gas forming, salt leaching, and solvent-casting on 3D templates [147]. The porosity of the scaffolds is intimately related to its mechanical performance, for instance, higher porosities, in most cases, lead to decreased Young's modulus, but on the other side lead to an improved surface area that is available for cell attachment, so once again, the technique to be chosen is highly dependent on the application [169]. Porous scaffolds are often used for bone TE due to their resemblance with the trabecular bone [170, 171], but are also studied for other tissues such as neuronal [170, 171], muscle [172], and cardiovascular [173].

Because cells are surrounded by complex 3D microenvironments, porous 3D scaffolds are a relevant morphology for TE approaches [174]. These 3D structures can be obtained using the previously mentioned conventional methods but due to the scaffolds' complex 3D structure, these methods can be disadvantageous in terms of causing cytotoxicity, because the solvents are more difficult to completely evaporate during processing, and scaffolds' microstructure and resolution are more difficult to control [175]. As an alternative, rapid prototyping approaches can be used to develop 3D scaffolds. Rapid prototyping technologies, or additive manufacturing, include selective laser sintering, fused deposition molding, stereolithography, and 3D printing. These technologies are integrated with computer-aided design (CAD) software and scaffolds can be designed with controlled macro- (size and shape), micro- (pore size and shape, porosity, distribution, and porous interconnection), and nano-architecture (e.g., surface roughness and patterning). The controllable architecture of scaffold porosity allows for the

manipulation of cellular dynamics and the facilitation of cell attachment, elongation and proliferation, nutrient diffusion, and vascularization, with the potential to revolutionize TE and regenerative medicine by designing 3D scaffolds to meet the needs of individual patients in the scope of personalized medicine [176]. Porous scaffolds are being studied as delivery systems in addition to tissue repair and regeneration. Their interconnected porosity enables for relatively significant cargo loading, such as proteins and live cells [177]. Combining porous architecture with electroactive materials can be beneficial in controlling the molecule release through electrical stimulation [178], and even be used simultaneously for electrical therapy [179].

1.5.4 Hydrogels

With respect to materials that can be processed in a variety of morphologies with tailored properties and responses, particular mention must be devoted to hydrogels. Throughout the past two decades, hydrogel-based matrices have been among the most popular scaffolds for TE. While not confined to a specific architecture or morphology, hydrogels encompass natural, synthetic, or composite polymeric cross-linked networks. Their resemblance to the native ECM in terms of high water content, flexibility, and elasticity contributes to their increasing use. Hydrogels offer easy customization regarding mechanical characteristics, enabling processing into diverse forms such as films, fibers, and 3D structures. Additionally, they can be loaded with biochemical factors, molecules, and materials to fine tune their performance, can fill any space, and are designed to be implanted through injection, avoiding invasive surgical procedures [133, 180]. These great advantages make hydrogels highly studied for the regeneration of all types of tissues.

Hydrogels can be synthesized using physical or chemical cross-linking, leading to a 3D network structure with unique properties suitable for a wide range of applications [181]. Physical cross-linking methods (e.g., temperature-induced, ionic, molecular entanglement) involve the use of non-covalent interactions between the polymer chains, such as hydrogen bonding, crystallization, protein, hydrophobic or ionic interactions, among others. Hydrogels cross-linked physically are reversible and responsive to external stimuli, such as pH, temperature, and ionic concentrations. On the other hand, chemical cross-linking involves the formation of covalent bonds between polymer chains, resulting in permanent and stable networks with lower responsiveness to external stimuli but higher mechanical strength. Chemical cross-linking methods include the use of chemical reactions, cross-linking agents, and radiation. Both physical and chemical methods possess their advantages and limitations, and their selection is dependent on the specific requirements of the hydrogel application [182].

One or more elements within a hydrogel's polymeric network structure can retain electroactive properties, resulting in a stimuli-responsive hydrogel, more specifically an electroresponsive hydrogel [183]. Electroresponsive hydrogels are frequently used for drug delivery purposes [184–186], but they can also be found in regeneration applications [187, 188].

1.6 Final Remarks

Electroactive biomaterials have emerged as a highly potential and needed approach to complement traditional methods for tissue repair and regeneration Given the presence of electrical and mechanoelectrical stimuli in various tissues of the human body, the use of smart materials, and in particular electrically conductive and piezoelectric ones, has shown to be a promising approach for tissue regeneration. This kind of biomaterials not only provide cellular support but also actively interact with the surrounding cells, leading to a more biomimetic recreation of the natural tissue microenvironment.

In this context, the primary focus should be on tailoring the biomaterial's morphology to match the specific tissue type being treated and the specific electrical signals to be statically and/or dynamically delivered. This approach considers the unique characteristics of the targeted tissue. In this way, developing an active biomaterial with an appropriate morphology capable of delivering physical stimuli to the target tissue, presents a needed and promising option for tissue repair treatments of specific tissues.

Acknowledgments

This work was supported by national funds through the Fundação para a Ciência e Tecnologia (FCT) and by ERDF through COMPETE2020 - Programa Operacional Competitividade e Internacionalização (POCI): strategic funding UID/FIS/04650/2020 unit, research grant SFRH/BD/145455/2019 (EOC), and contract under the Stimulus of Scientific Employment, Individual Support (CEECIND) doi.org/10.54499/2020.04163.CEECIND/CP1600/CT0019 (CR). This study forms part of the Advanced Materials program and was supported by MCIN with funding from European Union NextGenerationEU (PRTR-C17.I1) and by the Basque Government under the IKUR program. The authors also acknowledge funding by the Department of Education of the Basque Government (PIBA program, Grant number 2022333047), from the Basque Government Industry Department under the ELKARTEK program, and by the Spanish Ministry of Science and Innovation (Grant number PID2022-138572OB-C42).

References

1 O'Brien, F.J. (2011). Biomaterials & scaffolds for tissue engineering. *Materials Today* 14 (3): 88–95.
2 Bielfeldt, M., Rebl, H., Peters, K. et al. (2022). Sensing of physical factors by cells: electric field, mechanical forces, physical plasma and light—importance for tissue regeneration. *Biomedical Materials & Devices* 19: 221–223.
3 Esmaeili, H., Patino-Guerrero, A., Hasany, M. et al. (2022). Electroconductive biomaterials for cardiac tissue engineering. *Acta Biomaterialia* 139: 118–140.

4 Casella, A., Panitch, A., and Leach, J.K. (2021). Endogenous electric signaling as a blueprint for conductive materials in tissue engineering. *Bioelectricity* 3 (1): 27–41.

5 Ning, C., Zhou, Z., Tan, G. et al. (2018). Electroactive polymers for tissue regeneration: developments and perspectives. *Progress in Polymer Science* 81: 144–162.

6 Zhang, X., Li, L., Ouyang, J. et al. (2021). Electroactive electrospun nanofibers for tissue engineering. *Nano Today* 39: 101196.

7 Pelaez, D., Hare, J.M., and Cheung, H.S. (2011). The role of mechanical forces in the cardiomyogenic differentiation of stem cells. *Stem Cell Bioengineering and Tissue Engineering Microenvironment* 8: 85–118.

8 Thrivikraman, G., Boda, S.K., and Basu, B. (2018). Unraveling the mechanistic effects of electric field stimulation towards directing stem cell fate and function: a tissue engineering perspective. *Biomaterials* 150: 60–86.

9 Funk, R.H. (2015). Endogenous electric fields as guiding cue for cell migration. *Frontiers in Physiology* 6: 143–146.

10 Bresadola, M. (1998). Medicine and science in the life of Luigi Galvani (1737–1798). *Brain Research Bulletin* 46 (5): 367–380.

11 Snyder, S., DeJulius, C., and Willits, R.K. (2017). Electrical stimulation increases random migration of human dermal fibroblasts. *Annals of Biomedical Engineering* 45 (9): 2049–2060.

12 Harris, M.P. (2021). Bioelectric signaling as a unique regulator of development and regeneration. *Development* 148 (10): dev180794.

13 Walker, B.W., Lara, R.P., Yu, C.H. et al. (2019). Engineering a naturally-derived adhesive and conductive cardiopatch. *Biomaterials* 207: 89–101.

14 McCaig, C.D., Rajnicek, A.M., Song, B., and Zhao, M. (2005). Controlling cell behavior electrically: current views and future potential. *Physiological Reviews* 85 (3): 943–978.

15 Lu, P., Wang, Y., Graham, L. et al. (2012). Long-distance growth and connectivity of neural stem cells after severe spinal cord injury. *Cell* 150 (6): 1264–1273.

16 deVet, T., Jhirad, A., Pravato, L., and Wohl, G.R. (2021). Bone bioelectricity and bone-cell response to electrical stimulation: a review. *Critical Reviews in Biomedical Engineering* 49 (1): 1–19.

17 Ribeiro, C., Moreira, S., Correia, V. et al. (2012). Enhanced proliferation of pre-osteoblastic cells by dynamic piezoelectric stimulation. *Royal Society of Chemistry Advances* 2 (30): 11504–11509.

18 Adadi, N., Yadid, M., Gal, I. et al. (2020). Electrospun fibrous PVDF-TrFe scaffolds for cardiac tissue engineering, differentiation, and maturation. *Advanced Materials Technologies* 5 (3): 1900820.

19 Gomes, M.R., Castelo Ferreira, F., and Sanjuan-Alberte, P. (2022). Electrospun piezoelectric scaffolds for cardiac tissue engineering. *Biomaterials Advances* 137: 212808.

20 Kim, S., Jang, L.K., Jang, M. et al. (2018). Electrically conductive polydopamine-polypyrrole as high performance biomaterials for cell stimulation

in vitro and electrical signal recording in vivo. *ACS Applied Materials & Interfaces* 10 (39): 33032–33042.

21 Ribeiro, S., Gomes, A.C., Etxebarria, I. et al. (2018). Electroactive biomaterial surface engineering effects on muscle cells differentiation. *Materials Science and Engineering: C* 92: 868–874.

22 Du, S., Zhou, N., Gao, Y. et al. (2020). Bioinspired hybrid patches with self-adhesive hydrogel and piezoelectric nanogenerator for promoting skin wound healing. *Nano Research* 13 (9): 2525–2533.

23 Das, R., Le, T.T., Schiff, B. et al. (2023). Biodegradable piezoelectric skin-wound scaffold. *Biomaterials* 301: 122270.

24 Ribeiro, M., Elghajiji, A., Fraser, S.P. et al. (2020). Human breast cancer cells demonstrate electrical excitability. *Frontiers in Neuroscience* 14: 404.

25 Ryan, C.N.M., Doulgkeroglou, M.N., and Zeugolis, D.I. (2021). Electric field stimulation for tissue engineering applications. *BMC Biomedical Engineering* 3 (1): 1–9.

26 Liu, Q., Telezhkin, V., Jiang, W. et al. (2023). Electric field stimulation boosts neuronal differentiation of neural stem cells for spinal cord injury treatment via PI3K/Akt/GSK-3β/β-catenin activation. *Cell & Bioscience* 13 (1): 4.

27 Smith, R.S., Kenny, C.J., Ganesh, V. et al. (2018). Sodium channel SCN3A (NaV1.3) regulation of human cerebral cortical folding and oral motor development. *Neuron* 99 (5): 905–913.e7.

28 Sakellariou, P., O'Neill, A., Mueller, A.L. et al. (2016). Neuromuscular electrical stimulation promotes development in mice of mature human muscle from immortalized human myoblasts. *Skeletal Muscle* 6: 1–14.

29 Ribeiro, S., Marques-Almeida, T., Cardoso, V.F. et al. (2023). Modulation of myoblast differentiation by electroactive scaffold morphology and biochemical stimuli. *Biomaterials Advances* 151: 213438.

30 Zhou, P., He, F., Liu, B. et al. (2019). Nerve electrical stimulation enhances osseointegration of implants in the beagle. *Science Reports* 9 (1): 4916.

31 Ribeiro, C., Correia, D.M., Rodrigues, I. et al. (2017). In vivo demonstration of the suitability of piezoelectric stimuli for bone reparation. *Materials Letters* 209: 118–121.

32 Wang, X.F., Li, M.L., Fang, Q.Q. et al. (2021). Flexible electrical stimulation device with Chitosan-Vaseline® dressing accelerates wound healing in diabetes. *Bioactive Materials* 6 (1): 230–243.

33 Hernández, D., Millard, R., Sivakumaran, P. et al. (2016). Electrical stimulation promotes cardiac differentiation of human induced pluripotent stem cells. *Stem Cells International* 2016.

34 Hirt, M.N., Boeddinghaus, J., Mitchell, A. et al. (2014). Functional improvement and maturation of rat and human engineered heart tissue by chronic electrical stimulation. *Journal of Molecular and Cellular Cardiology* 74: 151–161.

35 Valls-Margarit, M., Iglesias-García, O., Di Guglielmo, C. et al. (2019). Engineered macroscale cardiac constructs elicit human myocardial tissue-like functionality. *Stem Cell Reports* 13 (1): 207–220.

36 Iyer, M., Venugopal, A., Chandrasekhar, M. et al. (2022). Electrical based cancer therapy for solid tumours—theranostics approach. *Biosensors and Bioelectronics: X* 11: 100214.

37 Love, M.R., Palee, S., Chattipakorn, S.C., and Chattipakorn, N. (2018). Effects of electrical stimulation on cell proliferation and apoptosis. *Journal of Cellular Physiology* 233 (3): 1860–1876.

38 Messerli, M.A. and Graham, D.M. (2011). Extracellular electrical fields direct wound healing and regeneration. *Biological Bulletin* 221 (1): 79–92.

39 Wright, S.H. (2004). Generation of resting membrane potential. *American Journal of Physiology - Advances in Physiology Education* 28 (4): 139–142.

40 Liu, Z., Wan, X., Wang, Z.L., and Li, L. (2021). Electroactive biomaterials and systems for cell fate determination and tissue regeneration: design and applications. *Advanced Materials* 33 (32): e2007429.

41 Zaszczynska, A., Sajkiewicz, P., and Gradys, A. (2020). Piezoelectric scaffolds as smart materials for neural tissue engineering. *Polymers* 12 (1): 412.

42 Szedlak, P., Steele, D.S., and Hopkins, P.M. (2023). Cardiac muscle physiology. *BJA Education* 23 (9): 350–357.

43 McCaig, C.D., Song, B., and Rajnicek, A.M. (2009). Electrical dimensions in cell science. *Journal of Cell Science* 122 (23): 4267–4276.

44 Kadir, L.A., Stacey, M., and Barrett-Jolley, R. (2018). Emerging roles of the membrane potential: action beyond the action potential. *Frontiers in Physiology* 9 (Nov): 1661.

45 Talikowska, M., Fu, X., and Lisak, G. (2019). Application of conducting polymers to wound care and skin tissue engineering: a review. *Biosensors and Bioelectronics* 135: 50–63.

46 Liang, Y. and Goh, J.C.H. (2020). Polypyrrole-incorporated conducting constructs for tissue engineering applications: a review. *Bioelectricity* 2 (2): 101–119.

47 Guibert, C., Ducret, T., and Savineau, J.P. (2008). Voltage-independent calcium influx in smooth muscle. *Progress in Biophysics and Molecular Biology* 98 (1): 10–23.

48 Suzuki, Y., Ohya, S., Yamamura, H. et al. (2016). A new splice variant of large conductance Ca^{2+}-activated K^+ (BK) channel α subunit alters human chondrocyte function. *Journal of Biological Chemistry* 291 (46): 24247–24260.

49 Church, P.J. and Stanley, E.F. (1996). Single L-type calcium channel conductance with physiological levels of calcium in chick ciliary ganglion neurons. *Journal of Physiology* 496 (1): 59–68.

50 Matta, C., Fodor, J., Szíjgyártó, Z. et al. (2008). Cytosolic free Ca^{2+} concentration exhibits a characteristic temporal pattern during in vitro cartilage differentiation: a possible regulatory role of calcineurin in Ca-signalling of chondrogenic cells. *Cell Calcium* 44 (3): 310–323.

51 Gong, X., Li, G., Huang, Y. et al. (2019). Synergistically regulated spontaneous calcium signaling is attributed to cartilaginous extracellular matrix metabolism. *Journal of Cellular Physiology* 234 (6): 9711–9722.

52 Bartolák-Suki, E., Imsirovic, J., Nishibori, Y. et al. (2017). Regulation of mitochondrial structure and dynamics by the cytoskeleton and mechanical factors. *International Journal of Molecular Sciences* 18 (8): 1812.

53 Cho, M.R., Thatte, H.S., Silvia, M.T. et al. (1999). Transmembrane calcium influx induced by ac electric fields. *FASEB Journal* 13 (6): 677–683.

54 Pall, M.L. (2013). Electromagnetic fields act via activation of voltage-gated calcium channels to produce beneficial or adverse effects. *Journal of Cellular and Molecular Medicine* 17 (8): 958–965.

55 Nakano, T., Moore, M.J., Wei, F. et al. (2012). Molecular communication and networking: opportunities and challenges. *IEEE Transactions on Nanobioscience* 11 (2): 135–148.

56 Kaestner, L., Wang, X., Hertz, L. et al. (2018). Voltage-activated ion channels in non-excitable cells-a viewpoint regarding their physiological justification. *Frontiers in Physiology* 9 (Apr): 132.

57 Leppik, L., Oliveira, K.M.C., Bhavsar, M.B. et al. (2020). Electrical stimulation in bone tissue engineering treatments. *European Journal of Trauma and Emergency Surgery* 46 (2): 231–244.

58 Hoop, M., Chen, X.Z., Ferrari, A. et al. (2017). Ultrasound-mediated piezoelectric differentiation of neuron-like PC12 cells on PVDF membranes. *Science Reports* 7 (1): 4028.

59 Van Westering, T.L.E., Betts, C.A., and Wood, M.J.A. (2015). Current understanding of molecular pathology and treatment of cardiomyopathy in Duchenne muscular dystrophy. *Molecules* 20 (5): 8823–8855.

60 Cooper, D.M., Mons, N., and Karpen, J.W. (1995). Adenylyl cyclases and the interaction between calcium and cAMP signalling. *Nature* 374 (6521): 421–424.

61 Huang, J., Ye, Z., Hu, X. et al. (2010). Electrical stimulation induces calcium-dependent release of NGF from cultured Schwann cells. *Glia* 58 (5): 622–631.

62 Fields, R.D., Eshete, F., Stevens, B. et al. (1997). Action potential-dependent regulation of gene expression: temporal specificity in Ca^{2+}, cAMP-responsive element binding proteins, and mitogen- activated protein kinase signaling. *Journal of Neuroscience* 17 (19): 7252–7266.

63 Ma, T., Ding, Q., Liu, C. et al. (2023). Electromagnetic fields regulate calcium-mediated cell fate of stem cells: osteogenesis, chondrogenesis and apoptosis. *Stem Cell Research & Therapy* 14 (1): 133.

64 Zhang, K., Guo, J., Ge, Z., and Zhang, J. (2014). Nanosecond pulsed electric fields (nsPEFs) regulate phenotypes of chondrocytes through Wnt/β-catenin signaling pathway. *Science Reports* 4: 5836.

65 Sun, S., Liu, Y., Lipsky, S., and Cho, M. (2007). Physical manipulation of calcium oscillations facilitates osteodifferentiation of human mesenchymal stem cells. *FASEB Journal* 21 (7): 1472–1480.

66 Díaz-Vegas, A., Campos, C.A., Contreras-Ferrat, A. et al. (2015). ROS production via P2Y1-PKC-NOX2 is triggered by extracellular ATP after electrical stimulation of skeletal muscle cells. *PLoS One* 10 (6).

67 Srirussamee, K., Mobini, S., Cassidy, N.J. et al. (2019). Direct electrical stimulation enhances osteogenesis by inducing Bmp2 and Spp1 expressions from macrophages and preosteoblasts. *Biotechnology and Bioengineering* 116 (12): 3421–3432.

68 Sart, S., Song, L., and Li, Y. (2015). Controlling redox status for stem cell survival, expansion, and differentiation. *Oxidative Medicine and Cellular Longevity* 2015: 1215.

69 Wolf-Goldberg, T., Barbul, A., Ben-Dov, N., and Korenstein, R. (2013). Low electric fields induce ligand-independent activation of EGF receptor and ERK via electrochemical elevation of H+ and ROS concentrations. *Biochimica et Biophysica Acta - Molecular Cell Research* 1833 (6): 1396–1408.

70 Titushkin, I. and Cho, M. (2009). Regulation of cell cytoskeleton and membrane mechanics by electric field: role of linker proteins. *Biophysical Journal* 96 (2): 717–728.

71 Zhao, M., Dick, A., Forrester, J.V., and McCaig, C.D. (1999). Electric field-directed cell motility involves up-regulated expression and asymmetric redistribution of the epidermal growth factor receptors and is enhanced by fibronectin and laminin. *Molecular Biology of the Cell* 10 (4): 1259–1276.

72 Fraden, J. (2004). *Handbook of Modern Sensors: Physics, Designs, and Applications*. Springer Science & Business Media.

73 Mohammed, M.A., Basirun, W.J., Rahman, N.M.M.A. et al. (2021). Chapter 13—Electrochemical applications of nanocellulose. In: *Nanocellulose Based Composites for Electronics* (ed. S. Thomas and Y.B. Pottathara), 313–335. Elsevier.

74 Le, T.H., Kim, Y., and Yoon, H. (2017). Electrical and electrochemical properties of conducting polymers. *Polymers* 9 (4).

75 Ravanbakhsh, H., Bao, G., and Mongeau, L. (2020). Carbon nanotubes promote cell migration in hydrogels. *Scientific Reports* 10 (1): 2543.

76 Fu, C., Yang, X., Tan, S. et al. (2017). Enhancing cell proliferation and osteogenic differentiation of MC3T3-E1 pre-osteoblasts by BMP-2 delivery in graphene oxide-incorporated PLGA/HA biodegradable microcarriers. *Scientific Reports* 7 (1): C7–C12549.

77 Wu, S., Qi, Y., Shi, W. et al. (2022). Electrospun conductive nanofiber yarns for accelerating mesenchymal stem cells differentiation and maturation into Schwann cell-like cells under a combination of electrical stimulation and chemical induction. *Acta Biomaterialia* 139: 91–104.

78 Tang, M., Song, Q., Li, N. et al. (2013). Enhancement of electrical signaling in neural networks on graphene films. *Biomaterials* 34 (27): 6402–6411.

79 Saberi, A., Jabbari, F., Zarrintaj, P. et al. (2019). Electrically conductive materials: opportunities and challenges in tissue engineering. *Biomolecules* 9 (9): 448.

80 Hall, N. (2003). Twenty-five years of conducting polymers. *Chemical Communications* 1: 1–4.

81 Li, M. and Guo, B. (2019). Conductive polymeric biomaterials for tissue regeneration applications. *Kexue Tongbao/Chinese Science Bulletin* 64 (23): 2410–2424.

82 Lu, X., Zhang, W., Wang, C. et al. (2011). One-dimensional conducting polymer nanocomposites: synthesis, properties and applications. *Progress in Polymer Science (Oxford)* 36 (5): 671–712.

83 Rai, R., Roether, J.A., and Boccaccini, A.R. (2022). Polyaniline based polymers in tissue engineering applications: a review. *Progress in Biomedical Engineering* 4 (4): 042004.

84 Kheilnezhad, B., Safaei Firoozabady, A., and Aidun, A. (2020). An overview of polyaniline in tissue engineering. *Journal of Tissues and Materials* 3 (4): 6–22.

85 Dixon, D.T. and Gomillion, C.T. (2023). 3D-printed conductive polymeric scaffolds with direct current electrical stimulation for enhanced bone regeneration. *Journal of Biomedical Materials Research - Part B Applied Biomaterials* 111 (7): 1351–1364.

86 Burnstine-Townley, A., Eshel, Y., and Amdursky, N. (2020). Conductive scaffolds for cardiac and neuronal tissue engineering: governing factors and mechanisms. *Advanced Functional Materials* 30 (18): 225–229.

87 Isaacson, B.M. and Bloebaum, R.D. (2010). Bone bioelectricity: what have we learned in the past 160 years? *Journal of Biomedical Materials Research - Part A* 95 (4): 1270–1279.

88 Walsh, F.C. (1991). Electrochemical cell reactions in metal finishing. *Transactions of the Institute of Metal Finishing* 69 (Pt 3): 111–116.

89 Aoki, K., Ogihara, N., Tanaka, M. et al. (2020). Carbon nanotube-based biomaterials for orthopaedic applications. *Journal of Materials Chemistry B* 8 (40): 9227–9238.

90 Dixon, D.T. and Gomillion, C.T. (2022). Conductive scaffolds for bone tissue engineering: current state and future outlook. *Journal of Functional Biomaterials* 13 (1): 1.

91 Cheng, J., Liu, J., Wu, B. et al. (2021). Graphene and its derivatives for bone tissue engineering: in vitro and in vivo evaluation of graphene-based scaffolds, membranes and coatings. *Frontiers in Bioengineering and Biotechnology* 9: 734688.

92 Dong, R., Ma, P.X., and Guo, B. (2020). Conductive biomaterials for muscle tissue engineering. *Biomaterials* 229: 119584.

93 Palmieri, V., Sciandra, F., Bozzi, M. et al. (2020). 3D graphene scaffolds for skeletal muscle regeneration: future perspectives. *Frontiers in Bioengineering and Biotechnology* 8: 383.

94 Dalla Colletta, A., Pelin, M., Sosa, S. et al. (2022). Carbon-based nanomaterials and skin: an overview. *Carbon* 196: 683–698.

95 Lasocka, I., Jastrzębska, E., Szulc-Dąbrowska, L. et al. (2019). The effects of graphene and mesenchymal stem cells in cutaneous wound healing and their putative action mechanism. *International Journal of Nanomedicine* 14: 2281–2299.

96 Kiyotake, E.A., Martin, M.D., and Detamore, M.S. (2022). Regenerative rehabilitation with conductive biomaterials for spinal cord injury. *Acta Biomaterialia* 139: 43–64.

97 Farokhi, M., Mottaghitalab, F., Saeb, M.R. et al. (2021). Conductive biomaterials as substrates for neural stem cells differentiation towards neuronal lineage cells. *Macromolecular Bioscience* 21 (1): 2000123.

98 Kumar, R., Rauti, R., Scaini, D. et al. (2021). Graphene-based nanomaterials for neuroengineering: recent advances and future prospective. *Advanced Functional Materials* 31 (46): 241.

99 Wang, S.X., Lu, Y.B., Wang, X.X. et al. (2022). Graphene and graphene-based materials in axonal repair of spinal cord injury. *Neural Regeneration Research* 17 (10): 2117–2125.

100 Scott, L., Jurewicz, I., Jeevaratnam, K., and Lewis, R. (2021). Carbon nanotube-based scaffolds for cardiac tissue engineering—systematic review and narrative synthesis. *Bioengineering* 8 (6): 80.

101 Li, Y., Wei, L., Lan, L. et al. (2022). Conductive biomaterials for cardiac repair: a review. *Acta Biomaterialia* 139: 157–178.

102 Savchenko, A., Yin, R.T., Kireev, D. et al. (2021). Graphene-based scaffolds: fundamentals and applications for cardiovascular tissue engineering. *Frontiers in Bioengineering and Biotechnology* 9: 156.

103 Wang, Y. (2018). Electrical conductivity of carbon nanotube- and graphene-based nanocomposites. In: *Micromechanics and Nanomechanics of Composite Solids* (ed. S.A. Meguid and G.J. Weng), 123–156.

104 Rawat, N.K. and Ahmad, S. (2019). Chapter 11—Unveiling nanoconducting polymers and composites for corrosion protection. In: *Nanomaterials-Based Coatings* (ed. P.N. Tri and C.M. Ouellet Plamondon), 373–395. Elsevier.

105 Zare, E.N., Agarwal, T., Zarepour, A. et al. (2021). Electroconductive multi-functional polypyrrole composites for biomedical applications. *Applied Materials Today* 24.

106 Curie, J. and Curie, P. (1880). Développement par compression de l'électricité polaire dans les cristaux hémièdres à faces inclinées. *Bulletin de minéralogie* 3 (4): 90–93.

107 Lippmann, G. (1881). Principe de la conservation de l'électricité, ou second principe de la théorie des phénomènes électriques. *Journal de Physique Théorique et Appliquée* 10 (1): 381–394.

108 Kochervinskiĭ, V.V. (2003). Piezoelectricity in crystallizing ferroelectric polymers: poly(vinylidene fluoride) and its copolymers (a review). *Crystallography Reports* 48 (4): 649–675.

109 Ribeiro, C., Sencadas, V., Correia, D.M. et al. (2015). Piezoelectric polymers as biomaterials for tissue engineering applications. *Colloids and Surfaces B: Biointerfaces* 136: 46–55.

110 Costa, C.M., Cardoso, V.F., Martins, P. et al. (2023). Smart and multifunctional materials based on electroactive poly(vinylidene fluoride): recent advances and opportunities in sensors, actuators, energy, environmental, and biomedical applications. *Chemical Reviews* 123 (19): 11392–11487.

111 Ribeiro, C., Correia, D.M., Ribeiro, S. et al. (2018). Piezo-and magnetoelectric polymers as biomaterials for novel tissue engineering strategies. *MRS Advances* 67: 1556.

112 Ribeiro, C., Pärssinen, J., Sencadas, V. et al. (2015). Dynamic piezoelectric stimulation enhances osteogenic differentiation of human adipose stem cells. *Journal of Biomedical Materials Research - Part A* 103 (6): 2172–2175.

113 Marques-Almeida, T., Fernandes, H.J.R., Lanceros-Mendez, S. et al. (2022). Surface charge and dynamic mechanoelectrical stimuli improves adhesion, proliferation and differentiation of neuron-like cells. *Journal of Materials Chemistry B* 34: 1224.

114 Hoop, M., Chen, X.Z., Ferrari, A. et al. (2017). Ultrasound-mediated piezoelectric differentiation of neuron-like PC12 cells on PVDF membranes. *Scientific Reports* 7 (1): 224.

115 Gao, S. (2019). Functional-material-based touch interfaces for multidimensional sensing for interactive displays: a review. *Semiconductor Science and Information Devices* 1: 199.

116 Jarkov, V., Allan, S.J., Bowen, C. et al. (2022). Piezoelectric materials and systems for tissue engineering and implantable energy harvesting devices for biomedical applications. *International Materials Reviews* 67 (7): 683–733.

117 Sood, A., Desseigne, M., Dev, A. et al. (2023). A comprehensive review on barium titanate nanoparticles as a persuasive piezoelectric material for biomedical applications: prospects and challenges. *Small* 19 (12): 2206401.

118 Liu, P., Wang, K., Li, L. et al. (2023). Lead-free piezoelectric materials for musculoskeletal tissue engineering. *Materials Today Sustainability* 23: 100393.

119 Ma, J. and C. Wu. Bioactive inorganic particles-based biomaterials for skin tissue engineering. *Exploration,* 2022. 2 (5): 20210083.

120 Samadi, A., Salati, M.A., Safari, A. et al. (2022). Comparative review of piezoelectric biomaterials approach for bone tissue engineering. *Journal of Biomaterials Science, Polymer Edition* 33 (12): 1555–1594.

121 Ribeiro, C., Correia, D.M., Ribeiro, S. et al. (2015). Piezoelectric poly(vinylidene fluoride) microstructure and poling state in active tissue engineering. *Engineering in Life Sciences* 15 (4): 351–356.

122 Goonoo, N. and Bhaw-Luximon, A. (2022). Piezoelectric polymeric scaffold materials as biomechanical cellular stimuli to enhance tissue regeneration. *Materials Today Communications* 31: 103491.

123 Fukada, E. and Ando, Y. (1986). Piezoelectric properties of poly-β-hydroxybutyrate and copolymers of β-hydroxybutyrate and β-hydroxyvalerate. *International Journal of Biological Macromolecules* 8 (6): 361–366.

124 Shin, D.M., Hong, S.W., and Hwang, Y.H. (2020). Recent advances in organic piezoelectric biomaterials for energy and biomedical applications. *Nanomaterials* 10 (1): 123.

125 Liang, X., Matyushov, A., Hayes, P. et al. (2021). Roadmap on magnetoelectric materials and devices. *IEEE Transactions on Magnetics* 57 (8): 1–57.

126 Pradhan, D.K., Kumari, S., and Rack, P.D. (2020). Magnetoelectric composites: applications, coupling mechanisms, and future directions. *Nanomaterials* 10 (10): 1–22.

127 Ribeiro, S., Garcia-Astrain, C., Fernandes, M.M. et al. (2019). Multidimensional biomechanics approaches though electrically and magnetically active microenvironments. *Advances in Biomechanics and Tissue Regeneration* 21: 253–267.

128 Curie, P. (1894). Sur la symétrie dans les phénomènes physiques, symétrie d'un champ électrique et d'un champ magnétique. *Journal de physique théorique et appliquée* 3 (1): 393–415.

129 Astrov, D. (1960). The magnetoelectric effect in antiferromagnetics. *Soviet Physics—JETP* 11 (3): 708–709.

130 Marques-Almeida, T., Correia, V., Fernandez Martin, E. et al. (2022). Piezoelectric and magnetically responsive biodegradable composites with tailored porous morphology for biotechnological applications. *ACS Applied Polymer Materials* 29: 123.

131 Hermenegildo, B., Meira, R.M., Correia, D.M. et al. (2022). Poly(lactic-co-glycolide) based biodegradable electrically and magnetically active microenvironments for tissue regeneration applications. *European Polymer Journal* 171: C7–C111197.

132 Ribeiro, S., Ribeiro, C., Carvalho, E.O. et al. (2020). Magnetically activated electroactive microenvironments for skeletal muscle tissue regeneration. *ACS Applied Bio Materials* 3 (7): 4239–4252.

133 Carvalho, E.O., Ribeiro, C., Correia, D.M. et al. (2020). Biodegradable hydrogels loaded with magnetically responsive microspheres as 2d and 3d scaffolds. *Nanomaterials* 10 (12): 1–12.

134 Correia, D.M., Fernandes, L.C., Cruz, B.D.D. et al. (2016). Processing and size range separation of pristine and magnetic poly(l-lactic acid) based microspheres for biomedical applications. *Journal of Colloid and Interface Science* 476: 79–86.

135 Reizabal, A., Brito-Pereira, R., Fernandes, M.M. et al. (2020). Silk fibroin magnetoactive nanocomposite films and membranes for dynamic bone tissue engineering strategies. *Materialia* 24: 100709.

136 Nguyen, T., Gao, J., Wang, P. et al. (2021). In vivo wireless brain stimulation via non-invasive and targeted delivery of magnetoelectric nanoparticles. *Neurotherapeutics* 18 (3): 2091–2106.

137 Zhang, Y., Chen, S., Xiao, Z. et al. (2021). Magnetoelectric nanoparticles incorporated biomimetic matrix for wireless electrical stimulation and nerve regeneration. *Advanced Healthcare Materials* 10 (16): 2100695.

138 Brito-Pereira, R., Martins, P., Lanceros-Mendez, S. et al. (2023). Polymer-based magnetoelectric scaffolds for wireless bone repair: the fillers' effect on extracellular microenvironments. *Composites Science and Technology* 243: 123.

139 Varma, M.V. and Kandasubramanian, B. (2021). The tactics of thermoelectric scaffolds with its advancements in engineering applications. *Polymer-Plastics Technology and Materials* 60 (1): 1–24.

140 Seebeck, T.J. (1822). Ueber den Magnetismus der galvanischen Kette.

141 Sarma, D.D. (2021). Essential considerations for reporting thermoelectric properties. *ACS Energy Letters* 6 (10): 3715–3718.

142 Shalan, A.E., Peřinka, N., Serea, E.S.A. et al. (2021). Advances in thermochromic and thermoelectric materials. In: *Advanced Lightweight Multifunctional Materials* (ed. P. Costa, C.M. Costa, and S. Lanceros-Mendez), 153–186. Elsevier.

143 Hofmann, A.I., Kroon, R., Müller, C. et al. (2019). 13—Doping and processing of organic semiconductors for plastic thermoelectrics. In: *Handbook of Organic Materials for Electronic and Photonic Devices*, 2e (ed. O. Ostroverkhova), 429–449. Woodhead Publishing.

144 Hu, B., Shi, X.L., Zou, J. et al. (2022). Thermoelectrics for medical applications: progress, challenges, and perspectives. *Chemical Engineering Journal* 437: 134.

145 Leonov, V. and Vullers, R.J.M. (2009). Wearable thermoelectric generators for body-powered devices. *Journal of Electronic Materials* 23: 1224.

146 Rao, Y., Bechtold, T., and Hohlfeld, D. (2022). Design optimization of a packaged thermoelectric generator for electrically active implants. *Microelectronics Reliability* 139: 1226.

147 Ribeiro, C., Costa, C.M., Correia, D.M. et al. (2018). Electroactive poly(vinylidene fluoride)-based structures for advanced applications. *Nature Protocols* 13 (4): 681–704.

148 Berni, A., Mennig, M., and Schmidt, H. (2004). Doctor blade. In: *Sol-Gel Technologies for Glass Producers and Users* (ed. M.A. Aegerter and M. Mennig), 89–92. Boston, MA: Springer US.

149 Martins, P.M., Ribeiro, S., Ribeiro, C. et al. (2013). Effect of poling state and morphology of piezoelectric poly(vinylidene fluoride) membranes for skeletal muscle tissue engineering. *RSC Advances* 3 (39): 17938–17944.

150 Marques-Almeida, T., Ribeiro, C., Irastorza, I. et al. (2023). Electroactive materials surface charge impacts neuron viability and maturation in 2D cultures. *ACS Applied Materials and Interfaces* 15 (26): 31206–31213.

151 Unnikrishnan, G., Joy, A., Megha, M. et al. (2023). Preparation and characterizations of antibacterial and electroactive polymeric composites for wound healing applications. *Polymer Composites* 45 (1): 267–285.

152 Zhu, S., Mbugua, J., Chase, M., et al. (2012). Electronically-controlled drug release system to promote wound healing. In: *Technical Proceedings of the 2012 NSTI Nanotechnology Conference and Expo, NSTI-Nanotech 2012* 18–21 June, Santa Clara, California, USA.

153 Aycan, D., Selmi, B., Kelel, E. et al. (2019). Conductive polymeric film loaded with ibuprofen as a wound dressing material. *European Polymer Journal* 121: 109308.

154 Cardoso, V.F., Costa, C.M., Correia, D.M. et al. (2021). Solution processing of piezoelectric unconventional structures. *Organic Ferroelectric Materials and Applications* 204: 375–439.

155 Yang, G., Li, X., He, Y. et al. (2018). From nano to micro to macro: electrospun hierarchically structured polymeric fibers for biomedical applications. *Progress in Polymer Science* 81: 80–113.

156 Wu, S., Chen, M.S., Maurel, P. et al. (2018). Aligned fibrous PVDF-TrFE scaffolds with Schwann cells support neurite extension and myelination in vitro. *Journal of Neural Engineering* 15 (5).

157 Demir, U.S., Shahbazi, R., Calamak, S. et al. (2018). Gold nano-decorated aligned polyurethane nanofibers for enhancement of neurite outgrowth and elongation. *Journal of Biomedical Materials Research - Part A* 106 (6): 1604–1613.

158 Narayanan, N., Jiang, C., Wang, C. et al. (2020). Harnessing fiber diameter-dependent effects of myoblasts toward biomimetic scaffold-based skeletal muscle regeneration. *Frontiers in Bioengineering and Biotechnology* 8: 203.

159 Quan, Q., Meng, H.Y., Chang, B. et al. (2019). Aligned fibers enhance nerve guide conduits when bridging peripheral nerve defects focused on early repair stage. *Neural Regeneration Research* 14 (5): 903–912.

160 Huang, C., Ouyang, Y., Niu, H. et al. (2015). Nerve guidance conduits from aligned nanofibers: improvement of nerve regeneration through longitudinal nanogrooves on a fiber surface. *ACS Applied Materials and Interfaces* 7 (13): 7189–7196.

161 Lee, J.H., Lee, Y.J., Cho, H. et al. (2014). Guidance of in vitro migration of human mesenchymal stem cells and in vivo guided bone regeneration using aligned electrospun fibers. *Tissue Engineering - Part A* 20 (15–16): 2031–2042.

162 Park, C.H., Rios, H.F., Jin, Q. et al. (2012). Tissue engineering bone-ligament complexes using fiber-guiding scaffolds. *Biomaterials* 33 (1): 137–145.

163 Costa, C.M., Cardoso, V.F., Martins, P. et al. (2023). Smart and multifunctional materials based on electroactive poly(vinylidene fluoride): recent advances and opportunities in sensors, actuators, energy, environmental, and biomedical applications. *Chemical Reviews* 21: 11392–11487.

164 Mohammadi Amirabad, L., Massumi, M., Shamsara, M. et al. (2017). Enhanced cardiac differentiation of human cardiovascular disease patient-specific induced pluripotent stem cells by applying unidirectional electrical pulses using aligned electroactive nanofibrous scaffolds. *ACS Applied Materials and Interfaces* 9 (8): 6849–6864.

165 Zhang, Y., Le Friec, A., and Chen, M. (2021). 3D anisotropic conductive fibers electrically stimulated myogenesis. *International Journal of Pharmaceutics* 606: C7-120841.

166 Farkhondehnia, H., Amani Tehran, M., and Zamani, F. (2018). Fabrication of biocompatible PLGA/PCL/PANI nanofibrous scaffolds with electrical excitability. *Fibers and Polymers* 19 (9): 1813–1819.

167 Song, J., Sun, B., Liu, S. et al. (2016). Polymerizing pyrrole coated poly (l-lactic acid-co-ε-caprolactone) (PLCL) conductive nanofibrous conduit combined with electric stimulation for long-range peripheral nerve regeneration. *Frontiers in Molecular Neuroscience* 9 (Nov 2016): C7–C117.

168 Accardo, A., Cirillo, C., Lionnet, S. et al. (2019). Interfacing cells with micro-engineered scaffolds for neural tissue reconstruction. *Brain Research Bulletin* 152: 202–211.

169 Flores-Jiménez, M.S., Garcia-Gonzalez, A., and Fuentes-Aguilar, R.Q. (2023). Review on porous scaffolds generation process: a tissue engineering approach. *ACS Applied Bio Materials* 6 (1): 1–23.

170 Girão, A.F., Sousa, J., Dominguez-Bajo, A. et al. (2020). 3D reduced graphene oxide scaffolds with a combinatorial fibrous-porous architecture for neural tissue engineering. *ACS Applied Materials and Interfaces* 12 (35): 38962–38975.

171 Wang, S., Sun, C., Guan, S. et al. (2017). Chitosan/gelatin porous scaffolds assembled with conductive poly(3,4-ethylenedioxythiophene) nanoparticles for neural tissue engineering. *Journal of Materials Chemistry B* 5 (24): 4774–4788.

172 Sun, Y., Zou, L., and Liu, J. (2015). Applications of porous scaffolds in muscle tissue engineering. *Sheng wu yi xue Gong Cheng xue za zhi = Journal of Biomedical Engineering = Shengwu Yixue Gongchengxue Zazhi* 32 (6): 1343–1347.

173 Watanabe, T., Sassi, S., Ulziibayar, A. et al. (2023). The application of porous scaffolds for cardiovascular tissues. *Bioengineering* 10 (2): 121–125.

174 Loh, Q.L. and Choong, C. (2013). Three-dimensional scaffolds for tissue engineering applications: role of porosity and pore size. *Tissue Engineering - Part B: Reviews* 19 (6): 485–502.

175 Abdelaziz, A.G., Nageh, H., Abdo, S.M. et al. (2023). A review of 3D polymeric scaffolds for bone tissue engineering: principles, fabrication techniques, immunomodulatory roles, and challenges. *Bioengineering* 10 (2).

176 Nikolova, M.P. and Chavali, M.S. (2019). Recent advances in biomaterials for 3D scaffolds: a review. *Bioactive Materials* 4: 271–292.

177 Xu, C., Thiruvadi, V.S., Whitmore, R. et al. (2019). 5—Delivery systems for biomedical applications: basic introduction, research frontiers and clinical translations. In: *Biomaterials in Translational Medicine* (ed. L. Yang and T.J. Webster), 93–116. Academic Press.

178 Servant, A., Leon, V., Jasim, D. et al. (2014). Graphene-based electroresponsive scaffolds as polymeric implants for on-demand drug delivery. *Advanced Healthcare Materials* 3 (8): 1334–1343.

179 Xie, C., Li, P., Han, L. et al. (2017). Electroresponsive and cell-affinitive polydopamine/polypyrrole composite microcapsules with a dual-function of on-demand drug delivery and cell stimulation for electrical therapy. *NPG Asia Materials* 9 (3): C7–e358.

180 Mantha, S., Pillai, S., Khayambashi, P. et al. (2019). Smart hydrogels in tissue engineering and regenerative medicine. *Materials* 12 (20).

181 Akhtar, M.F., Hanif, M., and Ranjha, N.M. (2016). Methods of synthesis of hydrogels—a review. *Saudi Pharmaceutical Journal* 24 (5): 554–559.

182 Nele, V., Wojciechowski, J.P., Armstrong, J.P.K., and Stevens, M.M. (2020). Tailoring gelation mechanisms for advanced hydrogel applications. *Advanced Functional Materials* 30 (42): 2002759.

183 MohanKumar, B.S., Priyanka, G., Rajalakshmi, S. et al. (2022). Hydrogels: potential aid in tissue engineering—a review. *Polymer Bulletin* 79 (9): 7009–7039.

184 Zhou, X., Zhang, N., Kandalai, S. et al. (2023). Dynamic and wearable electroresponsive hydrogel with robust mechanical properties for drug release. *ACS Applied Materials and Interfaces* 15 (13): 17113–17122.

185 Ha, J.H., Lim, J.H., Lee, J.M. et al. (2023). Electro-responsive conductive blended hydrogel patch. *Polymers* 15 (12): C7-2608.

186 Aycan, D., Karaca, F., Koca, A., and Alemdar, N. (2023). Electro-stimulated drug release by methacrylated hyaluronic acid-based conductive hydrogel with enhanced mechanical properties. *International Journal of Biological Macromolecules* 231: C7-123297.

187 Rogers, Z.J., Zeevi, M.P., Koppes, R. et al. (2020). Electroconductive hydrogels for tissue engineering: current status and future perspectives. *Bioelectricity* 2 (3): 279–292.

188 Liu, W., Luo, Y., Ning, C. et al. (2021). Thermo-sensitive electroactive hydrogel combined with electrical stimulation for repair of spinal cord injury. *Journal of Nanobiotechnology* 19: 1–16.