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Journal of Composites and Compounds

Piezoelectric composites in neural tissue engineering: material and fabrication techniques

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A B S T R A C T

To date, there is no effective treatment for central or peripheral nervous system damage, which results in cognitive and/or sensory impairment. After a neural injury, tissue engineering can provide a scaffold for either transplanted or native cells. With the recent focus on stimuli sensitive scaffolds, sometimes referred to as smart scaffolds, tissue engineering is highly dependent on scaffolds for supporting cell differentiation and growth. Piezoelectric scaffolds are a representative of this class of materials because they can generate electrical charges when mechanically stimulated, creating a prospect their possible use in non-invasive therapy for neural tissue. Research on piezoelectric materials that can be utilized to enhance neural tissue engineering is summarized in this study. The most common employed materials for tissue engineering strategies are discussed, as well as the most significant accomplishments, difficulties, and unmet research and treatment needs that will be needed in the future. As a result, this study compiles the most relevant findings and strategies, and it serves as a starting point for new research in the most relevant and difficult related issues.

Peer review under responsibility of JCC Research Group

A R T I C L E I N F O R M A T I O N

Article history:
Received 1 January 2022
Received in revised form 13 January 2022
Accepted 25 February 2022

Keywords:
Piezoelectric composite
Neural tissue engineering
β-phase induction
Electrical stimulation

Table of contents

1. Introduction

There are several applications for piezoelectric materials in a wide range of electronic devices, including sensors, actuators, energy harvesting (EH), and others. Although ceramics are frequently associated with the piezoelectric phenomenon, several polymers also exhibit piezoelectric behavior [1]. Despite their lower piezoelectric coefficients [2], piezoelectric polymers are commonly preferred for particular applications because of their flexibility, simplicity of production, and biocompatibility. Numerous polymer families exhibit piezoelectric properties. Some polyamides [3], polyesters [4], polypeptides [5], and polyureas also exhibit piezoelectric properties [6].

Among piezoelectric polymers, Polyvinylidene fluoride (PVDF) is one of the most extensively utilized and exhibits a high degree of piezoelectricity [7]. PVDF with Chemical formula of CH2-CF2- has useful special properties like high chemical resistance, high mechanical properties, antioxidation, thermal and hydrolytic stabilities, and notable piezoelectricicity [8]. Electrical stimulation of cells (such as stem cells)

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https://doi.org/10.52547/jcc.4.1.5

has gained greater interest in biocompatible PVDF materials [9]. When electrical poling induces the surface charge of PVDF, many biological reactions such as fibronectin adsorption, osteoblast adhesion and proliferation have been investigated [10].

Over the last few decades, a wide range of methods for producing PVDF fibers have been developed. Electrospinning has been shown to improve the piezoelectric property of PVDF. polyvinylidene fluoride-co-trifluoroethylene (PVDF-TrFE) nanofibers' superior biocompatibility and piezoelectricity have resulted in their successful applications as bioactive electrically charged functional scaffolds for bone and neural tissue engineering [11]. PVDF-TrFE exhibits great piezoelectric and electrochemical coefficient for specified monomer concentrations. This is due to the increased chain mobility of PVDF-TrFE, which causes the lamella thickness to increase [12].

For tissue engineering, PVDF-TrFE does not need the special processing procedures to create the crystalline structure of the β-phase [13]. PVDF-TrFE electrospun fibers can be used to control cell growth and behavior in a three-dimensional (3D) matrix [14]. Mechanical deformation of nerve and bone cells could produce an electric charge that could accelerate the repair of neuron and bone cells injured by mechanical deformation [15]. Tissue engineering is a primary use for electrospun fibrous polymer-based composites. Electrospun scaffolds have porous architectures with fiber sizes that replicate the extracellular matrix naturally found in the body, hence facilitating cell growth and adhesion [16].

Tissue engineering utilizes regeneration to repair or replace damaged organs and tissues. This method is excellent for rehabilitating body parts injured by congenital abnormalities, trauma, or cancerous diseases where traditional therapies have failed. Scaffolds made of piezoelectric materials are ideal for tissue regeneration because they stimulate cells electrically. Electric stimulation causes phenotypic and genetic changes in cells that speed up tissue regeneration. Piezoelectric materials have gained popularity as a non-invasive alternative to exogenous electric stimulation. They can now regenerate cartilage, tendons, nerves, skin, ligaments, and muscle [17].

Various natural and synthetic polymers have been investigated in the form of electrospun scaffolds for neural tissue engineering [17-20]. The treatment of patients with nervous system injuries is complicated because of the difficulty of localized and complex nerve regeneration. As a result, using piezoelectric polymers as nerve guidance conduits enables for direct electrical stimulation of the cell's ingrowth with its electrical activity during mechanical deformation without requiring an external power source. Neurons are particularly sensitive to electrical impulses; as recent research has demonstrated [21-24]. The objective of this study is to present a summary of research on piezoelectric materials utilized in neural tissue engineering. The most commonly utilized materials for neural tissue engineering strategies are discussed, as well as the major problems, accomplishments, and future research and therapy requirements. As a result, this study presents a compilation of the most important discoveries and tactics, as well as a starting point for innovative research pathways in the most relevant and challenging issues.

2. Piezoelectricity in biological tissues

Piezoelectricity was first explored in biological tissue in the 1950s [25], and generated considerable interest at the time due to the newly discovered link between stress electrical stimulation, stress, and fracture healing. The association between piezoelectricity and bone healing was investigated in a number of research. While the concept of piezoelectricity-driven remodeling was supported in dry bone investigations, multiple experiments in hydrated bone contradicted this process [26]. Because of the magnitudes of charge created by both pathways, these investigations claimed that streaming potentials were the major mechanism in mediating the strain generated potential (SGP). While there was less interest in the role of biological piezoelectricity in SGPs recently, there has been a lot more interest in piezoelectric energy harvesting in materials engineering. Studies on the application of zinc oxide and other inorganic piezoelectric materials in *in-vivo* energy harvesters for implant monitoring, nanosensors, and self-powered nanosystems have been increased. To aid in the development of drug delivery methods, research into the piezoelectric characteristics of organic biomolecules has accelerated [27, 28]. This mechanism of piezoelectricity in SGPs was hypothesized by Ahn and Grodzinsky (2009) in that piezoelectric effects work in conjunction with bone streaming potentials. This revived interest in biological piezoelectricity, resulting in more recent observations of tendon [29] and intervertebral disc (IVD) piezoresponses [30]. Most biological structures have been shown to have piezoelectric properties, while collagen is the primary molecule responsible for this action in bone and other connective tissues [27]. However, the physiological relevance of piezoelectricity in such hydrated tissues remains a mystery when comparing with ionic- and fluid-driven processes.

3. Neural cell response to electrical stimulation

The life quality and productivity of patients are greatly impacted by peripheral nerve damage caused by tumors, trauma, and other disorders [31]. These treatments, which have been referred to as "the gold standard" for nerve restoration, require further surgical procedures and are limited by the loss of sensory loss, a limited supply and probable neuroma formation [32]. With the rapid progress of regenerative medicine, it has been established that designed nerve guidance conduits (NGCs) can overcome these constraints and enhance neurological healing. However, fabricating perfect nerve grafts with exceptional qualities that meet therapeutic needs remains extremely difficult [33]. The peripheral nerve, which is composed of sensory and motor neurons, is one of the most electrically sensitive tissues, with conductivity varying between several and hundreds of mV/mm depending on the nerve component [34].

As a result, externally applied electrical stimulation (ES) has been shown to be a useful approach for promoting nerve regeneration. External electric fields have been shown to enhance galvanotaxis in cells, so directing cell movement toward the anode or cathode [35]. Although the precise mechanism is still unknown, several recent studies have demonstrated that ES may extend neurites and stimulate neuron regeneration *in vivo*, indicating that it is a viable technique for nerve engineering [36].

Many conductive materials for tissue engineering have been developed for facilitating ES, with Polypyrrole (PPy) being one of the most promising materials and neural prosthesis due to its ease of synthesis and processing, suitability of mechanical properties, and good conductivity [37, 38]. Because of its low solubility and degradation rate, PPy is unable to be used in more advanced applications. In comparison, despite their low conductivity, a variety of natural polymers have been employed due to their regulated biodegradability, unique bioactivity, and great biocompatibility. For example, chitosan-based scaffolds were effectively used to repair a 30-mm-long human median nerve lesion [39], while Zhang et al. reported promising neural regeneration following spinal cord damage using collagen-based scaffolds [40].

The self-assembled β-sheet architecture of silk fibroin (SF), a natural protein, has both remarkable biological and mechanical properties [41]. SF scaffolds may be molded into suitable shapes using a variety of processing techniques and have shown promising results in peripheral nerve regeneration [42]. As a result, it is thought that the combination of PPy with SF results in an advantageous water-soluble conductive material with high electroactivity [43]. NGC structure and surface features must also be taken into account when designing a conduit capable of promoting an abundance of nerve regeneration [44]. Longitudinally oriented

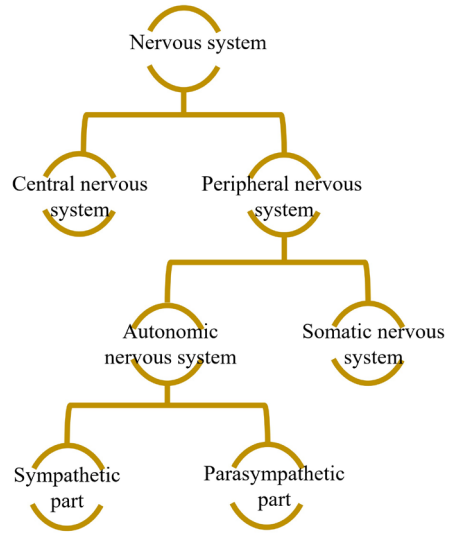

Fig. 1. Nervous system classification.

fibers have been demonstrated to facilitate Superior Colliculus (SC) migration, Büngner band formation, and axon extension in both *in vitro* and *in vivo* studies [45].

Biomimetic structures by changing design, material composition, and cell support may now be produced on-demand using 3D-printing technology. However, due to a lack of adequate resolution, the fabrication of matrixes with micro-nanostructures that accurately mimic the nanostructural features of natural tissues remains a major challenge. An additional benefit of this technique is that it allows the fabrication of fiber-aligned scaffolds with variable porosity and mechanical properties by electrospinning them into nano/microscale structures with interconnecting pores, which are more closely related to native extracellular matrix [46, 47]. As a result, a recent study conducted effectively combined 3D printing and electrospinning to design and fabricate 2D substrates based on SF and PPy with aligned structure [48].

4. Piezoelectric composite in neural tissue engineering

Bioengineering research on nerve injuries is hindered by the specific characteristics of the nervous system. The nervous system is divided into two parts: the central nervous system (CNS), which includes the brain and spinal cord, and the peripheral nervous system (PNS), which is comprised of nerves that leave the CNS (Figure 1). The somatic system of the PNS provides sensory and motor information to the CNS, whereas the autonomic system governs automatic functions (such as heartbeat and blood pressure) [49, 50]. Piezoelectric materials must have excellent electromechanical coupling and strong biocompatibility in order to meet biomedical application requirements. Other requirements may include being able to react physiologically, being sensitive to stimuli, being capable of adapting to changing conditions, and having great mechanical flexibility and strength. Bio-piezoelectric ceramics, bio-piezoelectric polymers, biomolecular piezoelectric materials, and bio-piezoelectric nanomaterials are all examples of piezoelectric materials for bio-piezoelectric systems that can be divided into four groups based on their chemical structure [51].

As with other cells, neuronal development is characterized by a variety of biochemical and morphological changes. To determine the amount of a culture's differentiation, these must be identified and quantified, as seen in Figure 2. Axonal growth is a critical morphological sign that may be evaluated by labeling certain neuronal markers immunofluorescently. Immunostaining is a technique used in biochemistry that employs antibody-based approaches to detect specific proteins in

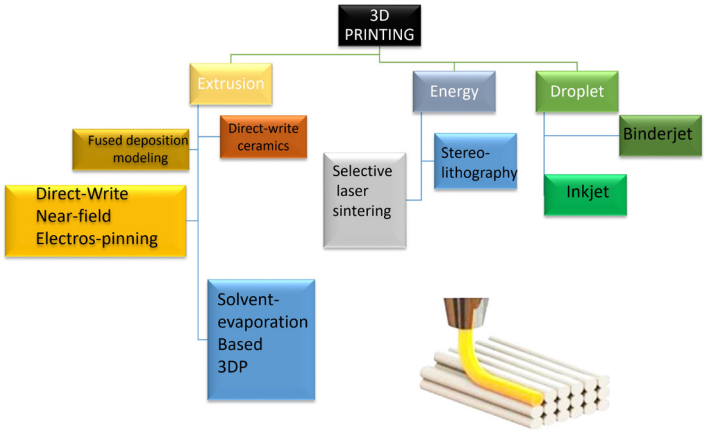

Fig. 2. Differentiation of neural, bone, and skeletal cells using piezoelectric scaffolds.

a sample. Wen et al. [52] employed this technique to characterize the expression of neuron-specific markers microtubule-associated protein 2 (MAP2), netrin-1, and its related receptor, DCC, while modulating axonal development in rat cortical neurons using highly piezoelectric Lead zirconate titanate (PZT). MAP2 is involved in microtubule assembly during neuritogenesis (the process by which new neurites emerge into axons and dendrites) and is expressed only in the dendrites of neuronal cells; netrin-1 is a protein involved in cell migration and axonal guidance [53]. Immunohistochemistry staining of cells identified axons, allowing the axonal length and cell density to be estimated from fluorescence microscopy images. 3-tubulin is another neuron-specific marker, which is abundant in the cytoplasm and neurites of diffusing neuroblastoma [54]. Similarly, 3-tubulin-containing cells can be stained immunofluorescently, and the percentage of immunopositive 3-tubulin cells is calculated by dividing the total number of cell nuclei identified using a 4′,6-diamidino-2-phenylindole (DAPI) stain by the total number of cell nuclei observed using a DAPI stain. Finally, inhibitory experiments can be performed in conjunction with immunofluorescent labeling to elucidate the underlying mechanism of stimulation (see to Mechanism of Piezoelectric Stimulation in Mammalian Cells). Inhibitory agents can be utilized to inhibit specific biochemical processes; this can be used to determine which biochemical pathways are likely to be involved in the effects generated by piezoelectric stimulation. The most frequently utilized examples are K252a, which inhibits neural growth factor (NGF)-specific receptors, and LaCl3, which inhibits calcium ion channels [55].

4.1. Piezo-ceramics

The piezoceramics were the first piezoelectric material group to be researched. The initial applications originate from around 1950, and they have been frequently employed in industry since then [56]. Porous piezoceramics were pioneered by Wersing et al. [57]as well as the theory and initial measurements [58]. Lead-free piezoelectric materials are urgently needed at the moment, yet the most feasible ceramics are still based on lead zirconate titanate. In spite of a reduction in the number of cells, rat cortical neurons grown on PZT slides coated with poly-L-lysine developed noticeably longer axons. Additional evidence that piezoelectricity may have increased neuronal activity occurs in the form of an increase in excitatory postsynaptic current frequency and amplitude. Notably, piezoceramics are employed in medical applications, including transducers, sensors, and actuators. Piezoceramics are not used in pure solution for medical implants due to allergic responses. Composites based on polymer matrices with ceramic fillers in the form of fibers are being developed for medical purposes [59, 60].

Barium Titanate (BT): Since then, ceramics have become a popular

component to scaffolds, particularly for medical applications because of their piezoelectric properties [61]. BT-based piezoceramics are less hazardous than lead-based piezoelectric materials. These piezoceramics have a lot of research going into them because of their high strain. At greater doses of 100 g/mL, BT nanoparticles have shown cytocompatibility. It has been shown that the addition of BT nanoparticles to poly lactic-co-glycolic acid (PLGA) matrix enhances the growth and attachment of osteocytes and osteoblast cells. Aside from improving the mechanical characteristics of the composite scaffold, barium titanate nanoparticles can also enhance cellular activity in tissue engineering [62].

Boron Nitride (BN): Because of their mechanical strength, high thermal stability and cinductivity, BN-based nanomaterials have a significant impact on nanotechnology. There are many types of piezo-materials, but the most common are nanotubes, which can be used in tissue engineering and drug delivery because of their high piezoelectric properties. boron nitride nanotubes have been shown to have a positive effect on cell adhesion [63]. There are numerous advantages by using BN nanotubes as nano vectors for the delivery of electrical or mechanical signals within cells, but their exceptional piezoelectricity stands out as the most critical [62].

Following that, a more detailed study of the piezoelectric stimulation of SH-SY5Y neuroblastoma cells was undertaken utilizing tetragonal BT nanoparticles. They established for the first time in this study that when BT nanoparticles are activated by USs, they can elicit a considerable cellular response in terms of Ca^{2+} and Na^{+} influx. The absence of cellular response when ultrasounds (USs) were combined with non-piezoelectric nanoparticles strengthened the idea of piezoelectric stimulation, which was further substantiated by an electroelastic model of BT nanoparticles subjected to USs [64].

Zinc Oxide: Due to their asymmetric hexagonal structure and polar crystal surface, zinc oxide-based piezoceramics are widely used. Due to their ease of manufacture, they have found application as piezoelectric nanogenerators. ZnO nanostructures are biocompatible [65]. It has been reported that as the size of ZnO grows, its cytotoxicity increases, affecting the amounts of reactive oxygen species, lowering the mitochondrial membrane potential, and inducing interleukin production in human cells. Additionally, chemical modification has been shown to minimize toxicity, allowing for usage in biomedical applications [66].Table 1 summaries some experimental works in neural tissue engineering with piezoelectric ceramics.

4.2. Piezo-polymers

PVDF is a piezoelectric material, with the chemical formula (CH2–CF2)n. The fluorine atoms in its molecular chain are more electronegative than the carbon and hydrogen atoms. Thus, polar C–F bonds are formed, and these C–F bonds each have a substantial dipole moment. PVDF possesses five crystalline polymorphs, including the nonpolar "α- and ε-phases, and three polar phases (β, γ, δ), depending on crystallization and processing conditions [70, 71]. This polymorph, α-PVDF, crystallizes easily from the melt and is the most prevalent form of PVDF. There are two chains in the unit cell of trans-gauche-trans-gauche0 nonpolar α-PVDF, which alternate in a TGTG0 conformation. Its antiparallel molecular chain arrangement cancels out the net dipole moment. An electric field, an annealing treatment, or mechanical stress (cold drawing) can all be used to change the α-PVDF into one of three polymorphic forms. All dipoles in the δ-phase are placed parallel to one another, leading to ferroelectric activity despite the same TGTG0 macromolecular chain conformation. Poling α-PVDF at an applied electric field of 100–150 MV/m can yield this phase. With regard to the carbon backbone, the hydrogen and fluorine atoms rotate. However, the electrode and polymer are frequently destroyed in this procedure [72]. Furthermore, given a high electric field (about 500 MV/m), the δ-phase can change to the β-phase, with the fluorine, carbon, and hydrogen atoms all moving to produce the all-trans configuration [73]. Its orthorhombic phase has an all-trans (TTTT) planar zigzag conformation, with all dipoles aligned in the same direction normal to the chain axis, and it has an orthorhombic phase with an all-trans (TTTT) planar zigzag conformation. As a result, the β-PVDF phase has the greatest ability to create spontaneous polarization while also exhibiting strong piezoelectric and ferroelectric capabilities. Because of the large energy barrier associated with the all-trans conformation, it is improbable that the β-phase will emerge from the melt [74].

Several processes such as annealing treatment at high pressure, electrical poling, and mechanical drawing can change the α-phase into the β-phase. There is also an orthorhombic unit cell in the γ-phase, which is distinguished by a sequence of trans and gauche conformational transitions (T3GT3G0). It is possible to obtain this phase through the high-temperature drawing of ultrahigh molecular weight PVDF [75].

PVDF can be copolymerized in a random order with TrFE, i.e.,–(CHF–CF2)–, and tetrafluoroethylene (TeFE) [– (CF2–CF2)–. Without mechanical stretching or drawing, P(VDF-TrFE) crystallizes quickly from the melt and forms the β- phase through copolymerization. This is because the extra fluorine atoms in the molecular chain cause a steric hindrance effect, inhibiting the development of the α-phase. Interchain distance is increased and activation energy for α-phase to β-phase is reduced by the addition of co-monomer. A higher degree of crystallinity and alignment of the dipoles in CF2 can be achieved through additional annealing procedures as well as mechanical stretching or electrical poling [76]. P(VDF-TrFE) normally has a TrFE concentration of 20–50 mol % in its trans conformation. P(VDF-TrFE) has mixed phases of α, β, and γ and at a TrFE content of 20 mol %. As a result, the amount of TrFE in P(VDF-TrFE) affects its ferroelectric, piezoelectric, and structural properties [77, 78]. Table 2 summarizes piezoelectric polymers in nerve tissue engineering.

4.2.1. Enhancing method to increase β-phase

PVDF is a semicrystalline polymer with five major crystalline polymorphs, including α, ε- (non-polar), β, γ, δ- (polar) [84]. The conformations of the polymeric chains determine these polymorphs. The PVDF non-polar α-phase transforms to the strongest polar moment of β-phase when the polymeric jet stretches during the electrospinning process. The β-phase content, rather than other crystallinity phases present in the structure, determines the piezoelectric and mechanical properties of PVDF nanofibers, which define their biomedical uses. By improving

Table 1.
Piezoelectric ceramic used in neural tissue engineering studies

Piezoelectric material	Method	Mechanical stimulation	Cells	Biological observations	Ref.
Boron nitride nanotube	Cell electrical stimulation by nanotubes	Ultrasounds	PC12 neural-like cells	Neurite elongation enhancement	[67]
Barium titanate nanoparticles	BTPNs as trancducer for indirect cell stimulation	Ultrasounds	SH-SYSY neural-like cells	Sodium and Calcium transients	[68]
PVDF-Trfe- barium titanate nanoparticles composite	Polymer/ceramic composite films for direct piezoelectric stimulation	Ultrasounds	SH-SYSY neural-like cells	Neurite elongation enhancement, Calcium transients	[69]

the electrospinning conditions and creating highly directed nanostructures, the -phase content could be greatly increased [85]. Because of the specific interactions induced by the incorporation of nanofillers such as multiwall carbon nanotubes (MWCNTs), metal nanoparticles, modified nanoclays, and graphene derivatives into PVDF nanofibers, the β-phase content and its associated stability in PVDF Nanofibers is increased and their associated stability is improved [86]. The electrical poling and mechanical stretching that occur during the electrospinning process allow the twisted PVDF chains to be extended and their orientation along the fiber axis to be promoted. The relaxation that occurs following electrospinning, on the other hand, results in the migration of PVDF polymeric chains back to their stable coiled form. Electrospinning of PVDF nanofibers can be used to integrate a range of particles, organic or inorganic fillers, to address this difficulty. PVDF, for example, can be reinforced by the addition of graphene oxide (GO) lamellae to the PVDF polymeric solution. Core-shell GO/PVDF nanofibers with enhanced β-phase content and four-fold greater piezoelectricity were produced by distributing GO lamellae parallel to the fiber axis on the outer shell and PVDF chains self-orientation at the fiber inner section. One of the most effective methods for orienting dipoles in the desired direction, increasing β-phase content, and thus increasing piezoelectricity, has been found to be after-treatment heat treatment (annealing) and stretching (pulling) [87]. The combined electromechanical properties can be improved more effectively through thermal annealing. The piezo responsiveness and elastic modulus of PVDF-TrFE nanofibers were improved by 2.0 and 1.7 times, respectively, after thermal annealing and stretching [76]. Samples of polymers are heated to a high temperature and then quenched with cold water to begin the process known as “annealing” [88]. Without additional treatment (as spun), the PVDF-TrFE nanofibers could be used for sensing applications; alternatively, further heat treatment (annealing) is an option [87]. The nanofibers’ shape, mechanical properties, and crystallinity are profoundly affected by the chilling procedure that follows the heat treatment [89]. Nanofiber crystallinity was improved by 70% by annealing PVDF-TrFE in the Curie and melting temperature range. After this procedure was completed, they saw a three-fold increase in elastic modulus and a 70% rise in piezoelectric constant in the nanofibers that we manufactured [90].

4.3. Natural piezo-biopolymers

Because of their biodegradability and low toxicity, natural polymers are becoming increasingly important in tissue engineering. The piezoelectricity of several biopolymers is prominent. We’ll use proteins and polysaccharides with high piezoelectricity as an example. Cellulose, a natural polymer having a piezoelectric value of 0.10 pC/N, has been extensively studied. Cellulose is a highly biocompatible linear glucose homopolymer [91, 92]. Microspheres, membrane sponges, and non-woven, woven, or knitted textiles are all examples of their application. Some of the uses of cellulose include connective tissue development, bone and cartilage tissue engineering [93, 94]; the growth of viable cardiac cell constructions in vitro, and drug delivery. Because of its high solubility in water, especially at low temperatures, methylcellulose (MC) is an essential cellulose derivative. The amount of methyl substitution and the distribution of methoxy groups determine how soluble it is in water. We can conclude from this that MC is well-suited for use in the healing of brain lesions [95]. In vitro and in vivo studies showed that gelatin-coated nanoparticles in cellulose acetate/ poly lactic acid (PLA) scaffolds were more effective at promoting cell viability than uncoated nanoparticles. However, PC12 cells were able to grow new, longer neurites when they were attached to a gelatin/chitosan/ poly(3,4-ethylenedioxythiophene) (PEDOT) hybrid scaffold. This helped neuron-like cells stick together and grow [96]. In nature, chitin is a polysaccharide that has a piezoelectric structure and a low piezoelectric coefficient in the range of 0.2 to 1.5 pC/N [97]. It is found in the shells of insects, mollusks, and crustaceans. Because chitin is hydrophilic and biocompatible, it is used in biomedical applications to help cells grow, change, and stick together [98]. Chitosan is a biodegradable and biocompatible linear polysaccharide that is made by partially deacetylating chitin, which is a type of chitin. It has been used a lot to make porous scaffolds for cartilage tissue engineering [99]. But the low mechanical characteristics of chitosan scaffolds make their practical use difficult. Blending chitosan with synthetic polymers is an

Table 2.
Piezoelectric polymers in nerve tissue engineering

Piezoelectric polymer	Method	Scaffold type	Cell type	Results	Ref
Polyvinylidene Fluoride (PVDF)	-	Nanosheets	Rat neuronal cell line	Providing a scaffold similar to neural natural ECM	[79]
	Polyglycidyl methacrylate (PGMA) immobilization on PVDF- porous and dense membranes	Membranes	Neuronal cells	Neuron aggregation on pristine PVDF membrane with neurite branching Neuron aggregation on PVDF-PGMA membrane without neurite branching	[80]
	Culturing neurons on electrically charged polymer growth substrates	Film	Mouse neuroblastoma cells	Nb2a cells grown on piezoelectric substrates exhibited neurite lengths Importance of surface characteristic as bulk properties for film scaffold	[81]
Poly[(vinylidene fluoride-co-trifluoroethylene] (PVDF-TrFE)	hNSC/NPC differentiation on piezoelectric fibers	Fibers	Poietics normal human neural progenitors	Great neurite length on micron-sized, annealed (more piezoelectric), aligned scaffolds, make it appropriate for neural tissue engineering	[82]
	hNSC/NPC differentiation on piezoelectric fibers	Films	Poietics normal human neural progenitors	• Great neurite length on micron-sized, annealed (more piezoelectric), aligned scaffolds, make it appropriate for neural tissue engineering	[82]
	Impact of polling on output voltage and neural regeneration	Tubes	In vivo implementation: rat sciatic nerves	When compared to chemically identical, unpoled tubes, piezoelectrically active vinylidene-fluoride-trifluoroethylene copolymer tubes significantly improve nerve regeneration. Polarity of the corona poling procedure used to fabricate piezoelectric materials may play a role in determining biological responses.	[83]

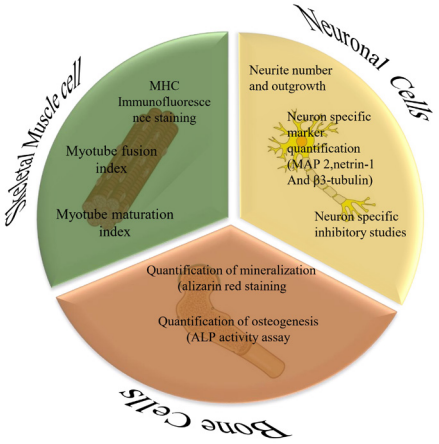

Fig. 3. 3D printing techniques classification for piezoelectric materials in tissue engineering.

efficient way to address its disadvantages [100]. It has been reported that the medication piperine, which has been shown to have neuroprotective potential against Alzheimer's disease, may be delivered to injured parts of the CNS using biocompatible chitosan microspheres designed by Skop et al [97]. For the delivery of therapeutic drugs to the brain, chitosan nanoparticles have been developed. Adhesion and proliferation of PC12 cells along the fiber orientation were enhanced when Polycaprolactone (PCL)/chitosan fibers were aligned [101]. Peripheral nerve regeneration can be facilitated by PLGA/chitosan scaffolds in vitro and in vivo. Collagen from 0.2 pC/N to 2.0 pC/N, this natural piezoelectric material has an excellent piezoelectric coefficient. Collagen scaffolds have been used in bone healing research [102]. The development of cartilage tissue using collagen-calcium phosphate composites has also been reported [103]. It has also been shown that piezoelectric composite scaffolds made of collagen and hydroxyapatite are effective for cell development. Chitosan-coated collagen scaffolds have been explored in the regeneration of adipose tissue. It was found that the scaffolds were cytocompatible in vitro and in vivo, and that adipocytes were planted into the scaffolds. Using magnetically aligned type I collagen gel, generated by subjecting the forming collagen gel to a strong magnetic field, as a filler for collagen tubes is an innovative application of collagen. Small peripheral nerve injuries, such as a 6-mm gap in a mouse's nerve, were successfully repaired using this approach *in vitro* and *in vivo* [104, 105]. Table 3 summarizes the piezoelectric characteristics of the most relevant natural materials.

5. Fabrication techniques

We need tissue engineering scaffolds to be bioactive and biocompatible as well as possessing suitable porosity and pore size to facilitate tissue regeneration and repair. Cell development, proliferation, and adhesion as well as the transportation of nutrients and metabolic waste products, require great mechanical strength [109, 110]. A low-cost, easily-processed porous scaffold made of PVDF is an excellent choice for tissue engineering. Conventional processing techniques, such as solvent-casting and 3D polymer template, solvent-casting/particulate leaching, thermally induced phase separation (TIPS), and non-solvent induced phase separation (NIPS) can be used to generate functional scaffolds based on PVDF and its copolymers [111-113].

5.1. 3D printing

The fabrication of 3D piezoelectric structures can be done using three main processes (extrusion, energy, and droplet based) [114]. To achieve the desired dipole alignment in extrusion-based printing, anisotropic

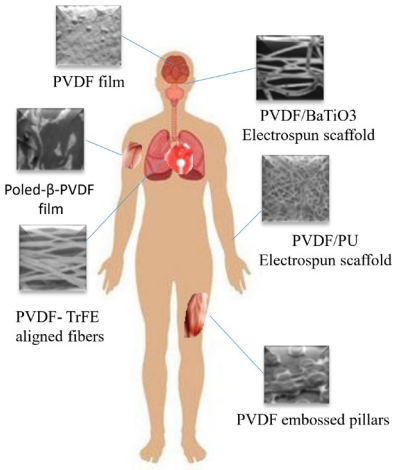

Fig. 4. Application of electrospun piezoelectric nanofibers in tissue engineering.

structures can be used to push the material through a tiny nozzle, which requires mechanical effort. Direct writing (DW) [115], direct-write assisted near field electrospinning (DW-NFES), and Fusion deposition modeling (FDM) [116]are the three types of extrusion-based methods (Brown et al., 2012) (Figure 3). For various applications, piezoelectric PVDF combined with 3D printing and melt electro writing processes is becoming more prevalent. However, due to the non-biodegradability of most of these materials, their use in tissue engineering is currently limited. Because of the piezoelectric charge generated by repeated external stress, the fabricated 3D structures may aid tissue regeneration. By combining intelligence and 3D printing techniques, piezoelectric materials could increase precision and customization, making them particularly attractive for tissue engineering applications. As a result, a 3D-printed PVDF ear prosthesis for use with hearing aids was created using the FDM process. For example, in 2016 it was determined how the prosthesis reacted to pressure and temperature changes. For pressures ranging from (0 Pa–16.35 MPa) and temperature ranging from (2°C–90°C), this piezoelectric prosthesis generated an electrical potential. To mimic human hearing, this intelligent PVDF prosthesis used the piezoelectric effect [117].

5.2. Solvent casting

Polymers are dissolved in an organic solvent and then mixed with particles of appropriate size, such as salt or sugar, before being cast onto a glass plate for membrane manufacturing or into a 3D mold for scaffold construction, depending on the procedure used. The mold can be cleaned in a water bath in order to dissolve the particles trapped inside. No special equipment is required for this procedure, which makes it quick and straightforward to complete. One disadvantage of this technique is the low pore interconnectivity, whereas variation in pore sizes and shapes, which are governed by the amount, size, and shape of the added particles, can be controlled by sieving the particles within a specific size range, and the crystallinity of the porous foam can be controlled by ap-

Table 3.
List of piezoelectric natural polymers.

Polymer	Piezoelectric coefficient -d14(Pc/N)	Ref
Fibrin	DNA of salmon (0.07)	[106]
Keratin	Wool (0.1)	[107]
	Horn (1.8)	[107]
Collagen	Tendon (2.0)	[106]
	Skin (0.2)	[108]
	Bone (0.7)	[108]

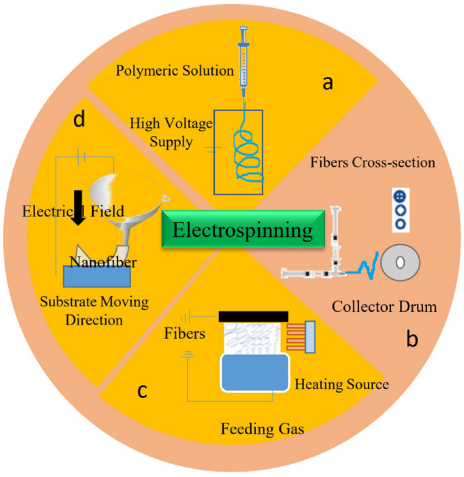

Fig. 5. Schematic of electrospinning techniques: a) Conventional b) Coaxial c) Bubble d) Near field

plying appropriate thermal treatment before leaching (The crystallization behavior of porous poly (lactic acid) prepared by modified solvent casting/panning). Alternatively, porous poly (lactic acid) prepared [118]. PVDF 3D scaffolds were created using a variety of techniques, including 3D nylon, freeze extraction, and solvent casting with particle leaching using poly vinyl alcohol (PVA) templates. In all of the processing procedures, scaffolds with high crystallinity (33–47 %) and a large amount of phase (86–94 %) were formed. The scaffold's tensile strength and Young's modulus may be tailored for various tissues based on quasi-static mechanical studies that indicated that increasing the pore size within the scaffold decreased these properties[119] .

5.3. Phase separation

Phase separation is a straightforward process that may be applied to both synthetic and natural materials. The core idea of phase separation is based on the fact that two or more polymers have different solubilities and hence separate into their respective solvents. The separated polymer can then be moulded around a mandrel to generate a tubular structure suitable for use as a vascular transplant. Thermally induced phase separation (TIPS) is accomplished by lowering the temperature as the polymer separates from the solvent, whereas diffusion induced phase separation (DIPS) is accomplished by immersing the polymer in an antisolvent bath to leach away the polymer solvent. Phase separation enables greater control over scaffold thickness and porosity, which are crucial elements in the success of vascular grafts due to their effect on both cell infiltration and vessel mechanics. There are three ways that polymer solutions can undergo phase transitions mass exchange with a non-solvent, solvent removal, and, temperature change [120]. For non-solvent induced phase separation, the polymer solution is immersed in a coagulation bath of non-solvent, which causes the polymer solution to become thermodynamically unstable, resulting in the phase separation into a polymer-rich and a polymer-lean phase. An interconnected porous network can be formed by eliminating solvents and polymer lean phases from the matrix and replacing them with a polymer-rich layer. To create PVDF membranes with piezoelectric capabilities, Wang et al. recently used 1-Butyl-3-methyl-imidazolium-hexafluorophosphate (BMIMPF6) as environmentally friendly solvents and utilized TIPS. Since the PVDF molecular chain and the ionic liquid have strong electrostatic interactions, PVDF membranes have spherulite structures with the beta phase crystalline phase [121].

5.4. Electrospinning

Piezoelectric materials at the nanoscale have been examined as a real possibility for various applications because of the increased interest in nanofabrication. Because the length scales at which biological interactions take place are close to those of cellular and extracellular components, piezoelectric materials have received significant attention for the generation of biomedical nanodevices. This provides control and activation of electro-mechanically sensitive cells [122]. Consequently, electrospinning is an intriguing technique for fabricating 1D nanostructures that allows for fine control of key parameters (i.e., diameter, composition, and morphology). PVDF nanofibers for biological applications can be made using an electrospinning process[123] . Piezoelectric nanofiber scaffolds currently target a wide range of tissues, as depicted by the diagram in Figure 4. A strong electric field is used to ionize the polymer solution in this procedure. As a result of whipping solvent evaporation, and bending, random or aligned fibers can be formed on a collector's surface when electrostatic force overcomes the polymer droplet's surface tension and one continuous charged polymer jet is blasted from the needle tip toward the collector. Bio-functional nanofibers with increased piezoelectricity and mechanical performance have been generated by conventional electrospinning (Figure. 5) in recent years. PVDF nanofibers with a highly orientated core-shell, hollow or porous structure have been synthesized using an electrospinning method that has had its feeding systems and collector geometry [124]. Post-drawing components were also developed into an electrospinning collection system to improve the crystallinity and β-phase content of PVDF nanofibers, which in turn improved their piezoelectric, mechanical functions and performance capabilities, and biological properties. PVDF nanofibers are more piezoelectric when electrospun because of a strong electrostatic force supplied to the solution jet [123]. Electrospun fibers can be made from a variety of polymers, including emulsions, molten states, and solvent solutions. In order to electrospinning, the PVDF polymer has been dissolved in a 12–25 weight percent Dimethylacetamide (DMA) mixture or a DMS/ACE, DMA/ACE, Dimethylformamide (DMF)/water, or DMF/ACE mixture with 12–25 weight percent DMF [125]. The most significant advantage of melt-electrospinning over solution electrospinning is the ability to create fibrous structures without the use of organic solvents. Low conductivity of the polymer melt helps to avoid whipping motion, resulting in a jet that travels directly from needle to collector, resulting in fibers with larger diameters being formed during this process. 236 As "smart" scaffolds, piezoelectric ultrafine or nanofibers can deliver electrical stimulation in response to mechanical input or the other way around, stimulating tissue regeneration [126]. In fact, piezoelectric electrospun fibers can promote cell differentiation, proliferation, and tissue function repair by supplying topographical, physico-chemical, and mechano-electrical stimuli. The ultrafine fibers can replicate the fibrillar ECM's architecture, allowing cells to communicate and receive mechanical support [127].

6. Conclusions and future perspectives

Clinicians and researchers are increasingly interested in smart materials because of their potential use in the development of temporary implants, drug delivery systems, and biomedical devices. A piezoelectric scaffold may renew and repair tissues in a way that is similar to the natural processes occurring inside the extracellular matrix. Neurite extension and increased cell adhesion and proliferation were reported lately in in vitro conditions when the piezoelectric scaffolds were deformed by mechanical or ultrasound activation. Most recent tests with piezoelectric scaffolds have not used stimulation in any way, and as a result, no piezoelectricity or associated electrical charges have been observed. There are only a limited number of charges that can be activated from a cellular perspective if the cells are permanently polarized, as well as if they contract and protrude during the contraction and expansion of their connect-

ed cells. To get a true piezoelectric response, it is necessary to simulate the in vivo conditions of internal macro- and micro-deformations employing mechanical agitation (ultrasounds) in vitro conditions for investigations involving piezoelectric scaffolds. The non-biodegradability of polymers with the highest piezoelectric coefficients, such as PVDF and its copolymers, is the next difficulty in piezoelectric scaffolds. As a result, biodegradable piezoelectric polymers like PHB or PLLA should be considered. Composite scaffolds including an electro-conductive polymer like PANi and a piezoelectric polymer are an intriguing alternative that should be investigated more in the future. Electro-conductive polymer added to piezoelectric matrix increased piezoelectricity, according to the results. When considering biodegradable piezoelectric polymers with initially low piezoelectricity, this type of composite scaffold should be considered.

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