Piezoelectric Nano-Biomaterials for Biomedicine and Tissue Regeneration

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Among various classes of biomaterials, the majority of non-centrosymmetric crystalline materials exhibit piezoelectric properties, i.e., the accumulation of charge in response to applied mechanical stress or deformation. Due to the growing interest in nanomaterials, piezoelectric nano-biomaterials have been widely investigated, leading to remarkable advancements throughout the last two decades. Piezoelectric properties, high surface energy, targeting properties, and intricate cell–material interactions render piezoelectric nanomaterials highly attractive for application in therapeutics as well as regenerative medicine. Herein, the major focus is to highlight the wide range of applications of piezoelectric nano-biomaterials in drug delivery, theranostics, and tissue regeneration. After a brief introduction to piezoelectricity, an overview is provided on the major classes of piezoelectric biomaterials as well as a description of the origin of biopiezoelectricity in different tissues and macromolecules. Subsequently, relevant properties and postfabrication strategies of nanostructured piezoelectric biomaterials are discussed aiming to maximize piezoresponse. Finally, recent studies on nano-piezoceramics and piezopolymers are presented, with specific focus on barium titanate, zinc oxide, and polyvinylidene fluoride.

1. Introduction

Biomaterials exhibiting piezoelectric properties (piezoelectric biomaterials) are a specific class of smart materials which display electromechanical behavior by transforming mechanical energy into electric polarization without the application of an external voltage. In Greek, “piezo” means “pressure.” Accordingly, piezoelectricity can be translated as “pressure-induced electricity.” Piezoelectricity in the physiological environment originates from structural anisotropy or transient deformations developing a net dipole moment greater than zero. Transient deformation of crystalline materials (highly ordered crystal lattices) occurs under mechanical stress leading to an atomic position shift within unit cells. This phenomenon leads to loss of the center of symmetry and induces accumulation of charge through an ordered dipole distribution.

Bioelectricity is an integral part of living systems where endogenous electric fields (EFs) play a vital role in early embryonic development to tissue regeneration. In the embryonic phase, developmental defects may arise due to minor deviations from the steady-state potential of the embryo. Endogenous electric fields may influence cellular processes such as chemotaxis, migration, proliferation and differentiation of cells. In addition, EFs also affect cell division, intracellular communication, neuronal activities, mechanotransduction, ion transport, as well as bone and epithelial healing. Due to the importance of bioelectricity, electrotherapy has been developed for accelerated wound healing, deep brain stimulation, tissue regeneration, improved musculoskeletal conditions and recovery of bone fractures. To this end, external devices or electrodes are employed to supply low level electric currents across the skin.

Nevertheless, the complexity and inconvenience to patients associated with electrotherapy have triggered the development of piezoelectric biomaterials possessing a built-in capacity for electric signaling. Such piezoelectric biomaterials offer numerous advantages over conventional biomaterials as they can easily transduce electricity to living systems in response to processes such as cell migration, body movements or external stimulation (e.g., ultrasound (US), vibration, etc.). These materials can also transform electrical stimuli to mechanical stresses via geometric deformation, known as reverse piezoelectricity.

Various piezoelectric biomaterials have been explored for different biomedical applications (Table 1). Piezoelectric inorganic materials (e.g., lead zirconate titanate (PZT), barium titanate (BT), boron nitride (BN), zinc oxide (ZnO), and hydroxyapatite (HA)) are more versatile compared to organic alternatives as they usually possess an extremely high piezoelectric coefficient and favorable mechanical properties. Piezopolymers can be of natural (e.g., collagen or silk) or synthetic origin,
e.g., poly(l-lactic acid) (PLLA), poly(vinylidene fluoride) (PVDF), poly(vinylidene fluoride-trifluoro ethylene) (P(VDF-TrFE)).

Recently, novel piezoelectric materials were developed such as gallium nitride (GaN), lithium niobate (LN), lithium potassium sodium niobate (LNKN), lead magnesium niobate (PMN), bismuth sodium titanate (BNT), polyhydroxybutyrate (PHB), peptide nanotubes (PNTs), and diphenylalanine (FF). Their suitability for biomedical applications remains unexplored. Hybrid piezoelectric materials such as HA/BT, HA/LNKN, PVDF/BT are also extensively investigated for applications in the biomedical field due to their multifunctionality and improved material properties.

Due to the nanosize effect, piezoelectric nanomaterials find wide applications in guided locomotion and controlled drug delivery to the targeted tissues, especially in the area of cancer chemotherapeutics. As therapeutic nanocarriers, they offer advantages such as easy delivery by minimally invasive procedures and reduced toxicity by an improved biodistribution with minimum off-target effects. A triggered drug release can be achieved by applying a mechanical stimulus from an external source. However, they also have shortcomings, including inadequate loading of therapeutic molecules or image contrast agent and in vivo stability. Piezoelectric nanomaterials often combine magnetic, optical, and plasmonic properties. BT-, ZnO-, or bismuth ferrite (BiFeO3 or BFO)-based 1D nanomaterials are useful as therapeutic nanocarriers as well as diagnostic agents. They can be used for in vivo imaging as they are not limited by luminescence blinking and photobleaching, unlike conventional fluorophores. Compared to bulk materials, piezoelectric nanomaterials are advantageous in terms of eliciting cell-specific response due to nanodimension, surface nanotopography and high surface-area-to-volume ratio resulting in high surface energy. For instance, ZnO nanoflowers support osteoblast growth and osseointegration, while ZnO nanorods inhibit fibroblasts, endothelial cells, and macrophages. 2D and 3D piezoelectric nanomaterials when used as scaffolds can guide in vitro cell activity and tissue regeneration. They play an instructive role in tissue regeneration, especially for bone and cartilage, where mechanical pressure induces electric (i.e., piezoelectric) response, or for nerve, skin and muscle where electric field regulates tissue function. P(VDF-TrFE) scaffolds with embedded piezoelectric BN nanotubes support more osteogenic activity as compared to electrospun BT nanoparticles/poly(3-hydroxybutyric acid-co-3-hydroxy valeric acid) (PHBV) scaffolds, which support more chondrogenic activity. Piezoelectric nanofibers can deliver differential outcomes based on fiber morphology, e.g., PVDF or PVDF-TrFE nanofibrous scaffolds with aligned fibers exhibit preferentially higher neuronal activity compared with random fibers.

Dermal patches prepared from piezoelectric ZnO nanorods are able to generate piezoelectric potentials required for wound healing. Piezoelectric scaffolds produce interesting results in the presence of mechanical stimulus, e.g., osteogenically induced human mesenchymal stem cells (hMSCs) cultured on PLLA nanofibrous scaffolds exhibit osteogenic activity, but under cyclic tensile loading, show tenogenic activity. Furthermore, materials processing has significant influence on their performance. Therefore, it is worth critically reviewing piezoelectric nano-biomaterials for therapeutic and tissue engineering applications from different perspectives such as materials processing, physicochemical characteristics, and external stimuli, which can greatly influence material properties, cellular chemistry, and drug release.

Several reviews have been dedicated to piezoelectric biomaterials, but these reviews focus on either extremely specific types of piezoelectric biomaterials (e.g., BT nanoparticles for drug delivery and label-free imaging) or specific application areas such as sensing and actuation, tissue engineering, or drug delivery using electroactive biomaterials (including...
Table 1. Applicability of piezoelectric nano-biomaterials.

<table>
<thead>
<tr>
<th>Piezoelectric device</th>
<th>Operating principle</th>
<th>Outcome</th>
<th>Ref.</th>
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<tbody>
<tr>
<td><strong>Drug delivery</strong></td>
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<tr>
<td>P(VDF-TrFE)/Ni nanoring-PPy nanowires (Piezoelectric Nanoeels)</td>
<td>Magnetic manipulation for locomotion (5–15 mT, 1–16 Hz) and pulsatile drug release (10 mT, 7 Hz)</td>
<td>35% Human epithelial breast cancer cell death</td>
<td>[21]</td>
</tr>
<tr>
<td>FeGa@P(VDF-TrFE) core–shell nanowires</td>
<td>3D propulsion actuated by conical rotating magnetic field</td>
<td>≈40% Cancer cell death</td>
<td>[22]</td>
</tr>
<tr>
<td>BT-DOX nanoparticles</td>
<td>Enhanced doxorubicin (DOX) internalization due to complexation with BT nanoparticles</td>
<td>Significant cytotoxicity on SH-SYSY cells</td>
<td>[23]</td>
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<tr>
<td><strong>Theranostics (applicability facilitated by optical properties)</strong></td>
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<tr>
<td>BT/Au core–shell nanoparticles</td>
<td>Photothermal ablation of human SH-SYSY cells using pulsed near infrared (NIR)</td>
<td>Photoinduced SH-SYSY cell damage</td>
<td>[24]</td>
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<tr>
<td>ZnO-Gd-DOX nanoparticles</td>
<td>DOX release in tumor acidic environment; imaging with strong red emission at 600–800 nm</td>
<td>Remarkable decrease of tumor volume in BxPC-3 nude mice</td>
<td>[25]</td>
</tr>
<tr>
<td>IR-680-FCPZnO nanoparticles</td>
<td>≈75% PTX release within 6 h; folic acid increased ≈30% tumor tissue specific NPs accumulation</td>
<td>&lt;5% Inhibition of MCF-7 cell proliferation</td>
<td>[13a]</td>
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<td>BFO-APTES-CM-Trp SHG nanoparticles</td>
<td>Trp release in response to SHG at 395 nm induced by NIR irradiation at 790 nm</td>
<td>15 min Irradiation induced 60% Trp release</td>
<td>[13b]</td>
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<tr>
<td><strong>Bone and cartilage regeneration</strong></td>
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<tr>
<td>BiFeO3 nanofilm-coated SrTiO3 implants</td>
<td>Provided a constant built-in electropositive field (+75 mV)</td>
<td>Faster bone healing and osseointegration in rat femoral defects</td>
<td>[6a]</td>
</tr>
<tr>
<td>Poled β-PVDF films</td>
<td>Vibration (1 Hz frequency, 1 mm amplitude) to mimic in vivo conditions</td>
<td>Enhanced cell adhesion and osteogenic differentiation on negatively poled surface</td>
<td>[7d]</td>
</tr>
<tr>
<td>Annealed P(VDF-TrFE) electrospun scaffolds</td>
<td>Significantly higher streaming potential (61.1 ± 1.5 µV) under dynamic compression</td>
<td>More pronounced osteogenesis for annealed P(VDF-TrFE) versus chondrogenesis on as-spun scaffolds</td>
<td>[26]</td>
</tr>
<tr>
<td>BN nanotubes/P(VDF-TrFE) films</td>
<td>≈20–60 mV Surface potential after US stimulation</td>
<td>Promoted differentiation of SaOS-2 on cast-annealed substrates</td>
<td>[16a]</td>
</tr>
<tr>
<td>HA/PLLA, Col I/HA/PLLA electrospun scaffolds</td>
<td>Enhanced piezoresponse due to aligned nanofibers; functional synergy between Col I and HA</td>
<td>Enhanced osteogenic activity of human fetal osteoblasts</td>
<td>[27]</td>
</tr>
<tr>
<td>PLLA-g-HA/PLLA scaffolds</td>
<td>Improved strength through reinforcement and toughening synergy; microenvironment change due to PLLA-g-HA release</td>
<td>Increased adhesion and proliferation of chondrocytes</td>
<td>[28]</td>
</tr>
<tr>
<td>BT Nanoparticles/HA</td>
<td>Synergistic effects of piezoelectricity and dynamic loading</td>
<td>Accelerated MG63 cell activity and bone formation</td>
<td>[29]</td>
</tr>
<tr>
<td>Ormocomp/BT nanoparticles (Osteo-Print)</td>
<td>Combined effects of BT nanoparticles and US stimulation</td>
<td>Significantly promoted SaOS-2 differentiation</td>
<td>[30]</td>
</tr>
<tr>
<td>Laser irradiated KNN (K0.5Na0.5NbO3)</td>
<td>Interspersed domains of high and low piezoelectricity producing differential electrical signals</td>
<td>Higher in vitro and in vivo osteogenesis without osteogenic medium</td>
<td>[31]</td>
</tr>
<tr>
<td>Electrospun sheets of BT nanoparticles/PHBV</td>
<td>d33 values (1.4 pC N−1) of poled samples were similar to native bone and cartilage (0.2–0.7 pC N−1)</td>
<td>Higher chondrocyte activity and Col II gene expression on polarized scaffolds</td>
<td>[16b]</td>
</tr>
<tr>
<td><strong>Nerve regeneration</strong></td>
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<tr>
<td>Poled PVDF</td>
<td>2–3 mV Potential generation when vibrated at 1200 Hz</td>
<td>Enhanced neurite outgrowth using Nb2a cells</td>
<td>[33]</td>
</tr>
<tr>
<td>GO nanosheets/PVDF</td>
<td>Enhanced β-phase, superior hydrophilicity, electromechanical and piezoelectric properties</td>
<td>Higher PC12 cells activity</td>
<td>[34]</td>
</tr>
<tr>
<td>Positively poled P(VDF-TrFE)</td>
<td>High piezoresponse in response to slight mechanical deformations</td>
<td>Complete bridging of 10 mm nerve gaps</td>
<td>[35]</td>
</tr>
<tr>
<td>Electrospun PVDF and P(VDF-TrFE)</td>
<td>Advanced piezoelectric properties due to high β-crystallinity</td>
<td>Aligned fibers significantly promoted neurite outgrowth of DRGs</td>
<td>[17]</td>
</tr>
<tr>
<td>Electrospun P(VDF-TrFE)</td>
<td>Cells migration induced substrate deformation generating piezoelectric potential</td>
<td>Higher human neural stem/precursor cell differentiation (hNSC/NPC)</td>
<td>[7a]</td>
</tr>
<tr>
<td>BT nanoparticles</td>
<td>Developed 0.07–0.19 mV surface potential; under US stimulation of the BNNTs</td>
<td>Accelerated SH-SYSY cell differentiation; neuronal networking; neurite outgrowth</td>
<td>[36]</td>
</tr>
<tr>
<td>BN nanotubes</td>
<td>US stimulation</td>
<td>30% Enhancement of neurite outgrowth in PC12 cells</td>
<td>[23]</td>
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</tbody>
</table>
sections on piezoelectric biomaterials) or bone and cartilage regeneration. However, these latter reviews do not elaborate on nanostructured piezoelectric biomaterials. Therefore, the current review provides a comprehensive overview of research on piezoelectric nano-biomaterials with a focus on applications in biomedicine and tissue regeneration as well as a perspective on the applicability of piezoelectric nano-biomaterials as theranostics. Applications in sensing and actuation are excluded from this review.

### 2. Piezoelectric Biomaterials: General Classification

In this section, synthetic biomaterials with piezoelectric properties are broadly classified into four different categories, i.e., i) naturally occurring piezocrystals, ii) piezoceramics (titanates, lead-based, and lead-free ceramics), iii) piezopolymers, and iv) piezocomposites. Natural crystals and piezoceramics can exist as single crystals (anisotropic, monocristalline, defect-free crystal lattice without grain boundaries), polycrystals (random crystallite orientation with grain boundaries or twin boundaries), solid solutions (homogeneous dispersion of minor dispersed phase in the crystal lattice of the matrix phase), and thin films (subnano- to micrometer-thick layer deposited on solid support).

The development of piezoelectric materials originally started with the discovery of piezoelectric natural crystals (single crystal α-quartz) by the Curie brothers in 1880 as well as the observation of piezoelectric properties for Rochelle salt (NaKC₄H₄O₆⋅4H₂O). Pressure-induced electric polarization was termed direct piezoelectricity. Reverse piezoelectricity (mechanical straining of piezoelectric materials under the influence of an applied electric field) was demonstrated by Gabriel Lippmann in 1881. Until World War I, the application of piezoelectric single crystals (quartz and Rochelle salt) was limited to acoustic devices, since quartz exhibits low electromechanical coupling, narrow frequency bandwidth, inefficient signal transduction, whereas water-soluble Rochelle salt exhibits a temperature dependent performance.

Water-insoluble, polycrystalline barium titanate (BaTiO₃, BT) ceramics were developed during World War II. Gray, 

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**Table 1. Continued.**

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<tr>
<th>Piezoelectric device</th>
<th>Operating principle</th>
<th>Outcome</th>
<th>Ref.</th>
</tr>
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<tbody>
<tr>
<td>BT nanoparticles/P(VDF-TrFE)</td>
<td>US stimulation generated ≈2 mV local voltage and Ca²⁺ transients</td>
<td>Promoted neuronal maturation and neurite outgrowth in SH-SYSY</td>
<td>[37]</td>
</tr>
<tr>
<td>SWNTs/FN/Silk electrospun scaffolds</td>
<td>Piezoelectricity, electrical conductivity, neurite outgrowth regulated by silk, SWNTs, and FN</td>
<td>Functional recovery of myelinated axons after 5 weeks</td>
<td>[38]</td>
</tr>
<tr>
<td><strong>Skin regeneration</strong></td>
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<tr>
<td>Electrospun PVDF/PU</td>
<td>Up to 8% deformation through intermittent biaxial stretching</td>
<td>Cell migration almost doubled with higher wound healing rate in abdomen and back versus vertex region</td>
<td>[7b]</td>
</tr>
<tr>
<td>ZnO NRs/PDMS dermal patch</td>
<td>Potentials developed in the range of endogenous potential (300 and 900 mV)</td>
<td>Enhanced cell migration, metabolic activity, and differentiation</td>
<td>[18]</td>
</tr>
<tr>
<td>Electrospun Col I/PCL</td>
<td>Microporous scaffolds with required mechanical strength</td>
<td>Infiltration and growth of dermal fibroblasts within micro pores and healthy skin tissue regeneration when implanted in rat</td>
<td>[39]</td>
</tr>
<tr>
<td><strong>Ligament and tendon regeneration</strong></td>
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<tr>
<td>Electrospun PLLA</td>
<td>Braided aligned nanofibers; parallel orientation created similar pattern to tendon ECM</td>
<td>Tenogenic differentiation under cyclic tensile strain; spindle-shaped morphology of tendon progenitor cells; enhanced tendon-specific gene expression</td>
<td>[19,40]</td>
</tr>
<tr>
<td>Electrospun Col I /PLLA</td>
<td>Wrapped into bundles to mimic tendon hierarchy; nanofiber alignment along the axis of bundles</td>
<td>Continuous growth of human fibroblasts</td>
<td>[41]</td>
</tr>
<tr>
<td>Electrospun SF–Col I</td>
<td>Biomimetic microenvironment; BMP-13 promoted cell migration</td>
<td>More pronounced attachment of hASCs, human tenocytes</td>
<td>[42]</td>
</tr>
<tr>
<td><strong>Muscle regeneration</strong></td>
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<tr>
<td>Tri-layered ZnO NPs/PDMS /pNIPAm system</td>
<td>Skeletal muscle mimetic microenvironment by repeated stretching and bending of the device (0.3 Hz, 10 d)</td>
<td>Myogenic cell sheets; muscle regeneration upon injection</td>
<td>[43]</td>
</tr>
<tr>
<td>Poled β-PVDF mats</td>
<td>Mechanoelectrical stimulation produced piezoelectric response</td>
<td>Negatively charged surface promoted C2C12 activity</td>
<td>[44]</td>
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<tr>
<td><strong>Cardiovascular Regeneration</strong></td>
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<tr>
<td>Electrospun P(VDF-TrFE)</td>
<td>Aligned microfibers simulate cardiac pacemaking of sinoatrial node cells</td>
<td>Adherence and aligned morphology of cardiomyocytes along nanofiber length</td>
<td>[45]</td>
</tr>
<tr>
<td>BT nanoparticles/PLGA</td>
<td>Nanotransducer effects of BT nanoparticles need detailed characterization</td>
<td>Supported proliferation and differentiation of H9c2 murine cardiomyoblasts</td>
<td>[46]</td>
</tr>
<tr>
<td>P(VDF-TrFE) coated SPIONs/PCL nanofilm</td>
<td>Transient potential on surface when mechanically deformed by external magnetic field</td>
<td>Promoted cardiomyocyte attachment, proliferation and alignment, preserving contractility</td>
<td>[47]</td>
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who is generally considered as “the father of piezoceramics,” demonstrated piezoelectricity of electrically poled polycrystalline BT for the first time in 1946.\textsuperscript{[49]} Subsequently, other perovskite ceramics (General formula: ABO$_3$ like CaTiO$_3$, SrTiO$_3$ (Figure 2a) were also found to be piezoelectric, exhibiting a reasonably high coupling coefficient similar to BT. However, high-temperature applications of BaTiO$_3$ were limited due to an ageing effect (tetragonal to centrosymmetric cubic phase transformation) caused by its low Curie temperature ($T_c = 120$ °C) above which piezoelectric properties disappear. In 1949, single crystals of LiTaO$_3$ (LT) and LiNbO$_3$ (LN) were developed from the ilmenite group, with much higher $T_c$ values (600 and 1140 °C, respectively) compared to BT ceramics.

The era of lead-based piezoceramics started when ABO$_3$ perovskites were doped with Pb resulting in polycrystalline Pb(Zr,Ti)O$_3$ based solid solutions. These compounds were commercialized under the trademark of “PZT” by Clevite Corporation.\textsuperscript{[50]} By tailoring zirconia (Zr) content and including doping acceptor (Mn) and donor (Nb) ions, a wide range of soft (PZT-5H), semihard (PZT-4) and hard (PZT-8) PZT materials were developed for different applications. Zr content in PZTs determines tetragonal or rhombohedral crystal symmetry, while PZTs prepared with a composition near the morphotropic phase boundary (MPB) of these two phases displayed the highest piezoelectric property due to easy dipole reorientation.

Single-crystal relaxor ferroelectrics which follow MPB exhibit high electrostriction, i.e., high strain output under electric poling of rhombohedral phases. Ternary solid solutions of perovskites, such as binary systems of Pb(Mg$_{1/3}$Nb$_{2/3}$)O$_3$ (PMN) and Pb(Zn$_{1/3}$Nb$_{2/3}$)O$_3$ (PZN) along with PbTiO$_3$ (PMN-PT and
Piezoelectricity of crystalline PVDF (β-form) was discovered by Kawai in 1969, but was only employed for peripheral nerve regeneration two decades later.[51] PVDF typically contains 50–60% crystalline fraction after crystallization from its molten state. Uniaxial or biaxial drawing increases the polar β-phase content, and subsequently electric poling leads to polarization due to dipole reorientation.[52] The piezoelectric coefficient of poled PVDF thin films is ~6–7 pC N⁻¹, which is about tenfold higher than other polymers. Random trifluoroethylene (TrFE) copolymer of PVDF (P(VDF-TrFE)) is mainly composed of the β-phase, which can be directly poled without stretching. Poly- i-lactic acid (PLLA), a biodegradable polymer derived from a vegetable (corn) base, exhibits pure piezoelectric effects without pyroelectricity. Poly(3-hydroxybutyrate) (PHB) is another biodegradable piezopolymer frequently used in tissue engineering. Recently, piezoelectric FF nanofibers and PNTs were developed for constructing hydrogels (through self-assembly and extensive π–π stacking) exhibiting mechanical similarity with human soft tissue.[19] Overall, piezoelectric polymers share a few similar characteristics, such as low piezoelectric charge constants (for actuators), low mechanical quality factors (Qm) (for broad resonance band width), large voltage constants (for sensors), similar acoustic impedance (Z) to water. In addition, piezoelectric polymers are generally elastic and light weight, which is beneficial for many biomedical applications.

Piezocomposites comprise of piezoceramic and piezopolymer phases. In 1972, Kitayama reported the hot-rolled PZT/PVDF composites which exhibited a comparable flexibility but a higher piezoelectric coefficient than native PVDF.[50] Piezocomposites are especially useful as ultrasonic transducers for medical diagnoses. Magnetoelastic composites, on the other hand, combine both magnetostriective (e.g., CoFe₂O₄) and piezoelectric (BaTiO₃) components facilitating the development of electric potentials upon application of magnetic fields. Such composites are useful as magnetic field sensor. Photostriactive composites (e.g., PLZT-based materials) exhibit converse piezoelectric effects (strain development through light-induced photoelectricity) and are therefore used for wireless photo- or micro-actuator or sensor applications.

In this review, nanomaterials are defined as materials comprising sub-micrometer building blocks (below 1000 nm) in at least one direction. Herein, examples were coined from a wide range of nanomaterials for different applications. These include, piezoelectric nanoparticles and nanotubes (1D and 2D nanomaterials exemplified with BT nanoparticles and BN nanotubes) for direct cell stimulation and drug delivery and piezoelectric nanowires (3D nanomaterials exemplified with P(VDF-TrFE)/Ni-PPy nanorobots and FeGa@P(VDF-TrFE) nanoeels) for drug delivery applications. Further, piezoelectric nanocomposites were referred to the heterogeneous materials with one component displaying sub-micrometer dimensions, such as polymer matrices containing a dispersed piezoelectric nanomaterial. Therefore, nanofibrous electrospun sheets and 3D hybrid materials composed of BT or BN nanoparticles/tubes have been included.

3. Electrical Properties of Piezoelectric Nanomaterials

Elongation (tension), twisting (shear), bending, or squeezing (compression) of a piezoelectric material leads to generation of surface charges (direct piezoelectric effects) or inversely, deformation occurs under an applied external electric field (reverse piezoelectric effects). In the deformed state, relative shifting of positive and negative charge centers cause polarization without affecting the overall charge neutrality. A typical stress-induced charge distribution in bulk piezoceramics is shown in Figure 2c. Compared to piezoelectricity, flexoelectricity is associated with electric polarization induced by a strain gradient (direct) or deformation induced by an electric field gradient (reverse). Interestingly, strain gradients can distort the centrosymmetric nature in non-piezoelectric crystals inducing flexoelectricity on a point-to-point basis. Here, it is important to mention that charge redistribution can occur in four possible ways: i) displacement of electronic clouds, ii) ion separation, iii) dipole reorientation, and iv) Maxwell–Wagner–Sillars interfacial polarization.[53] Polarization can also be induced via the poling process (quasi-unidirectional orientation of randomly aligned dipoles), which can be either corona poling (application of a high voltage (1–10 kV) at a fixed temperature) or thermal poling (applied at normal to very high temperatures (25–1000 °C).[11,54] Piezoelectric materials demonstrate a wide range of responses depending on their crystallinity. Generally, piezoresponses can be quantified by various parameters including piezoelectric strain coefficients or piezoelectric charge constants (d), piezoelectric voltage coefficients (g) and electromechanical coupling coefficients (k). The piezoelectric charge constant (d) is expressed as polarization (electric charge, m V⁻¹) per unit of mechanical stress (T) (Coulomb/Newton or C N⁻¹) or mechanical strain (S) experienced by the material per unit of applied electric field (E). In “d”, the subscript “i” indicates applied or induced electric field direction and “j” indicates the applied stress or induced strain direction. For example, as shown in Figure 2d, d₃₁ represents electric field generation in direction 3 which is perpendicular to the force applied in direction 1. Similarly, charges developed parallel to the applied force (compressive/tensile) in direction 3 are represented by d₃₃. If shear (rotational) forces (“j”) are applied along directions 1, 2, and 3, “i” values are represented by 4, 5, and 6, respectively. Alternatively, the piezoelectric voltage constant (g) represents electric charges generated by a piezoelectric material per unit of mechanical shear stress applied (or vice versa). The electromechanical coupling coefficient (k) is defined as the ratio of mechanical energy accumulated per unit of applied electric field (or vice versa).
Direct piezoelectric effects can be calculated by using Equation (1)

\[ D = d \times T + e \times E \]

where, \( D \) = displacement, \( d \) = piezoelectric coefficient, \( T \) = applied mechanical stress, \( e \) = material permittivity, \( E \) = field strength.

Alternatively, reverse piezoelectric effects can be expressed by Equation (2)

\[ X = s \times T + d \times E \]

where, \( X \) = strain and \( s \) = mechanical compliance.\[53\]

A higher d-value represents a higher voltage output against the same material deformation.\[55\]

The piezoelectric effects of nanomaterials considerably differ from bulk materials due to size or small-scale effects. Advanced techniques facilitated the measurement of theoretical and experimental values of effective piezoelectric coefficients (EPCs), by using Maxwell equations and piezoresponse force microscopy (PFM), respectively. In general, piezoelectric effects in nanomaterials are characterized by two piezoelectric coefficient tensors, \( e_{1i} \) and \( d_{1i} \) (\( i = 1, 2, 3 \) and \( \alpha = 1, 2, 3, ..., 6 \)), where \( e_{13} \) (often denoted as \( e_{11} \)) and \( d_{13} \) (often denoted as \( d_{11} \)) values are more valuable for quantification of axial/in-plane piezoelectricity in nanofilms and nanowires/tubes.\[56\] Their values are greatly influenced by nanoscale geometry, crystal orientation and temperature. In perovskite-based nanowires, \( e_{13} \) decreases with a decrease in cross-sectional thickness (\( t \)), whereas the opposite is observed in the case of wurtzite-based nanowires.\[57\] Interestingly, \( e_{13} \) increases with increasing diameter of BN nanotubes, but ZnO nanotubes show opposite effects.\[58\] Layer number (layer thickness) is another factor which plays crucial role in determining piezoelectric properties of multilayer BN nanosheets. An odd number of layers causes piezoelectric effects due to non-centrosymmetry, whereas an even number of layers does not induce piezoelectricity.\[59\] Therefore, size dependency of \( e_{13} \) can be an attractive choice for tailoring properties of piezoelectric nanomaterials.

Piezoelectric properties can vary depending on crystal orientation. \( d_{13} \) values of BaTiO\(_3\) thin films significantly vary from 14.3 to 54.0 pm V\(^{-1}\) due to a change in crystal orientation ([010], [001]).\[60\] ZnO nanowires commonly develop along the c axis ([0001]) or [0110]). EPC measured for the [2110] orientation was much higher than [0110] orientation.\[61\]

Piezoelectric materials are also pyroelectric. Temperature dependency of EPC is substantially enhanced for nanoscale materials as compared to their bulk counterparts. \( e_{13} \) values of GaN nanowires (wurtzite) decreases with increasing temperature.\[62\] On the other hand, \( d_{13} \) of PZT nanowires (perovskite) reaches maximum values when approaching \( T_c \).\[63\]

Although piezoceramic materials possess high piezoelectric coefficients, their brittleness limit their applicability in load-bearing applications. On contrast, piezoelectric polymers are flexible, mechanically robust and biocompatible. They exhibit substantial piezoelectric effects in an appropriate crystalline form, especially at the nanoscale level. PVDF [(CH\(_2\)-CF\(_2\))\(_n\)] is most frequently used piezopolymer for tissue engineering applications. Its dielectric property arises from the electronegativity difference between fluorine (F) and hydrogen (H) atoms, producing dipole moments in the F \( \rightarrow \) H direction. However, the net electric dipole moment of an entire polymer chain depends on its crystallinity. PVDF exists in five different crystalline forms (\( \alpha, \beta, \gamma, \delta, \epsilon \)), of which \( \alpha \) and \( \beta \) are predominant.\[64\] \( \alpha \)-PVDF does not display piezoelectricity due to a net cancellation of dipole moments formed in opposite directions, whereas, parallel orientation of dipole moments in \( \beta \)-PVDF contributes to piezoelectric properties (Figure 2e). Strategies to form highly aligned, polar \( \beta \)-PVDF with enhanced piezoelectric properties include mechanical stretching, electrospinning, directional growth, poling, and annealing. Electrospinning applies extremely high voltages (5–50 kV) to produce nanowires or nanofibers (100–1000 nm diameters). Template-assisted growth of nanowires is relatively mild and simple without the need for any harsh processing conditions. To this end, polymer solutions or melts are infiltrated within nanoporous templates at elevated temperature (to accelerate chain mobility). Due to confinement effects caused by high capillary shear forces, “self-poled” crystalline phases appear through crystallization, contributing to superior piezoelectric properties along the grown length without additional poling. P(VDF-TrFE) is a copolymer of PVDF with high contents of fluorine substitution. This polymer contains a very high fraction of polar \( \beta \)-phase due to steric effects of bulky TrFE groups. Electric poling of P(VDF-TrFE) nanowire-arrays exhibits almost tenfold higher piezoelectric coefficients than P(VDF-TrFE) thin films.\[65\] P(VDF-TrFE) nanotubes, synthesized by a template-etching approach, contains \( \beta \)-phase as main phase with preferable polarizability along the nanotube axis.\[66\]

In case of PLLA, four different crystal phases (\( \alpha', \alpha, \beta, \gamma \)) are observed.\[67\] Unlike PVDF which necessarily requires a polar \( \beta \)-phase for piezoelectricity, PLLA does not depend on the formation of a specific crystalline phase. Although elongated PLLA films do not show any spontaneous polarization, they still exhibit large shear piezoelectric constants.\[68\] Like PVDF, it is possible to control the molecular alignment and degree of crystallinity in PLLA by template-assisted nanowire growth, nanoimprinting, electrospinning, and postprocessing (annealing). PLLA nanofibers synthesized by template-etching possess higher degrees of crystallinity (up to 70% \( \pm \) 5%) than electrospun nanofibers (30–40%).\[69\]

Piezoelectric constants are usually positive in all piezoelectric materials, except for a few polymers (e.g., PVDF, PLLA). Under applied electric fields, such polymers, unlike others, undergo compression instead of expansion, which is attributed to the electronic redistribution within molecular orbitals, transfer of charged nuclei, and dipole reorientation.\[70\]

### 4. Biopiezoelectricity: Origin and Significance

Biopiezoelectricity is piezoelectricity observed in biological systems. Interestingly, many hard tissues (bone, dentin, cementum, etc.) as well as soft tissues (cartilage, ligament, tendon, muscle, hair, pineal gland, etc.) are piezoelectric in the human body.\[20c\] This property mainly originates from the nanocrystalline or liquid-crystalline ordered nature of complex
extracellular matrix components, such as collagen, keratin, elastin, glycosaminoglycans, and hydroxapatite. Electroactive living cells generally do not contribute substantially to the piezoelectricity of bone.[71]

Collagen is a biopolymer and the key component of biological tissue and substantially contributes to their piezoelectric properties. Collagen molecules (1.5 nm in diameter and 300 nm long) exist as a spiral triple helix (Figure 2f,i) which self-assembles through extensive hydrogen bonding of amine and carbonyl functionalities and packs into a quasihexagonal (C6) lattice of crystalline fibrils (50–200 nm diameter).[71] Under mechanical stress, displacement of hydrogen bonds redistributes dipole moments toward the longitudinal axis of collagen molecules, thereby inducing permanent polarization (Figure 2f ii). The crystalline symmetry and structural stability of collagen is maintained by crystalline water molecules bridged through carbonyl groups of polypeptide chains. However, piezoelectric behavior of collagen is less pronounced in the presence of water molecules.[72] Besides collagen, α-keratin (the major constituent of hair, wool, horn, and hoof) possesses right-handed α-helices which exhibit similar piezoelectric properties.[73] Spider and silkworm silk, in contrast, are piezoelectric due to the presence of β-sheets (Figure 2f,iii) formed through extensive hydrogen bonding.[74]

Bone is a natural hybrid nanocomposite which demonstrates piezoelectric properties due to its dense fibrillar packing of non-centrosymmetric collagenous matrix (~22 wt%) with embedded crystalline nanapatite crystals (~69 wt%) (size: 50 × 25 × 3 nm) (Figure 2g).[75] The highest piezoelectric coefficient ($d_{44}$) in femoral bone was reported to be 0.7 pC N⁻¹ measured in shear mode.[74] However, due to structural anisotropy, the bone displays anisotropic piezoresponse. The axial piezoelectricity and vertical or radial piezoresistivity of bone was suggested to be related to directional orientation of collagen fibrils as well as p–n junctions between collagen (n-type material) and apatite (p-type material).[77] As shown in Figure 2g, under physiological compression, polar hexagonal crystalline units of collagen in dry bone undergo dipolar reorientation resulting in a negatively charged surface. In wet state, applied pressure additionally drives a flow of fluid containing charged ions through the canalicular space, resulting in streaming potentials that provide electric signals to cells (i.e., mechanosensation).[78] Piezoelectricity of bone also indirectly contributes to the process of bone remodeling. According to Wolff’s law, mechanically weaker bone is strained more extensively than mechanically stronger bone. Consequently, stronger streaming potentials are developed in weak bone, which promotes bone formation by enhancing the influx of ions and biomacromolecules.[79]

5. Applications of Piezoelectric Nano-Biomaterials

Piezoelectric nanomaterials have widespread application in biomedicine. Herein, we focus the scope of this review to two major application areas: biomedicine and tissue regeneration (as listed in Table 1). In addition, a perspective is provided on potential applications of piezoelectric nano-biomaterials as theranostics.

5.1. Biomedicine

5.1.1. Drug Delivery

Nanorobotic systems are promising carriers owing to their precise locomotion, target specificity, and on-demand release mechanism driven by external parameters such as pH, temperature, etc.[80] Nevertheless, challenges need to be solved include i) control over locomotion and on-demand drug delivery, ii) prevention of off-target drug release in response to changes in the local environment, and iii) hindrances offered by cells and bodily fluid.[81] Smart nanorobots, similar to natural sperm and electric eels, are able to propel and navigate through biological fluid based on chemically driven forces as well as activation by electric, magnetic, optic, or acoustic stimuli.[20c] Nanorobots responsive to magnetic stimuli have been well explored for drug delivery applications. By switching magnetic fields from extremely low to high, three different types of nanorobotic motion can be produced including rolling, spintop, and swimming motions as shown in Figure 3a. Essentially, the design of nanorobots mimics flagella or ciliary motion of prokaryotes and eukaryotes.[82] Flagella exhibit traveling wave-type or corkscrew-type motions through their helical tail, and such nonreciprocal motion can be mimicked in nanorobots by constructing nonsymmetric chiral or helical structures and nonsymmetric actuation through field manipulation.[83] Magnetically driven nanorobots can exhibit dual effects by operating the power source at two different modes, i.e., rotating fields for propulsion and alternating fields for actuation of drug molecules.[20b]

Mushtaq et al. developed nanorobots (nanoelis) loaded with doxorubicin (DOX) by coaxial lithography using a coaxially aligned P(VDF-TrFE) tail and nickel (Ni) ring-polypyrrole (PPy) nanowire head.[21] The flexible tail served as a propeller, whereas rotating magnetic fields stimulated translational motion of the device. P(VDF-TrFE) surface was precoated with polydopamine (PDA) to load Dox via adsorption. The piezoresponsive polymer tail was able to develop a wavy motion which induced a spontaneous polarization-depolarization cycle in response to magnetic actuation of the nanowire head by varying strength and frequency of the applied magnetic field. Nanoeel locomotion was varied by switching the magnetic field from low to high strength (5 to 15 mT) and frequency (1 to 16 Hz) (Figure 3b). At the target site, pulsatile drug release was obtained through electrostatic desorption by tuning magnetic parameters in drug release mode (10 mT and 7 Hz). As shown in Figure 3c, drug release mode generates higher P(VDF-TrFE) strain and oscillation (13 Hz) to induce satisfactory release as opposed to the swimming mode (10 mT and 11 Hz) which lead to insufficient strain and tail oscillation (1 Hz). DOX delivered to cancer cells in drug release mode induced 35% cell death, whereas only 10% was noted in swimming mode (Figure 3d).

Besides nanorobots, piezoelectric nanowires have been investigated for the delivery of anticancer drugs. Recently, core/shell nanowires possessing a FeGa magnetostrictive core and a piezoelectric P(VDF-TrFE) shell have been explored for the delivery of paclitaxel (PTX).[22] FeGa@P(VDF-TrFE) nanowire shells were initially coated with PDA followed by surface adsorption of PTX. P(VDF-TrFE) nanotubes (~35 nm wall thickness) were first built-up by melt-wetting using anodic
aluminum oxide templates, followed by FeGa electrodeposition within nanotube cores (≈250 nm internal diameter) and template removal by etching (Figure 3e). Upon magnetic stimulation, the FeGa core with high magnetostrictive coefficient deforms and strain developed in this way is transferred to the piezoelectric P(VDF-TrFE) shell inducing surface polarization. To ensure effective strain transfer and maximum magnetoelectric output, the crystalline β-form of P(VDF-TrFE) and tight interfacial junctions between core–shell materials are crucial. Excitation of FeGa@P(VDF-TrFE) nanowires through an alternating magnetic field induces polarization on P(VDF-TrFE) surface, facilitating disruption of interaction between PDA and drug molecules (Figure 3f). As a result, drug release occurs in a controlled manner to successfully kill cancer cells.

Noncovalent complexation of DOX with barium titanate (BT-DOX) nanoparticles enhances the internalization of DOX over the free drug to show improved cytotoxicity to SH-SY5Y neuroblastoma cells in vitro.[23] Combination of DOX (1 and 1.5 µg mL⁻¹) with BT nanoparticles (15 µg mL⁻¹) showed significant cytotoxic effect on SH-SY5Y cells, whereas free DOX at similar concentrations did not show any cytotoxic effect. To achieve glioblastoma cell targeting by crossing in vitro blood-brain barrier, BT nanoparticles functionalized with the anti-TfR (transferrin) receptor were recently developed.[84] These nanoparticles showed dual targeting capability and were suitable for both imaging and wireless electric stimulation. Upon US stimulation, these nanoparticles could improve the therapeutic efficacy of the anticancer drug temozolomide. The authors suggest that their findings will open new perspectives in nanomedicine for remote therapy of brain cancer and neurodegenerative diseases.

5.1.2. Perspective on Applications in Theranostics

Piezoelectric BT-, ZnO-, and BFO-based nanomaterials can be applied in theranostic applications, since nanoparticles prepared from these piezoelectric materials can be loaded with both therapeutic molecules and contrast agents. For instance, drug release from nanoparticles can be pulsed at the site of action through the polarization-depolarization effects of piezoelectric materials in the presence of external stimulation (e.g., ultrasound). ZnO-Gd-DOX nanocarriers exhibited enhanced therapeutic efficacy, reduced toxicity and enabled high contrast for fluorescence imaging and MRI (Figure 4a–d).[25] Piezoelectric ZnO-nanocarriers conjugated with PTX, folic acid (FC), and IRDye-680 (IR-680-FCPZnO nanoparticles) allowed for simultaneous targeting and in vivo tracking of human breast cancer cells (MDA-MB-231) (Figure 4e).[13a] Optical excitation of certain piezoelectric crystals results in the emission of an
optical signal at an exact double frequency known as second-harmonic generation (SHG) (Figure 4f).\[85\] BT nanoparticles as SHG labeling probes offer ideal signal transfer, which is essential for in vivo imaging (Figure 4g).\[86\] NIR stimulation (at 790 nm for 15 min) of piezoelectric BFO harmonic nanoparticles loaded with l-tryptophan (Trp) has strong potential in treating diseases like depressive disorder (Figure 4h).\[13b\]

However, in the above-described applications, non-piezoelectric (e.g., optical) rather than piezoelectric properties are exploited to render the particles theranostic. Unfortunately, theranostic applications of these piezoelectric nano-biomaterials directly based on their intrinsic piezoelectric properties still remain to be realized. For these theranostic applications, the weight percentage of theranostic components must be high enough to allow for successful detection, which is not yet possible for commonly used piezoelectric nanocarriers. Moreover, their administration at very high concentrations is not yet possible since these since these materials may induce toxicity. Hence, it remains a challenge to achieve theranostic applications by exploiting piezoelectric properties. However, piezoelectricity might benefit control over drug release while in vivo tracking of nanocarriers might be facilitated by control over optical properties. Effective combination of both properties has strong potential to increase the functionality of future piezoelectric theranostic systems.

5.2. Tissue Regeneration

Tissue engineering attempts to repair or replace damaged, nonfunctional organs and tissues through regeneration. This technique is especially useful for rehabilitation of body parts that are damaged through congenital defects, trauma, or carcinogenic diseases where conventional therapies are no longer effective. For tissue regeneration, scaffolds based on piezoelectric materials are an attractive choice as they provide electrical stimulation to cells. When cells are subjected to electric stimulation, they undergo various phenotypic and genetic alterations that accelerate the process of tissue regeneration. Modern electrotherapy successfully begun centuries back to Christ's birth when electric shocks were given by torpedo fish for pain relief purpose. Over time, piezoelectric materials have gained more attention as an alternative to exogenous electric stimulation for repairing bone defects. Over the last few decades, their applicability has expanded to regeneration of other soft tissues such as cartilage, tendons, nerves, skin, ligaments,
and muscle. This section provides an overview of the state-of-the-art stimulation of soft and hard tissue regeneration using piezoelectric nano-biomaterials.

5.2.1. Bone and Cartilage Regeneration

In response to physiological loads, bone undergoes microfracture. As shown in Figure 2g, mechanical strain on bone generates electric fields in the bone. Generally, endogenous electronegative potentials are formed in areas of bone under compression, whereas electropositive potentials are developed where tensile force is experienced. Such potentials play an important role in the process of bone healing. Under loaded conditions, surface charges are developed on piezoelectric collagen and hydroxyapatite nanocrystals, which facilitate accumulation of charged macromolecules through electrostatic forces, induce conformational changes, stimulate migration, proliferation and differentiation of bone forming (osteoblasts, osteoclasts, and osteocytes) and progenitor cells. Electrotherapy commonly promotes healing through a cascade of cellular events, called galvanotaxis, involving cell membrane depolarization (facing cathode) and hyperpolarization (facing anode), \( Ca^{2+}/Mg^{2+} \) ions influx through hyperpolarized ends, actin depolymerization and cell protrusion toward the cathode. Attempts have been made to produce physiologically relevant electrical signals by replacing electrodes. In such cases, piezoelectric materials hold strong promise. Liu et al. developed piezoelectric BFO+ nanofilms (~10–20 nm thickness) on strontium titanate (SrTiO\(_3\) or STO) implants which generated a constant built-in potential of 75 mV measured by PFM and strongly interacted with the electronegative potential (~52 to ~87 mV) of bone (Figure 5a). This stimulation strategy triggered bone healing as well as osseointegration of implanted BFO+/STO in rat femoral defects (Figure 5b). BFO+ nanofilms promoted adsorption of adhesion protein fibronectin (isoelectric point 4.8–6.4) and upregulated expression of cadherin 16 (Cdh16) (mediating intercellular adhesion), \( I t g b 3 \), \( C d 4 4 4 \), \( V c l \), and \( A c t b \) (mediating cell–cell crossstalk) and osteogenic-related genes (\( B m p 2 \), \( S p p 1 \) and \( W n t 5 a \)) through activation of the PI3K-AKT signaling pathway (\( A k t 1 \), \( S r c \) and \( P t e n \) genes are responsible for osteogenesis, bone formation, and remodeling). Finally, the stimulation strategy triggered cortical-like bone deposition as well as osseointegration of implanted BFO+/STO in rat femoral defects. In response to mechanical stress arising from body movements, piezoelectric materials undergo polarization. This has considerable effects on bioactive protein adsorption and apatite deposition from \( Ca^{2+} \) and \( PO_4^{3–} \) ions. Due to the presence of electrostatic charges on piezoelectric materials surface, they also promote migration, attachment and activity of bone-forming cells.

Load-bearing implants having piezoelectric properties share a similar working principle of a nanotransducers acting on the basis of electromechanical coupling. When exposed to physiological loads (e.g., ultrasound, mechanical vibrations, etc.) at an implantation site, they generate electrical signals in a similar manner as collagen fibrils within bone and cartilage matrix by developing negative charges through dipole reorientation (Figure 2g). As shown in Figure 5c, the developed electrical signal subsequently triggers intricate cell signaling pathways, for instance by opening of voltage-gated \( Ca^{2+} \) channels in cell membranes. This increases intracellular \( Ca^{2+} \) concentrations which activate the calmodulin/calcineurin pathway following dephosphorylation of Nuclear Factor of Activated Cells (NF-AT), translocation of dephosphorylated NF-AT into the cell nucleus and cooperative binding with other transcription factors. This process regulates gene expression of signal proteins (bone morphogenetic proteins/BMPs), growth factors (TGF-\( \beta \)) which are crucial for cartilage and bone formation.

PVDF and its copolymers exhibit a more pronounced piezoresponses compared to other piezoelectric polymers. Recently, Reis et al. designed Li-battery powered PVDF actuators for electrical stimulation (5 V, at 1 and 3 Hz, 15 min daily for 1 month) of fractured bone in order to stimulate bone healing.\(^{[89]}\) Caused by converse piezoelectric effects (electricity-induced deformation), these newly designed actuators accumulated more bone (almost double) on the surface of PVDF compared to its static controls after implantation in sheep femur and tibia osteotomy cuts. As mentioned earlier, the piezoelectric property of PVDF can be amplified by a poling process. However, the cellular response depends on the nature of poling. For example, Ribeiro et al. showed that negatively poled \( \beta \)-PVDF samples \((d_{33} \approx 32 \text{ pC N}^{-1})\) have a stronger ability to stimulate protein adsorption and osteogenic differentiation (alkaline phosphatase/ALP activity) of human adipose stem cells (hASCs) compared to unpoled as well as positively poled substrates under dynamic conditions (1 Hz frequency, maximum amplitude 1 mm) (Figure 5d).\(^{[54]}\) Compared to PVDF, P(VDF-TrFE) possesses a higher piezoelectric coefficient, which can be amplified by annealing at 135 °C. Annealing above the Curie temperature \((T_c = 113 \degree C)\) but below the melting temperature \((T_m = 147 \degree C)\) significantly increases the amount of \( \beta \)-phase. Subsequently, under dynamic compression (1 Hz frequency, 10% deformation), surface charge density (piezoelectric potential) in annealed samples was significantly higher than as-spun P(VDF-TrFE) (without annealing). The scaffolds developed streaming potentials of 61.1 ± 1.5 µV and 25.2 ± 2.5 µV, respectively. A pronounced piezoelectric response on the surface of annealed P(VDF-TrFE) samples promoted osteogenic activity compared to as-spun samples. The former expressed higher osteogenic markers (ALP, osteocalcin, mineralization) as well as upregulated osteogenic related genes (ALP and Runx2 (early markers), OPN and OCN (late/mature bone markers), but downregulated collagen I (Col I) (immature fibrocartilage marker) was at day 28. On the other hand, as-spun samples promoted chondrogenic activity as compared to annealed P(VDF-TrFE). The as-spun samples promoted GAGs synthesis, collagen types II/I ratio and upregulated expression of chondrogenic genes, such as Sox9 (early chondrogenic transcription factor), chondroadherin (late/mature hyaline cartilage marker), collagen II and IX (late/mature hyaline cartilage and hypertrophic chondrocytes marker), downregulated expression of aggrecan (early hyaline cartilage marker) and Col I (increased for the other). Collagen II or IX expression increased until day 14 after which the expression decreased. However, dynamic compression of non-piezoelectric control did not produce any significant cellular response. In a similar study, ultrasound stimulation of cast-annealed BN nanotube/P(VDF-TrFE)
Figure 5. Bone regeneration using piezoelectric nano-biomaterials. a) Illustration of rapid osseointegration between electropositive implant and electronegative bone interaction. b) Histological evidence for pronounced osseointegration with BFO$^+$ coated STO implants compared to uncoated or BFO$^-$ coated STO implants (yellow arrows: bone-material interfaces; NB: nascent bone, FT: fibrous tissue). a,b) Reproduced with permission.[6a] Copyright 2017, Wiley-VCH. c) Schematic representation of mechanical strain induced electric charge generation on piezoelectric material surface triggering cell signaling pathways; d) Alkaline phosphatase activity after 15 d of static and dynamic culture of hASCs on different PVDF films using regular and osteogenic medium (*$p \leq 0.005$). Reproduced with permission.[6b] Copyright 2014, John Wiley & Sons, Inc. e) Effects of US stimulation to cultured SaOS-2 cells on P(VDF-TrFE) and P(VDF-TrFE)/BNNT films using differentiation medium (Bright-field microscopy after Alizarin Red staining). Reproduced with permission.[29] Copyright 1997, Elsevier.
nanocomposites films (80% higher $d_{31}$ value) stimulated differentiation of SaOS-2 osteoblast-like cell compared to plain P(VDF-TrFE) films, developed 20–60 mV potential on their surface (Figure 5e).[27]

PLLA has been extensively used for bone regeneration owing to its biocompatibility and tunable bioresorption properties. Earlier studies demonstrate that implantation of longitudinally drawn PLLA rods (length 5.0 cm, diameter 3.2 mm) remarkably increased the fracture healing rates compared to undrawn PLLA samples.[29] A pioneering study with PLLA which revealed that piezoelectric potentials developed through body movement play a crucial role in protein adsorption, conformational changes of adsorbed proteins, stimulation of cellular activity and callus formation.[31] PLLA nanofibrous electrospun samples display strong piezoelectric properties due to directionally aligned polymer chains along the fiber length. Since bone is a natural nanocomposite of nanocrystalline HA and collagen I (Col I), bioinspired nanofibrous scaffolds (HA/PLLA and Col I/HA/PLLA with fiber diameters of $845 \pm 140$ nm and $310 \pm 125$ nm, respectively) were fabricated by electrospinning and cultured with human fetal osteoblasts cells. Interestingly, Col I/HA/PLLA nanofibrous scaffolds exhibited 25% higher ALP activity and 57% higher mineral deposition than HA/PLLA nanofibers. ALP is commonly used as a marker of bone formation.[27] In bone, ALP hydrolyzes organic phosphate esters thus increasing the local phosphate ion concentration to stimulate matrix mineralization in bone.

In nanocomposites, interfacial bonding between the dispersed phase and matrix plays significant role in determining their mechanical strength. To ensure homogeneous distribution and intimate contact between dispersed HA nanoparticles and PLLA matrix in HA/PLLA nanocomposites, PLLA was grafted on HA nanoparticles (diameter 20–40 nm) (PLLA-g-HA) through their surface hydroxyl groups. Thereafter, grafted particles were incorporated in PLLA matrix to prepare PLLA-g-HA/PLLA composite scaffolds and annealed at 115 °C for 1 h to improve their crystalline phases. This modification drastically improved their mechanical strength. The highest mechanical strength was observed with 4 wt% filler (PLLA-g-HA) composition. The same composition also increased adhesion and proliferation of chondrocyte cells compared to PLLA and ungrafted HA/PLLA composite. Due to the loss of bioactive PLLA-g-HA particles in culture medium, there was an increase in surface roughness which stimulated cell adhesion. The released particles also raised the local pH which promoted cell activity.[28]

As mentioned earlier, piezoresponse varies anisotropically (directional manner) in any material. Jianqing et al. designed an in vivo study to establish this phenomenon in implanted materials.[92] Composite scaffolds of BT/HA ($d_{11}$ = 6.0 pC N$^{-1}$) were prepared by press sintering followed by electrical poling and implanted in dog jawbones. After one week of implantation, new bone formation occurred on the implant surface. Interestingly, bone formation occurred only on the surface which is vertical to the poling direction, whereas no bone formation was observed on the surface parallel to the poling direction, even after two weeks. Notably that masticatory loads played a potential role in surface charge development on poled BT/HA samples, which in turn regulated osteogenesis, cell proliferation and deposition of ECM.

Porous samples are equally effective in displaying piezoelectric response similar to dense samples. 3D porous scaffolds mimicking the trabecular bone (>50% porosity and pore size in 100–500 µm range) are essential for rapid bone ingrowth and osseointegration (In Latin, osseus “bone”; integra “to make whole”; establishing direct structural and functional connection between implant surface and living bone, viz., biological fixation) of load-bearing implants. Lack of adequate osseointegration is one of the major causes of implantation failure. Complex 3D bioinspired scaffolds can be prepared by advanced techniques, such as two-photon lithography (TPL). Nanocomposite 3D porous scaffolds, Osteo-Prints (OP), were fabricated by TPL using Ormocomp, a photore sist doped with 10% BT nanoparticles.[30] These scaffolds displayed eightfold higher piezoelectric coefficients ($d_{11} = 0.6$ pC N$^{-1}$) than that of BT nanoparticles-free scaffolds ($0.07 \pm 0.01$ pm V$^{-1}$). BT nanoparticles inside scaffolds were activated by US stimulation (0.8 W cm$^{-2}$ for 5 s every 4 h, 3 times a day), which not only promoted higher SaOS-2 osteoblast-like cell activity, but significantly induced cell differentiation. HA deposition was significantly higher (12.2 ± 3.3%) in OP/BT + US groups compared to OP groups. The cells also secreted a higher amount of collagen on OP/BTNP + US groups (0.7 ± 0.2%) compared to OP (0.3 ± 0.1%), OP + US (0.3 ± 0.1%) and OP/BTNP (0.2 ± 0.1%) groups. The results indicate that bioinstructive scaffolds having a combination of both topographic cues and piezoelectric properties can produce a better response than any of them in terms of bone regeneration. Periodic assembly of piezoelectric and non-piezoelectric domains in bone ECM formed by collagen nanofibrils and HA nanocrystals, respectively could be simulated by laser irradiated $K_0.5Na_0.5NbO_3$ (KNN) samples having interspersed domains of orthorhombic and tetragonal phases with high and low piezoelectric coefficients, respectively.[31] Such specially designed samples have pronounced effects on in vitro osteogenic response without involving any other osteogenesis-inducing factors. Compared to unirradiated KNN, they significantly promoted in vivo bone formation due to developed piezoelectric potential through body movements. Since bone formation involves bone-forming cells from different lineages (e.g., osteoblasts, osteoclasts, osteocytes), this study can be helpful for a future design of orthopaedic implants with built-in piezoelectric/nonpiezoelectric micro/nano domains exerting differential cellular response. Therefore, designing implantable devices consisting of either a dense or porous surface with embedded piezoelectric nanostructures, instead of regular bioactive titinate/HAP-based nanostructures, could be a more effective way of promoting osseointegration of titanium-based orthopedic implants.[93] In articulating joints, cartilage exists simultaneously with bone as a cushioning barrier to protect bone from stress induced damages through overloading or repetitive loading/unloading cycles. Articular (hyaline) cartilage is a hydrated, avascular, load-bearing tissue, mainly composed of collagen (type II, VI, IX, X, and XI) molecules and proteoglycans in addition to noncollagenous proteins and fluids. The piezoelectric property of cartilage tissue mainly arises from the collagen molecule, which develops streaming potential in response to mechanical stress, similar to bone. Therefore, piezoelectric materials could prove to be an interesting choice for developing engineered cartilage constructs, as...
cartilage is made up of piezoelectric components. Influences of mechanical stress (shear, compressive, or hydrostatic) and electromagnetic stimuli on the behavior of chondrocyte cells are already known. However, piezoelectric materials were rarely explored for establishing a direct link between piezoelectricity and cartilage regeneration.

In a study by Mitani et al., piezoelectric PVDF membrane was used as a support for growing multi-layered chondrocyte sheets. Properties of developed cell sheets were examined through the expression of SOX9, Col I, Col II, Col XXVII, integrin α10 and fibronectin. Chondrocytes adhered firmly to the PVDF surface as revealed by significantly higher gene expressions for fibronectin and integrin α10 (which function like a glue). They also maintained the phenotype of regular chondrocytes, since a significantly higher gene expression of SOX9 and Col XXVII was observed. However, the study did not highlight any specific roles and interplays between piezoelectric material and chondrocyte activity. Recently, Jacob et al. developed smart piezoelectric BT nanoparticles reinforced PHBV nanohybrid electrospun scaffolds for cartilage regeneration. The mechanical strength was remarkably augmented by the addition of 20% filler content. After poling the scaffolds showed $d_{33}$ values (1.4 pC N$^{-1}$) similar to native bone and cartilage (0.2–0.7 pC N$^{-1}$), but significantly higher than PHBV scaffolds (0.43 ± 0.02 pC N$^{-1}$). The BT/PHBV scaffolds demonstrated higher chondrocytes activity and Col II gene expression, especially on polarized scaffolds as compared to the control (PHBV) and unpoled BT/PHBV. Therefore, piezoelectric scaffolds alone can stimulate cartilage regeneration without introducing other chemical factors. For osteochondral healing, bilayer scaffolds can be constructed from piezopolymers or bone/cartilage mimetic matrix embedded with piezoelectric nanomaterials, which may induce simultaneous stimulation of cartilage- and bone-forming cells using the same source of mechanical stimulus (e.g., US).

5.2.2. Neural Regeneration

External electric fields have significant influence neurite outgrowth and nerve tissue regeneration. Since bone undergoes repetitive cycles of physiological loading, AC currents are usually applied to stimulate bone healing, whereas neural cell stimulation essentially require DC currents due to the direct nature of transmembrane potentials. Nevertheless, the applied field strength required for neurite outgrowth is lower (70–250 mV mm$^{-1}$) than the potential required for wound healing (150 to 1200 mV). Instead of applying direct current, conducting polymers (e.g., PPy and polyaniline (PANi)) can act as electrical transducers. For example, PPy, PPy-coated or PCL/gelatin/PANI scaffolds accelerate neurite outgrowth by transducing direct currents (DC) to the cultured PC-12 or neuronal stem cells.

Peripheral nerve regeneration through piezoelectric nerve guidance channels was first introduced by Aebscher et al. and validated in a transected mouse sciatic nerve model. The poled PVDF channels regenerated a higher number of myelinated axons after 4 weeks of implantation as compared to unpoled conduits. Later, PVDF and its copolymer (P(VDF-TrFE)) were extensively used by other researchers as well. Extruded P(VDF-TrFE) demonstrated a higher piezoelectric response as compared to PVDF due to a higher β-phase and all-trans configuration attributed to bulky TrFE groups which produced aligned dipoles. The nature of poling can regulate the behavior of myelinated axons. Positively poled P(VDF-TrFE) conduits stimulated the growth of myelinated axons more efficiently than negatively poled and unpoled substrates.

As discussed in Section 3, electrospinning can spontaneously reorient dipoles along the length of spun fibers, and their piezoelectric property can be enhanced by annealing. Electrospun PVDF and P(VDF-TrFE) scaffolds, annealed at 135 °C for 96 h, and expressed advanced piezoelectric properties due to high β-crystallinity. Scaffolds with aligned nanofibers significantly promoted neurite outgrowth and guidance of dorsal root ganglia (DRGs) compared to random fibers. Similar electrospun P(VDF-TrFE) scaffolds also provoked more pronounced differentiation of human neural stem/precursor cells (hNSC/NPC) into mature neuronal cells. β-III tubulin (mature neuronal marker associated with microtubules) gene expression was low, but abundant protein expression was observed on annealed nanorandom and micrometer-aligned scaffolds. The higher neuronal activity was attributed to developed piezoelectric potentials through cell induced deformation of substrates during their attachment and migration. Apart from fiber alignment, diameter of electrospun fibers played a crucial role in controlling neurite length and outgrowth. Neurite outgrowth increases when fiber diameter decreases from supracellular (100–500 μm) to cellular (30 μm) and subcellular size (5 μm). However, at submicrometer level, neurite length is significantly reduced (42%) as fiber diameter decreased from 759 + 179 nm to 293 + 65 nm.

Direct mechanical stimulation of piezopolymer conduits leads to the generation of surface charge. Mechanical vibration (50 Hz, 96 h) of PVDF films remarkably increased the piezoconstant value ($d_{33} \approx 20$ pC N$^{-1}$). Owing to such enhanced piezoelectric property, arborization of cultured rat spinal cord neuronal cells was significantly accelerated. Similar studies by Valentin et al. also reported higher neurite outgrowth from mouse neuroblastoma (Nb2a) cells cultured on poled PVDF substrates. Substrates were vibrated at 1200 Hz. A 2–3 mV surface potential was generated on poled PVDF, whereas unpoled PVDF produced no electrical output. Piezoelectric nanomaterials have an important role in noninvasive neuronal stimulation, which is useful for treating neurological diseases (e.g., epilepsy, Parkinson’s disease), since they bypass the risk of invasive procedures. Piezoelectric nanoparticles, can act as nanotransducers when stimulated by ultrasound. Ultrasound has limited tissue penetrability (tens of centimeters), but through piezoelectric nanoparticles this stimulation method can reach to much deeper tissue. Ultrasonic-driven PZT nerve-cuff stimulators were designed to wirelessly stimulate muscle-twitch in rats. However, high lead content (60%) of PZT led to several health issues. Alternatively, several studies explored BT nanoparticles, BN nanotubes and ZnO nanowires as nanotransducers. These nanomaterials interact with subcellular components more efficiently owing to their nanoscale dimensions and high surface energy associated with their high surface area. When stimulated by US, BT nanoparticles...
generate 0.07–0.19 mV surface charge which leads to accelerated differentiation of SH-SY5Y human neuroblastoma cells (Figure 6c). The same also enhanced neuronal networking under low-intensity pulsed ultrasound (LIPUS) stimulation (1 MHz with 2 s periodicity). However, direct stimulation or applied via non-piezoelectric nanoparticles did not trigger any networking response. Boron nitride nanotubes (BN nanotubes) were stimulated with US after delivery to PC12 cells, resulting in 30% enhancement of neurite outgrowth. At present, the efficacy of US/BT nanoparticle or US/BN nanotube based nanotransducers has been tested in an in vitro cell culture system. Therefore, it is necessary to validate obtained results using in vivo models. Targeting of these nanoparticles to nerve cells in the brain can be achieved by surface functionalization of these nanoparticles with suitable ligands.

Deafness or sensorineural dysfunction is an additional problem which arises from degeneration/dysfunction of cochlear hair cells. Auditory nerve fibers of brains are closely linked to outer and inner sensory hair cells of the cochlea for their integrated functioning. Here, piezoelectric nanomaterials play a crucial role. PVDF can effectively restore hair cell function owing to their in-built piezoelectric properties, they lack sufficiently high piezoelectric coefficients to produce adequate stimulation of auditory neurons. Their piezoelectric property

Figure 6. Piezoelectric nano-biomaterials for nerve tissue regeneration. a) Confocal images of dorsal root ganglion (DRG) cultured on nanosized i) random and ii) aligned PVDF scaffolds (actin staining with phalloidin) (Scale bar 300 μm). Reproduced with permission. Copyright 2011, Elsevier. b) Nb2a cells cultured on i) poled PVDF supported neurites growth in all directions and ii) unpoled PVDF supported fewer cells with extended neurites (Scale bar 100 μm). Reproduced with permission. Copyright 1992, Elsevier. c) Representative data for Ca²⁺ imaging of cultured SH-SY5Y neuroblastoma cells stimulated with 0.8 W cm⁻² ultrasound (US) intensities with/without BT nanoparticles. Reproduced with permission. Copyright 2015, American Chemical Society. d) Ca²⁺ imaging of differentiated SH-SY5Y on P(VDF-TrFE) and P(VDF-TrFE)/BT nanoparticles films following US stimulation. Y axis: Time courses of the ΔF/F₀ traces; arrows—initiation point of 5 s US pulse. e) SEM images of SH-SY5Y cells cultured on Ibidi (control), P(VDF-TrFE) and P(VDF-TrFE)/BT nanoparticles films with/without continuous US stimulation. d,e) Reproduced with permission. Copyright 2016, Wiley-VCH.
improved through formation of nanocomposites with BT nanoparticles, gold nanoparticles, carbon nanotubes (CNTs), or graphene oxide (GO) nanosheets.[100] Several studies established the advantageous effect of such piezoelectric nanocomposites to improve cochlear function through neuronal stimulation. Electrospin meshes composed of BT nanoparticle/PVDF nanofibers (also enriched with piezoelectric β-phase) were found to be noncytotoxic to cochlear epithelial cells. In addition, they promoted attachment and viability of neural-like cells of under mechanical stimulation. Nanocomposites films of BT nanoparticles/P(VDF-TrFE), prepared by cast annealing, showed enhanced piezoelectric properties. Upon US stimulation, they significantly promoted maturation and neurite outgrowth (Figure 6d,e).[37] Their piezoelectric property can be tailored by varying the percentages of BT nanoparticles in PVDF or P(VDF-TrFE) matrix. Therefore, such nanocomposites with enhanced piezoelectric properties can be materials of choice for developing next-generation cochlear implants.[101]

Alternatively, CNT- and graphene-based nanocomposites exhibit superior mechanical as well as electrical properties. GO nanosheets/PVDF nanocomposite scaffolds, prepared by phase separation, exhibit superior hydrophilicity, mechanical strength, electrical conductivity, and piezoelectricity due to an enriched β-phase. Nerve conduits can be prepared from GO nanosheets/PVDF nanocomposites which markedly enhance attachment and proliferation of PC12 cells compared to PVDF scaffolds.[34] To obtain a homogeneous dispersion, functionalization of GO is necessary. Biosynthetic nerve conduits prepared from electrospin single walled carbon nanotubes (SWNTs)/silk fibroin (SF)/fibronectin (FN) nanocomposites, led to functional recovery of rat sciatic nerve defects by regenerating myelinated axons after 5 weeks of implantation. Nanofiber alignment in the electrospin conduits mimicked elongated patterns of native nerve tissue. The matrix components of nanocomposites were selected based on functional properties. Cells induce scaffold deformation during migration and proliferation and surface charge was induced by piezoelectric silk, distributed throughout the scaffold by electrically conductive SWNTs, whereas FN maintained Schwann cell function in the myelin sheath of axons.[38]

The above-mentioned studies mainly focused on wireless, noninvasive, electric stimulation of neuronal cells either through a direct method (using US-driven piezoelectric nanotransducers) or culturing cells on piezoelectric substrates which occasionally contain US-responsive piezoelectric nanotransducers. Nanoparticles mainly act from the cell surface, and rarely penetrate the cell membrane. Therefore, the effects produced by these nanoparticles is indirect. Future studies should focus on developing nanovectors which can penetrate cells, encouraging direct stimulation for higher neuronal activity and neurogenic differentiation.

### 5.2.3. Skin Regeneration

Cutaneous wound healing largely depends on transepithelial potentials that spontaneously arise from the disruption of differential ionic gradients at wound beds. Living biological tissues can generate endogenous potentials up to 500 mV mm⁻¹, which accelerates the process of wound healing by modulating migration, proliferation and differentiation of keratinocytes, fibroblasts, epithelial as well as endothelial cells.[18,85] Such endogenous potential could be artificially developed by using piezoelectric nanomaterials which are commonly activated in response to natural body movement.

Piezoelectric effects on fibroblast migration and wound healing were investigated by Guo et al. using electrospun PVDF/PU scaffolds.[78] Flexible bottomed culture plates (Flexcell culture tension plus system, USA) were used for scaffold deformation up to 8% through intermittent biaxial stretching at 0.5 Hz for 24 h. The cell migration rate was almost doubled due to the piezoelectric effect as compared to scaffolds without any piezoactivity. Wound healing rates were also higher in scaffolds implanted in the abdomen and back of Sprague-Dawley (SD) rats as compared to implantation in vertex. Since body movements were less pronounced in the vertex region, piezoelectric potentials were reduced in this region.

Dermal patches enriched with piezoelectric nanomaterials such as ZnO nanorods (ZnO NRs) offer fascinating opportunities to promote skin regeneration. Bhang and coworkers developed multi-layered piezoelectric dermal patches (ZnO NRs/PDMS) through spin-coating of ZnO NRs dispersed in PDMS followed by rubbing for directional alignment of ZnO NRs.[18] Different piezoelectric potentials (300 and 900 mV) were developed for patches with different ZnO filling densities (54.8% and 95.2%, respectively) and applied on the back of mice to induce mechanical bending, as shown in Figure 7ai–iv. Obtained values were in the range of endogenous potentials (150–1200 mV) required for wound healing.[102] The patches significantly enhanced the expression of following markers: PCNA (cell proliferation), SM α-actin (myofibroblastic differentiation), TGF-β receptor (voltage gated Ca²⁺ channels, Col synthesis), Col III (granulation tissue), Col IV (keratinocyte migration) and positively stained with von Kossa (calcium deposition); upregulated genes: CD68 (macrophage, inflammation), VEGF (inflammation), integrin α5, TGF-β, and CD99 (angiogenesis); enhanced phosphorylation of Akt, P13K (electrotaxis), ERK1/2 (electrostatic migration), Rho-GTPase (electrotaxis), PCNA (signaling pathway). The schematic representation of endogenous electric field-induced intracellular signaling pathways involved in cell migration and proliferation during wound healing is shown in Figure 7av. This confirms that migration, metabolic activity, differentiation and electrotaxis by the cultured fibroblasts, keratinocytes and macrophages were markedly promoted by piezoelectric patches, which lead to faster wound healing.

Bonvallet et al. developed synthetic skin grafts by electrospinning of Col I/poly(t-caprolactone) (PCL) solutions.[39] Scaffolds were microporous (160μm average pore size) with highest mechanical strength (1.4 MPa, tensile) at a Col I/PCL ratio of 70 : 30. This allowed infiltration and growth of dermal fibroblasts, keratinocytes and macrophages which accelerates the process of wound healing by modulating migration, proliferation and differentiation of keratinocytes, fibroblasts, epithelial as well as endothelial cells.[18,85] Such endogenous potential could be artificially developed by using piezoelectric nanomaterials which are commonly activated in response to natural body movement.

Bone growth was examined by culturing cells on piezoelectric nanocomposites scaffolds. Cells showed enhanced proliferation and differentiation in response to mechanical stimulation. Scaffolds prepared by electrospinning of Col I/PCL solutions were microporous (160μm average pore size) with highest mechanical strength (1.4 MPa, tensile) at a Col I/PCL ratio of 70 : 30. This allowed infiltration and growth of dermal fibroblasts, keratinocytes and macrophages which accelerates the process of wound healing by modulating migration, proliferation and differentiation of keratinocytes, fibroblasts, epithelial as well as endothelial cells.[18,85] Such endogenous potential could be artificially developed by using piezoelectric nanomaterials which are commonly activated in response to natural body movement.
Electrospinning was the most useful and widely explored technique for synthetic dermal grafts. Numerous piezoelectric biopolymers have been investigated for wound healing including PHBV, collagen, silk, PVDF and PLLA. They often produced a remarkable outcome in terms of accelerated skin regeneration. However, these materials were either not classified as nanoscale materials or not adequately characterized for piezoelectric properties; they have therefore been excluded from the present scope of this review.

5.2.4. Ligament and Tendon Regeneration

The tendon is an important load-bearing tissue involved in transmission of tensile forces between muscle and bone, where the ligaments maintain the stability of joints. Similar to bone and cartilage, dense, highly aligned, piezoelectric collagen fibrils are mainly responsible for mechanotransduction (by developing streaming potential) in tendon/ligament (T/L) tissues. Therefore, structural hierarchy and integrity of T/L tissues are highly essential for smooth functioning, which is often disrupted by overloading, thereby leading to tearing or rupturing of tissue. Like cartilage, T/L possesses poor regenerative capacity since they lack adequate reparative cell population and blood vessels. Natural healing of injured tissues involves scar-like tissue formation, which results in mechanically inferior tissues. While synthetic grafts are used for T/L reconstruction, they often lack structural hierarchy, functionality and prolonged durability like native tissue. Owing to structural and mechanical similarity with collagenous T/L tissue, electrospun nanofibers hold promises to be an engineered scaffold for T/L repair/replacement. PLLA based synthetic grafts are preferable owing to their biomechanical compatibility and easy manufacturing. PLLA nanofibrous scaffolds, synthesized by electrospinning, showed mechanical strength (40.68 ± 2.99 MPa tensile strength; 91 ± 3.55 MPa flexural strength) similar to natural anterior cruciate ligament (ACL).

Barber et al. developed braided 3D scaffolds (3, 4, or 5 aligned bundles) from electrospun PLLA nanofibers of average diameter 702 ± 205 nm (Young's modulus 47.6–55.0 MPa). When scaffolds were seeded and cultured with hMSCs using growth medium, they exhibited upregulation of genes: Oct3/4 and Sox2 (stem cell pluripotency and self-renewal). In osteogenic medium, hMSCs showed: downregulation of Scleraxis (tenogenic differentiation), but upregulation of Runx2 (osteogenic differentiation) genes; under cyclic tensile loading: upregulation of Scleraxis, EphA4 (MSCs), Col I, Col III and downregulation of Runx2. Therefore, mechanical stimulation has potential role toward tenogenic differentiation. Role of fiber alignment on tenogenic differentiation was investigated by Yin et al. by culturing human...
tendon stem/progenitor cells (hTSPCs) on electrospun PLLA nanofibrous scaffolds. Cells grown on aligned nanofibers showed spindle-shaped morphology (Figure 7b,i,iii,v) with significantly higher expression of tendon-specific genes (integrin α1, α5, and β1 subunits, and myosin II B (leading to tenogenesis)) under both normal and osteogenic media as compared to random fiber orientation. However, cells grown on randomly oriented nanofibers showed stellate-shaped morphology with significantly higher alkaline phosphatase activity and mineralization (leading to osteogenesis) (Figure 7b,ii,iv,vi). Implantation of scaffolds in mouse skeletal muscles formed dense collagenous tendon-like tissue on aligned nanofibers, whereas only disorganized soft tissue was detected on randomly oriented scaffolds. Therefore, aligned nanofibrous morphology is essential for creating an instructive microenvironment for tenogenic differentiation. Sensini et al. prepared crosslinked Col I/PLLA nanofibers with diameter 0.35–0.40 µm by electrospinning. The nanofibers were wrapped into bundles to mimic the 3D structural hierarchy of a tendon, where nanofibers were aligned along the axis of bundles. The biomimetic morphology of those bundles was similar to that of a tendon, as also observed by SEM and high-resolution X-ray computed tomography (XCT) analysis. The bundles exhibited mechanical properties (Young’s modulus 222.8±84.6 MPa) similar to human tendon fascicles. Continuous growth of human fibroblasts (possessing similar characteristics of tendon fibroblasts) on scaffolds was assured, by an increase of metabolic activity. Therefore, cross-linked Col I/PLLA nanofiber bundles can be a suitable material for constructing bioengineered tendon fascicles. Although PLLA possess many advantageous properties as biomaterial, their acidic degradation byproducts trigger local inflammatory reactions. Hence, long-term follow-up studies are necessary to evaluate the in vivo performance of PLLA grafts.

Natural piezoelectric biopolymers, similar to tendon matrix proteins, can be stimulatory for ligament/tendon regeneration. Electrospun nanofibrous scaffolds prepared from SF–Col I was highly effective for enhancing attachment of hASCs as well as human tenocytes compared to pure SF scaffolds. Even after crosslinking, such scaffolds were mechanically weaker than the native T/L, but they were successfully able to provide a local biomimetic microenvironment.

5.2.5. Skeletal Muscle Regeneration

Skeletal muscles control our body movement as well as body posture. Up to a certain limit, they undergo self-repair upon injury, with the help of muscle satellite cells or circulating MSCs. However, with large traumatic defects, chronic degeneration, congenital diseases (e.g., muscular dystrophies) or age-related related dysfunction of satellite cells cannot be overcome. In such conditions, exogenous reconstruction is necessary for structural and functional repair. Autologous muscle transplantation, delivery of muscle progenitor cells produced limited success due to poor survival and integration of transplanted tissues as well as inadequate in vivo differentiation of delivered cells. Direct delivery of myogenically differentiated cells or development of cell–material constructs can be an interesting choice. Since effects of biophysical stimuli (mechanical stretching, cyclic strain, electrical stimuli) are already known to induce myogenic differentiation and functional maturation of muscle-like tissues, piezoelectric materials would perform better in response to physiological loading.

Yoon et al. developed a piezoelectric smart device which can transduce electrical and mechanical stimulation to cultured cells. The device involved a tri-layered stretchable piezoelectric substrate prepared from unidirectional aligned and dispersed ZnO NRs in polydimethylsiloxane (PDMS) matrix, which was further grafted with poly(N-isopropylacrylamide) (pNIPAAm). Devices subjected to repetitive cycles of stretching/bending (0.3 Hz) could generate electromechanical stimuli, which induced myogenic differentiation of human umbilical cord blood derived mesenchymal stem cells (hUCB MSCs) cultured for 10 d. Differentiation of hUCB MSCs induced gene overexpression: MyoD (early myogenesis and myoblast formation) (highest on day 2 and gradually decreased with progressive myogenic differentiation); protein expression: MyoD, myogenin (terminal myogenic differentiation), troponin I (late markers for skeletal myocytes), MEF2 (myogenic differentiation), the highest for hUCBMSCs group provided with myogenic medium, piezoelectric potential and mechanical stimulus. They also triggered the signalling pathway by upregulating Gp78 and RhoA (by mechanical strain), hsp27 (piezoelectric pulse), MRFs and phosphorylating p38 through IGF-1 (Figure 7c,i). After sheet formation by differentiated cells, they were removed from the device, sliced into small pieces and injected to injured skeletal muscle of mice. This substantially promoted muscle regeneration compared to undifferentiated or myogenically differentiated trypsinized hUCB MSCs (Figure 7c,ii).

The nature of surface charge determines the myogenic response. PVDF films and fiber mats were prepared by solvent casting and electrospinning, respectively, followed by stretching and electric poling to induce polarization (d33 ~ -32 pC N⁻¹). C2C12 cells (mouse myoblasts cell line) were cultured on different samples. Interestingly, aligned β-PVDF mats, after negative poling significantly promoted adhesion, proliferation, and directional growth of C2C12 cells as compared to randomly aligned substrates without or with positive poling. Therefore, a tailored surface charge with nanotopography (fiber alignment) of PVDF samples synergistically stimulate muscle regeneration. Piezoelectric nanofibrous PVDF-TrFE mats were prepared by electrospinning with 970 ± 480 nm average fiber diameter and 1.7 µm mean pore diameter. The scaffolds supported attachment and proliferation of human dermal fibroblasts which exhibited elongated, spread morphology with the expression of talin, FAK, paxillin, and vinculin related genes at day 7. Such nanostructured 3D PVDF-TrFE substrates with aligned fiber orientation are suitable for muscle regeneration.

5.2.6. Cardiovascular Regeneration

Cardiac tissue-engineered constructs prepared from electroactive materials are attractive candidates for cardiovascular regeneration, since contractile behavior of cardiomyocytes is regulated by the complex, dynamic electromechanical microenvironment in cardiac tissues. However, scaffolds must preserve cardiomyocytes contractility in the absence of external stimulation.
Piezoelectric properties of electrospun PVDF and P(VDF-TrFE) scaffolds have been explored in cardiovascular tissue engineering. Cultured embryonic stem cell-derived cardiomyocytes as well as endothelial cells strongly adhered onto P(VDF-TrFE) substrates and displayed an aligned morphology along the nanofiber axis. Cardiomyocytes also demonstrated impulsive contractility and expressed cardiac-specific markers. Alternatively, endothelial cells cultured on the substrate manifested mature phenotype and expression of classic endothelial markers associated with differentiation and angiogenesis. However, the role of piezoelectricity on cardiomyocytes and endothelial cell activity remains unclear. It is speculated that transmembrane piezo proteins, especially Piezo1, may regulate migration, organization, alignment of endothelial cell, as well as mechanotransduction.

Nanocomposites prepared by doping of piezoelectric BT nanoparticles (10 and 30 wt%) into poly(lactic-co-glycolic) acid (PLGA) matrix have been tested with H9C2 murine cardiac muscle cells. These supported proliferation and differentiation of cardiomyoblasts. However, detailed characterization of these materials is necessary to understand nanotransducer effects exerted by BT nanofibers toward cardiac tissue regeneration.

Magnetoelectric poly(caprolactone) (PCL) nanofilms (MNF) were prepared by dispersing superparamagnetic magnetite nanoparticles (SPIONs) and further coating with piezoelectric P(VDF-TrFE) microfibers in order to simulate cardiac pacemaking of sinoatrial node cells. MNF provide a flexible support for cell contraction and aligned fibers created a suitable environment for cell alignment by providing electrical stimulation to seeded cells. These scaffolds promoted attachment, proliferation and alignment of rat/human cardiac cells along the fiber length without affecting the contractility of cardiomyocytes. Magnetic stimulation of MNF induced mechanical deformation that facilitated generation of transient potentials on the piezoelectric microfiber surface.

Regardless of the cell source, more attention should be paid on directing the alignment of myoblasts within the defect site to facilitate seamless integration between native and the newly grown tissue. In vitro studies should be validated with suitable in vivo models. Finally, biomimetic design with an appropriate blend of structural and mechanical properties should be ensured as long-term support for muscle regeneration.

6. Conclusion
Piezoelectric nano-biomaterials are utilized in an extremely wide range of applications, including targeting of therapeutics, sensing, actuation, and tissue engineering. Such materials are an attractive choice for constructing cardiac pacemakers, cochlear implants, synthetic skin, osteochondral and T/L grafts, nerve conduits and nanotransducers. A wide range of piezoelectric nano-biomaterials have been explored for this purpose by varying material composition, crystallinity, nano- to macro-scale hierarchy through manipulation of processing and post-processing conditions, which significantly influenced their piezoelectric properties. Piezopolymers like PVDF, P(VDF-TrFE), PLLA and PHBV, and piezoceramics like BT, BN, ZnO, BFO, and HA and their composites were extensively used. Among them, several produce piezoelectric output in a range similar to that of endogenous potentials (150–1200 mV), which naturally exist at the wound healing site. In addition to this, piezoelectric nanocomposites combine the high piezoelectric coefficients of a piezoceramic material and the flexibility of a piezopolymer, thereby proving more suitable for load-bearing applications (such as, bone and cartilage regeneration). Electrospun scaffolds find application in nerve and T/L regeneration due to their inherent structural similarity to the native tissue; their functional properties have been improved by customizing morphological features (fiber diameter and alignment) as well as crystallinity. It has been found that appropriate selection of materials from this category can restore cochlear/auditory function by stimulating neurite outgrowth and formation of myelinated axons. While controlled locomotion and targeted drug release are challenges to conventional drug delivery systems, magnetically driven piezoelectric nanocarrier systems were found to be effective in vitro. However, their drug loading efficiency and site-specific targeting should be improved. Since piezoelectric BT, ZnO and BFO nanoparticles combine optical properties, they are also useful for theranostic application, especially cancer chemotherapy. However, their in vivo efficacy, as well as their prolonged in vivo stability, is yet to be established. In summary, piezoelectric nano-biomaterials evidently exhibit strong potential for a wide range of biomedical applications. Nevertheless, their clinical translation remains to be realized. Therefore, the biocompatibility, biodegradability and tissue accumulation of piezoelectric nano-biomaterials should be thoroughly evaluated.

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Conflict of Interest
The authors declare no conflict of interest.

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