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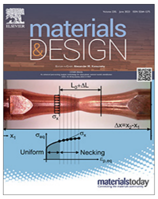


An overview of polymeric composite scaffolds with piezoelectric properties for improved bone regeneration

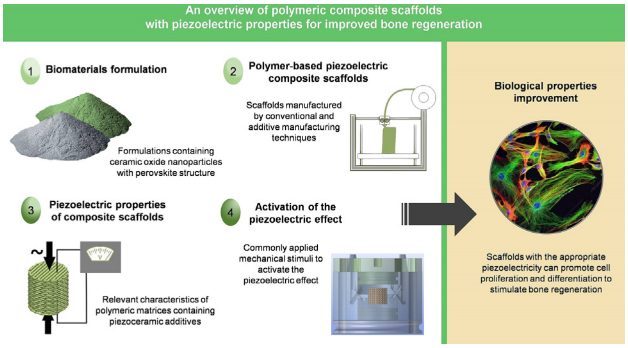
Ricardo Donate [a,*], Rubén Paz [a], Rocío Moriche [b], María Jesús Sayagués [c], María Elena Alemán-Domínguez [a], Mario Monzón [a]

[a] Departamento de Ingeniería Mecánica, Grupo de Investigación en Fabricación Integrada y Avanzada, Universidad de Las Palmas de Gran Canaria, Campus Universitario de Tafira s/n, 35017 Las Palmas, Spain
[b] Departamento de Física de la Materia Condensada, ICMS, CSIC-Universidad de Sevilla, Apartado 1065, 41080 Sevilla, Spain
[c] Instituto de Ciencia de Materiales de Sevilla (CSIC-US), Sevilla 41092, Spain

HIGHLIGHTS

- The development of piezoelectric composite scaffolds has shown promise for improved bone regeneration.
- Piezoelectric polymeric-based composite scaffolds intended for bone tissue engineering applications are reviewed.
- Special attention is given to the use of lead-free ceramic oxide nanoparticles with perovskite structure.
- Commonly applied mechanical stimuli to activate the piezoelectric effect of the developed materials are presented.
- Due its innovative character, there is still a large gap in understanding the potential of this strategy.

GRAPHICAL ABSTRACT



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ABSTRACT

Despite the dramatic change that Tissue Engineering or stem cell therapies have brought to current therapeutic strategies, there is a lack of functionalities in the available biomaterials for manufacturing scaffolds to treat several highly prevalent osseous diseases (osteochondral defects, osteoporosis, etc.). One promising approach to fill this gap involves the development of innovative piezoelectric scaffolds for improved bone regeneration. Scaffolds with the appropriate piezoelectricity can positively influence the proliferation and differentiation of mesenchymal stem cells to regenerate bone tissue, since surface electrical charges play a key role in the mechanotransduction process. In this work, polymeric-based composite scaffolds with piezoelectric properties intended for bone tissue engineering are reviewed. Special attention is paid to biocompatible, piezoelectric polymers that show suitable properties to be processed by additive manufacturing techniques. Previous works on composite scaffolds based of these polymeric matrices and containing piezoceramic additives are summarized. The use of piezoelectric nanostructured composite formulations containing lead-free ceramic oxide nanoparticles with perovskite structure is highlighted. Also, different commonly applied mechanical stimuli to activate the piezoelectric effect of the developed materials are presented. Finally, other applications of such scaffolds are mentioned, including their capabilities for real-time monitoring.

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* Corresponding author.
E-mail address: ricardo.donate@ulpgc.es (R. Donate).

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1. Introduction

Bone Tissue Engineering (BTE) aims to provide substitutes to restore, support or improve the functionality of bone tissue when it has been damaged due to disease or trauma. The key approach of Tissue Engineering (TE) is the development of scaffolds, which are structures that support and direct the growth of the surrounding tissue during the healing process. Manufacturing techniques and materials used to obtain functional scaffolds must be carefully designed in order to provide a suitable porosity of the final parts to ensure the vascularization of the tissue (and therefore, the supply of nutrients and the removal of biological residues). Additive Manufacturing (AM) techniques are powerful processes to manufacture 3D porous scaffolds because of their design flexibility to provide interconnectivity of the porosity and tuneable mechanical properties [1].

The balance between porosity and mechanical properties is an essential requirement in the development of scaffolds to be used in the BTE field. Nevertheless, this balance is not enough to ensure successful bone regeneration. The major challenge in this biomedical field is to obtain bioactive scaffolds that can improve the tissue remodelling process. In order to achieve this objective, one of the most common approaches is the introduction of growth factors. However, some of the main disadvantages of this strategy include the cost of these bioactive substances and the difficulty of accurate control over their *in vivo* release [2].

Another approach is the one related to the concept of mechanotransduction, which is a complex biological process through which the mechanical load applied to the bone is transformed into biochemical signalling that regulates the remodelling of the tissue. In this process, ion channels and proteins (specially integrins and focal adhesion kinases) configuration and polarization play a key role [3]. Regarding the implications of electrical currents in the ion channels, it has been proposed that the electrostimulation is able to increase the local concentration of Ca^{2+} and, afterwards, this increase regulates osteoblasts functions through calmodulin pathways [4]. The influence of electrostimulation can modulate a wide range of biological processes, affecting for example the migration, proliferation and differentiation of mesenchymal stem cells (MSCs), as discussed by authors such as Liu et al. [5], Pelto et al. [6] and Zhang et al. [2].

Bone is a piezoelectric material [7], as electrical currents are responsible for the transduction of mechanical stress to the biochemical reactions mentioned above. For this reason, the possibility of using electrical signalling to promote bone remodelation has been explored in the literature [8]. However, the complexity of deeply understanding how electricity is involved in the biological mechanisms of mechanotransduction, together with the technological challenges related to the effective utilization of piezoelectricity to obtain tissue-engineered products, have made of this topic a relatively unexplored but highly promising field of study.

Synthetic polymers, some of which possess piezoelectric properties, have been extensively used as base materials for bone scaffold manufacturing [9]. Piezoelectricity in polymers is attributed to the induction of a net dipole moment in response to mechanical stress and due to the inherent crystal or chemical structure of the material [7]. This feature, coupled with their predictable, reproducible and tuneable physicochemical and degradation properties, good processability by AM techniques and suitable mechanical properties for load-bearing applications [10], makes them great candidates for being used in BTE applications. Herein, we discuss the published work describing the developments on piezoelectric polymer-based scaffolds for bone tissue regeneration, with special attention to the use of inorganic materials with perovskite structure as additives to the polymeric matrix.

2. Piezoelectric polymeric scaffolds for BTE applications

Polymer-based scaffolds with piezoelectric properties have been explored in order to improve the biological response in BTE applications. Among them, polyvinylidene fluoride (PVDF) and its copolymers have been established as biomaterials with great potential to be used for the manufacturing of piezoelectric substitutes for hard TE applications [11–13]. Regarding PVDF copolymers, the most frequently used are poly(-vinylidene fluoride-trifluoroethylene) (P(VDF-TrFE)) and poly(-vinylidene fluoride-co-hexafluoropropylene) (PVDF-HFP) [12]. For example, Marques-Almeida et al. [14] have obtained micropatterned P(VDF-TrFE) structures by moulding solutions of this piezoelectric polymer on moulds manufactured by AM. The surface microstructure of the scaffolds tested, combined with the inherent electroactivity of the material, showed beneficial effects on the proliferation and differentiation of bone precursor cells.

One of the most widely used methods to develop piezoelectric PVDF-based scaffolds is electrospinning [15], in which an electric field is applied to an ejecting polymeric solution to generate a non-woven fibrous mat that is collected on a grounded plate [11]. In addition to being a relatively simple, fast and efficient method, a relevant advantage of electrospinning for the manufacturing of piezoelectric scaffolds is its induced self-polarization [16]: the stretching force applied on the electrified liquid promotes the alignment of dipoles and the formation of β crystalline phase in PVDF nanofibres, which ultimately enhances the piezoelectric properties. Highly-oriented nanostructures with improved β-phase content can be obtained by optimizing the electrospinning conditions [15]. For example, Damaraju et al. [17] evaluated the effect of accelerating voltage on the formation of the piezoelectric β-phase, concluding that higher voltages during the electrospinning process increased the crystalline phase and enhanced osteogenic differentiation of MSCs. After electrospinning, the PVDF chains could partially come back to a more stable curled state [15]. To reduce this effect, different fillers have been proposed to act as nucleating agents during crystallization. With this objective, Liu et al. [18] used graphene oxide to produce PVDF-based nanofibres with over 4-fold enhanced piezoelectric properties by increasing the content of the β-phase and adjusting its orientation. Graphene oxide was also used by Shuai et al. [19] as filler of PVDF in order to induce the conversion from α- to β-phase, thus drastically improving the piezoelectric performance of the developed scaffolds. Composite scaffolds containing 0.3% w/w of graphene oxide showed a significant enhancement in terms of hydrophilicity of the surface, mechanical properties and cell response.

Apart from the conventional manufacturing techniques mentioned above, the application of AM techniques to obtain piezoelectric scaffolds has recently gained attention. The use of smart materials and AM technologies to obtain specific functionalities (4D printing) is itself a very up-to-date topic. According to ISO/ASTM 52900:2021 standard, AM technologies are classified in 7 categories, as described in Table 1.

The combination of promising piezoelectric materials with AM technologies for 4D bone tissue regeneration is a research line of great interest in the field [20]. One strategy that is worth mentioning involves the application of PBF of polymers (PBF-LB/P, also known as selective laser sintering or SLS) to manufacture composite scaffolds based on the combination of synthetic polymers and piezoelectric additives. In this scope, examples can be found regarding the use of different ceramic additives embedded into a polylactic acid (PLA) [21] or PVDF [22–24] polymeric matrix. Qi et al. [22], for example, fabricated PVDF-based composite scaffolds by PBF-LB/P after mixing the polymer matrix with polyaniline-decorated molybdenum disulfide powder. Introduction of this

Table 1
AM processes based on process categories.

AM process category	Abbreviation	Description
Binder Jetting	BJT	A liquid bonding agent is selectively deposited to join powder materials
Directed energy deposition	DED	Focused thermal energy is used to fuse materials by melting as they are being deposited
Material extrusion	MEX	Material is selectively dispensed through a nozzle or orifice
Material jetting	MJT	Droplets of feedstock material are selectively deposited
Powder bed fusion	PBF	Thermal energy fuses regions of a powder bed
Sheet lamination	SHL	Sheets of material are bonded to form a part
Vat photopolymerization	VPP	Liquid photopolymer in a vat is selectively cured by light-activated polymerization

additive at 1% w/w led to a significant increase in β-phase (from 43% to 90%), providing improved mechanical and piezoelectric performance and subsequently promoting cell proliferation and differentiation. Regarding other AM techniques, the introduction of piezoelectric feedstock for MEX processes (commonly known as Fused Deposition Modeling, FDM) has been explored [25,26], but, to the best of our knowledge, not applied in TE. Extrusion-based AM techniques are versatile and cost-effective, so the possibility of processing piezoelectric materials with this set of technologies would allow increasing the potential applications of these systems.

As AM techniques lacks the induced self-polarization of electrospinning, a post-processing step is generally required to orient the dipoles in the preferred direction to promote β-phase content and, therefore, the electromechanical properties of the developed scaffolds. Thermal treatments, such as annealing [27], have been reported to be efficient methods to enhance piezoelectricity. In the case of the 3D piezoelectric fibrous P(VDF-TrFE) scaffolds manufactured by Damaraju et al. [11], after annealing, the piezoelectric properties of the 3D structures were enhanced by increasing the β-phase crystal content of the base material. The results of this work demonstrated increased chondrogenic differentiation of human MSCs on the P(VDF-TrFE) scaffolds without treatment (lower piezoelectric activity), while in the annealed P(VDF-TrFE) scaffolds, a higher MSCs osteogenic differentiation was observed.

Although studies comprising piezoelectric composites are mainly limited to PVDF-based materials, other organic piezoelectric materials have gained attention recently, including PLA, collagen, silk and graphene [27]. The piezoelectric effect in these organic materials is caused by the reorientation of the molecular dipoles that are present in the polymer molecular structure. In the case of PLA, and as mentioned above for PVDF, the β crystalline phase needs to be favoured in order to obtain the best piezoelectric properties. In this configuration, the C = O dipoles are not aligned randomly but in a parallel direction, thus generating the highest dipole moment [28].

Apart from annealing, poling (Fig. 1) is another process through which crystallinity of PLA, and therefore its piezoelectricity, can be altered; in this case via the reorientation of dipoles in relation to a strong electric field applied above the material's Curie temperature [29]. Specifically, corona poling has been used to improve the piezoelectric properties of scaffolds [30] and membranes [31] intended for BTE applications. The possibility of controlling the crystallinity of the manufactured PLA samples, and therefore enhance its piezoelectricity, together with the intrinsic biocompatibility, bioresorbability, biodegradability, low toxicity and strong mechanical performance of this biomaterial [32,33], make PLA a suitable candidate for its application in the TE field.

3. Composite scaffolds containing piezoceramic additives

3.1. Piezoceramics

The most common way to characterize the piezoelectricity of a material used for BTE is the piezoelectric strain constant d_{33}, which quantifies the amount of charge generated when a force is applied in a parallel direction to the sample [7]. Fig. 2 illustrates the way d_{33} and d_{31} coefficients are determined. In the case of d_{31}, another commonly used parameter for piezoelectric characterization, the force is applied in an axial direction, but the voltage is obtained in a perpendicular direction. Values of d_{33} in the range of 0.7 – 2.3 pC/N has been reported for human bone in shear mode [7,30,34]. Polymeric materials such as PVDF already possess a d_{33} constant of approximately 34 pC/N [13,35], and that value can be further increased by using piezoelectric additives or, as stated before, by applying a post-treatment (annealing, stretching, corona poling, etc.). Thus, reported d_{33} constants for piezoelectric scaffolds

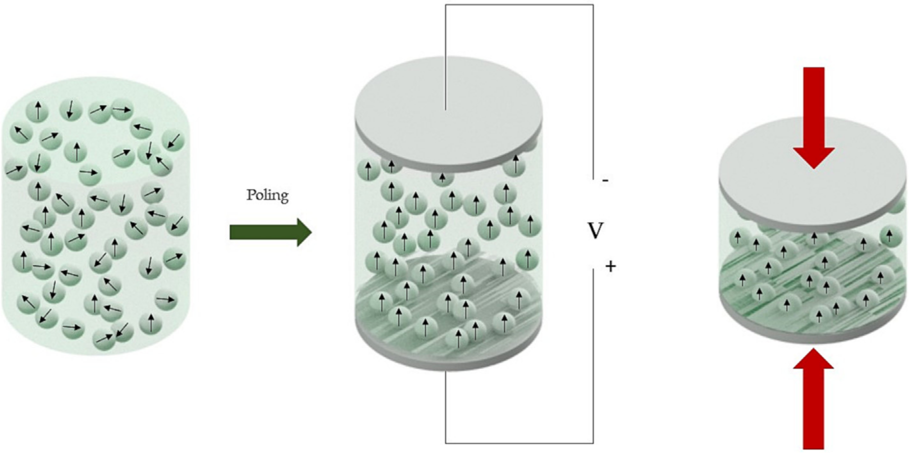

Fig. 1. Schematic representation of the poling process applied to a nanostructured piezoelectric material.

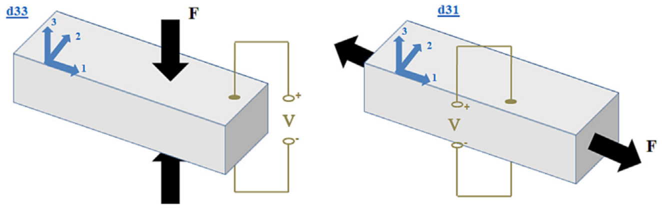

Fig. 2. Determination of piezoelectric coefficients d31 and d33.

are generally significantly higher than that of natural bone [15,24]. Even then, the most important outcome is that the piezoelectric engineered material are capable of inducing a positive cellular response [7].

If a larger piezoelectric response to mechanical loads is sought to achieve improved scaffold integration (with the consequent reduction of recovery time and/or healthcare cost after a surgical intervention), the introduction of inorganic materials with relatively higher piezoelectric coefficients into the biomaterial formulation should be considered. Inorganic materials with perovskite structure (ABO_3) have shown the best piezoelectric properties, being lead titano-zirconate ($PbZrxTi1\text{-}xO_3$, PZT) and barium titanate ($BaTiO_3$) of special interest; having d_{33} values of 600 pC/N and 450 pC/N, respectively [36,37].

The high toxicity of lead even at low doses has been reported to cause health problems such as pregnancy complications, neurotoxicity, attention deficit hyperactivity disorder and slow growth rate in children, thus hindering the biomedical application of PZT [38]. As a consequence, there is an increased research trend on lead-free piezoceramic alternatives. In fact, the number of studies focused on improving the piezoelectric properties of lead-free materials and their applications has grown exponentially over the past 15 years [39–41]. Thus, the $K_{0.5}Na_{0.5}NbO_3$ (KNN), $BaTiO_3$, $Bi_{0.5}Na_{0.5}TiO_3$ (BNT) and $BiFeO_3$ (BF) systems, all with perovskite-like structure, have attained the best results in terms of piezoelectric coefficient values [41], although they do not exceed those of PZT. Among them, $BaTiO_3$ is the most studied piezoceramic for implants since it has shown good biocompatibility and bioactivity with hard tissues [42]. Some electric properties of these systems are summarized in Table 2.

On the other hand, Liu and Ren [43] proposed the BTZ-BCT (or BCZT) system ($Ba(Ti_{0.8}Zr_{0.2})O_3$–$(Ba_{0.7}Ca_{0.3})TiO_3$) as a real lead-free alternative with piezoelectric coefficient values very similar to PZT. These authors highlighted the importance of looking for systems that present a morphotropic phase boundary (MPB) in their phase diagram. The high piezoelectricity of the BCZT system comes from the existence of a cubic-rhombohedral-tetragonal (C-R-T) triple point in the phase diagram that causes, in compositions close to the MPB region, a very low energy barrier for polarization rotation and lattice distortion.

3.2. Use of ceramic additives with perovskite structure in polymer-based scaffold formulations

Although piezoceramics can be shaped into porous structures, their brittleness is excessive to fulfil the requirements for BTE. For this reason, it is more adequate to incorporate the ceramic particles into a polymeric matrix, thus obtaining a composite material that can be easily processed to manufacture scaffolds with suitable mechanical properties. In addition to this, the mechanical properties of pure polymeric scaffolds can be also improved by the presence of the ceramic additives. For instance, it has been observed that PVDF piezoelectric properties can be considerably improved with the addition of ceramic particles with large piezoelectric coefficients without compromising its mechanical strength and flexibility [52–57]. For example, the PVDF-based composite scaffolds manufactured by Qi et al. [23], which contained a 1% of BaTiO3/carbon hybrid nanoparticles, showed an increase in terms of tensile strength and compressive strength of 22.6% and 71.4%, respectively, compared to PVDF scaffolds. Apart from improved mechanical properties, the core–shell structured nanoparticles developed conferred to the 3D structure an enhancement of piezoelectric properties that ultimately promoted cell response.

Even for those materials without lead in their formulation, the cytotoxicity of piezoceramics intended for BTE applications should be considered, as most of the abovementioned materials ($BaTiO_3$, KNN, etc.) show ion dissolution in biological fluids [38]. Since the toxicity of the ions released depends on their concentration, the incorporation of piezoceramics embedded into a polymeric matrix would help control ion dissolution. However, when working with biodegradable polymer-based formulations, the degradation products of the nanocomposite have to be removed from the body via human metabolism to avoid long-term risks [33].

Another important factor to consider in these composite systems is the size of the nanoparticle clusters (aggregation) that are dispersed into the polymer matrix, since it will have a great influence on the resulting properties [58,59]. When the ceramic particles are dispersed into the polymer matrix, the high surface energy of the nanoparticles can lead to their agglomeration and phase separation from the polymer. This process gives place to a heterogeneous composite and the decrease of the electrical properties.

The composites developed can then be processed by different manufacturing techniques to produce piezoelectric scaffolds for BTE applications. A feasible strategy to produce such scaffolds by AM is presented in Fig. 3. A first step would include the build-up of the composite to be used as feedstock for the AM processes

Table 2
Relevant piezoelectric properties of different ceramic materials with perovskite-like structure.

System	d_{33} (pC/N)	k_p (%)	ε_r	Ref.
$(0.825)BaTiO_3$–$0.10CaTiO_3$–$0.075(BaZr_{0.5}Hf_{0.5})O_3$	500	52	2800	[44]
$(Ba_{0.95}Ca_{0.05})(Ti_{0.88}Zr_{0.12})O_3$	200	17	–	[45]
$Ba_{0.9}Ca_{0.1}Ti_{0.9}Zr_{0.1}O_3$	390	50	2253	[46]
$(Ba_{0.85}\text{-}xSr_xCa_{0.15})(Zr_{0.1}Ti_{0.9})O_3$	534	47.7	–	[47]
$(BaCa)(ZrTi)O_3$	560–620	45–65	2900–4500	[48]
$Na_{0.5}K_{0.5}NbO_3$–$BiScO_3$	210	45	–	[49]
$(K_{0.5}Na_{0.5})NbO_3$	120	40	~500	[50]
$0.99(K_{0.45}Na_{0.52}Li_{0.03})(Nb_{1-x}Sb_x)O_3$–$0.01BiScO_3$	319–341	~50	–	[5]
$K_{0.5}Na_{0.5}NbO_3$	123	–	609	[51]

d_{33}: piezoelectric coefficient.
k_p: planar electromechanical coupling factor.
ε_r: dielectric constant.

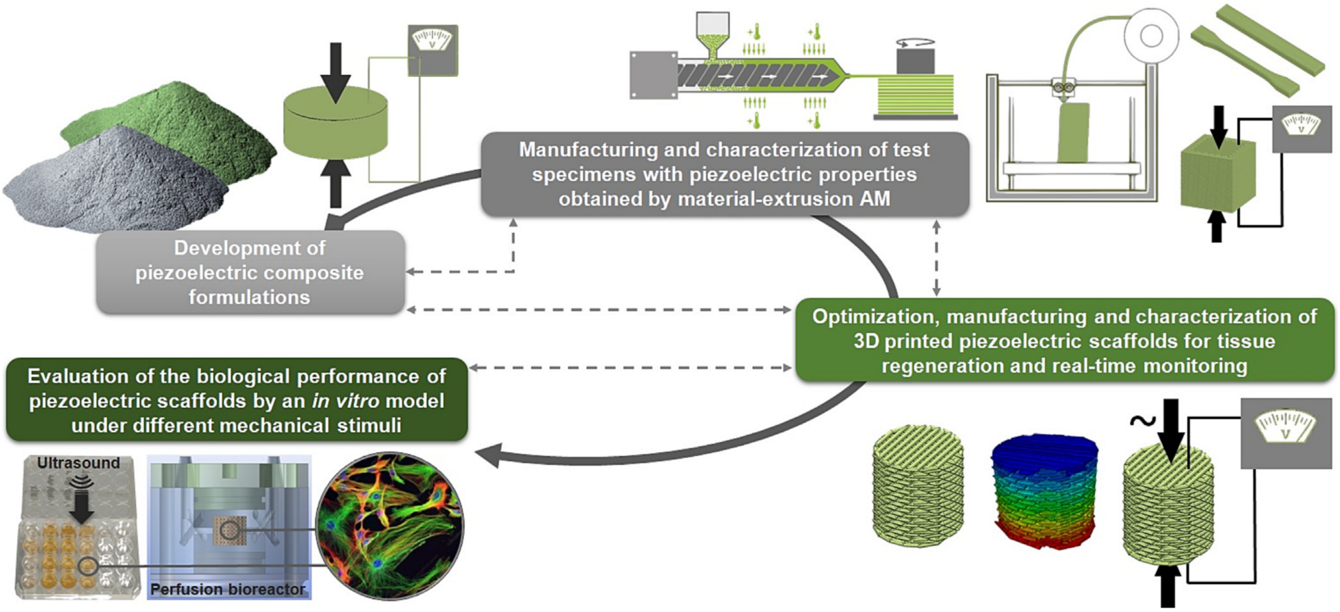

Fig. 3. Methodological approach for the development and testing of composite polymeric-based piezoelectric scaffolds obtained by Additive Manufacturing (AM) and intended for tissue engineering applications.

and the evaluation of its piezoelectric properties. Next will be the extrusion of the filament and the manufacturing of test specimens by MEX, during which the shear stress created can modify the filler distribution and the orientation of the macromolecular chains of the matrix. Both effects may affect the piezoelectric and physicochemical properties of the final part. Therefore, it is important to carefully characterize the material after each processing step (filament production and subsequent MEX). Following the methodological approach of Fig. 3, a design optimization process of the scaffolds (by using Finite Element Analysis, for example) could be the next step. The design optimization process should consider the manufacturing constraints and the mechanical and piezoelectric properties of the composite 3D printed materials. The samples obtained must be characterized at least in terms of mechanical properties and piezoelectricity. Finally, and in order to deeply understand the relationship between the piezoelectricity of the scaffolds and the biological response, the improvement in the biological performance of the composite structures developed should be confirmed by using different mechanical stimuli. The most commonly used options to stimulate the scaffolds and activate their piezoelectric effect will be reviewed in Section 4.

Several examples can be found in the literature where $BaTiO_3$ nanoparticles have been used as piezoelectric fillers for polymers, such as PVDF and PVDF-TrFE, to prepare piezoelectric membranes tested for bone regeneration [31,60,61]. These composite piezoelectric membranes showed a stable surface potential according to the level of endogenous biopotential that promoted cell adhesion, proliferation and differentiation. $BaTiO_3$ nanoparticles dispersed in biodegradable polymeric matrices have also shown good results in terms of piezoelectric properties induction and cell proliferation and differentiation. Li et al. [42] concluded that osteogenic response of bone marrow MSCs was significally promoted by their randomly oriented PLA/$BaTiO_3$ scaffolds manufactured by electrospinning. Noteworthy, aligned fibrous electrospun scaffolds having the same composition increased cell elongation and decreased cell differentiation. Therefore, the authors stated that both fibre orientation and piezoelectricity of the 3D scaffolds developed have a combining effect on osteogenic differentiation.

Also using a biodegradable matrix, Amrit Bagchi et al. [62] developed composite scaffolds based on poly(ε-caprolactone) (PCL) and containing ceramic additives with perovskite structure. Specifically, a comparison between calcium titanate ($CaTiO_3$), strontium titanate ($SrTiO_3$) and $BaTiO_3$ nanoparticles as additives to the PCL matrix was carried out. The electrospun composite scaffolds were assessed by mechanical, morphological, electrical and biological characterization. While $BaTiO_3$ imparts piezoelectric properties to the 3D polymeric structure, the interest of using $CaTiO_3$ and $SrTiO_3$ nanoparticles lies in the potential release of Ca^{2+} and Sr^{2+} ions respectively, which have shown to promote bone formation [63,64]. Piezoelectricity of the PCL/$BaTiO_3$ scaffolds was confirmed by piezo-force microscopy, whereas induction coupled plasma-optical emission spectroscopy revealed the release of Ca^{2+} and Sr^{2+} ions from PCL/$CaTiO_3$ and PCL/$SrTiO_3$ structures. All scaffolds tested supported osteoblast proliferation and enhanced osteogenic genes expression compared to neat PCL.

Another recent example is the work of Yang et al. [21], who developed AM-manufactured composite scaffolds based on the combination of polylactic acid (PLA) with $BaTiO_3$ (because of its piezoelectric properties) and graphene (to increase the conductivity of the matrix and improve the overall piezoelectricity of the composite). Improved electric properties of the developed scaffolds promoted proliferation and differentiation of MSCs. Shuai et al. [24] also used AM to obtain 3D scaffolds, in this case made of PVDF-based composite materials. The main innovation of these authors is the introduction of silver (Ag) in the $BaTiO_3$ nanoparticles structure, as this element is able to improve the electrical conductivity of the material and, at the same time, it has antibacterial properties which are interesting for the clinical translation of the approach.

4. Stimulation of scaffolds to activate the piezoelectric effect

Considering the mechanical stimulus to promote the piezoelectric effect, the most common practice during the preclinical evaluation of the scaffolds is the direct application of a compressive force [65] by using a mechanical bioreactor, or the shear stress

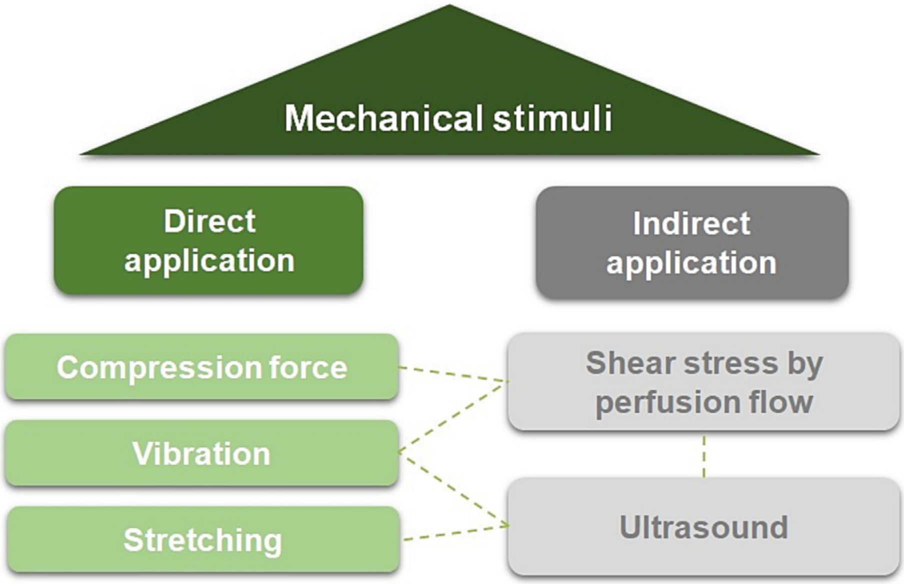

Fig. 4. Different methods to promote the piezoelectric effect of tissue-engineered scaffolds.

caused by perfusion flow [66]. A schematic of commonly used methods for activation of the piezoelectric effect is presented in Fig. 4. As for mechanical bioreactors, a specific dynamical mechanical stimulus applied during cell culture can be based on vibration, compression or stretching of the piezoelectric test sample [67]. The system used by Ribeiro et al. [68], for example, allows the application of a vibration frequency of 1 Hz to a culture plate containing poled or non-poled PVDF films seeded with osteoblast-like cells. While investigating cell response under these dynamic conditions, the authors concluded that the applied mechanical stimuli led to improved growth and proliferation of osteoblast-like cells. The same mechanical bioreactor was used to test the differentiation of human adipose stem cells cultured on PVDF films [69]. The best results in term of osteogenic differentiation were obtained for poled PVDF samples under dynamic conditions.

Regarding the use of perfusion bioreactors, this strategy is based on the use of a pump system that perfuses media through the samples to be tested in a controlled way. This type of system provides a good mixing of the media and allows suitable control of the experimental conditions and physical stimulation of the cells. The shear stress generated by perfusion flow bioreactors stimulates proliferation and differentiation of human osteoblastic cells [70]. One interesting example is the system developed by Montorsi et al. [71], which is in fact a multimodal perfusion bioreactor that allows the simultaneous application of hydrodynamic shear stress and ultrasound-driven nano-scaled vibrations. The differentiation of SaOS-2 bone-derived cells cultured on piezoelectric P(VDF-TrFE)/$BaTiO_3$ membranes was investigated under different stimuli individually or in combination. According to the results obtained, the later configuration (multimodal) was the most effective in terms of cell response, confirming the importance of mechanotransduction on the differentiation process.

On the other hand, recent studies have also tested the use of ultrasound (US) to stimulate growth and differentiation of cell populations seeded on piezoelectric materials. This approach has proven to be able to induce differentiation of neuron-like PC12 cells on PVDF membranes [72] and promote chondrogenesis of MSCs [73]. In the specific case of bone repair, Fan et al. [74] developed porous Ti6Al4V scaffolds with $BaTiO_3$ piezoelectric ceramic coating which was stimulated through Low Intensity Pulsed Ultrasound (LIPUS) in both *in vitro* and *in vivo* studies. The use of LIPUS stimuli and the piezoelectric ceramic coating demonstrated to be an effective method to promote bone regeneration in large bone defects, as their combination led to improved cell adhesion, proliferation and differentiation of MSCs *in vitro*, and also increased new bone formation *in vivo*.

A common way of applying LIPUS to the culture plate or dish that contains the cells is by placing it in contact with the US transducer, but with a thin layer of US gel [75,76] or distillate water [74] between them acting as a coupling medium. However, improved cell viability and more reproducible results can be obtained when using an experimental setup in which the US source and the biological target are separated by a greater distance (a schematic of this strategy is shown in Fig. 5). As an example, in the work of Poblete-Naredo et al. [77] a culture dish was placed at the water surface of a tank, in which the US transducer was submerged 1 cm below the dish. The authors concluded that the lower cell viability observed when applying LIPUS directly to the culture dish, in similar acoustic conditions to that applied in the water tank setup, could be explained by cell detachment produced by mechanical vibrations and resonance effects. Both factors are significantly reduced when the acoustic waves have to travel through a larger mass of water before reaching the culture dish. In fact, if the target is too close to the US source (near-field) extensive intensity distribution fluctuations may arise [78]. Following these recommendations, Cai et al. [79] applied LIPUS to $BaTiO_3$-coated titanium dishes, seeded with mouse pre-osteoblast MC3T3-E1 cells, using an immersed transducer placed 5 cm from the samples. The use of both $BaTiO_3$ coating and LIPUS synergistically promoted osteogenesis, as previously concluded by Fan et al. [74].

Regardless of the experimental configuration adopted, the effectiveness of US application would depend on the adequate selection of the process parameters, such as mode (continuous or pulsed), frequency, duration and intensity. Thermal effects are generated when US is applied in continuous mode, while the pulsed mode is used to produce biological effects [80]. The frequency range for therapeutic application of US goes from 0.75 to 3.3 MHz, being 1 MHz the most commonly selected value for *in vitro* stimulation of cells [78]. A great variability in the duration selected for US treatment is derived from the literature [77,78],

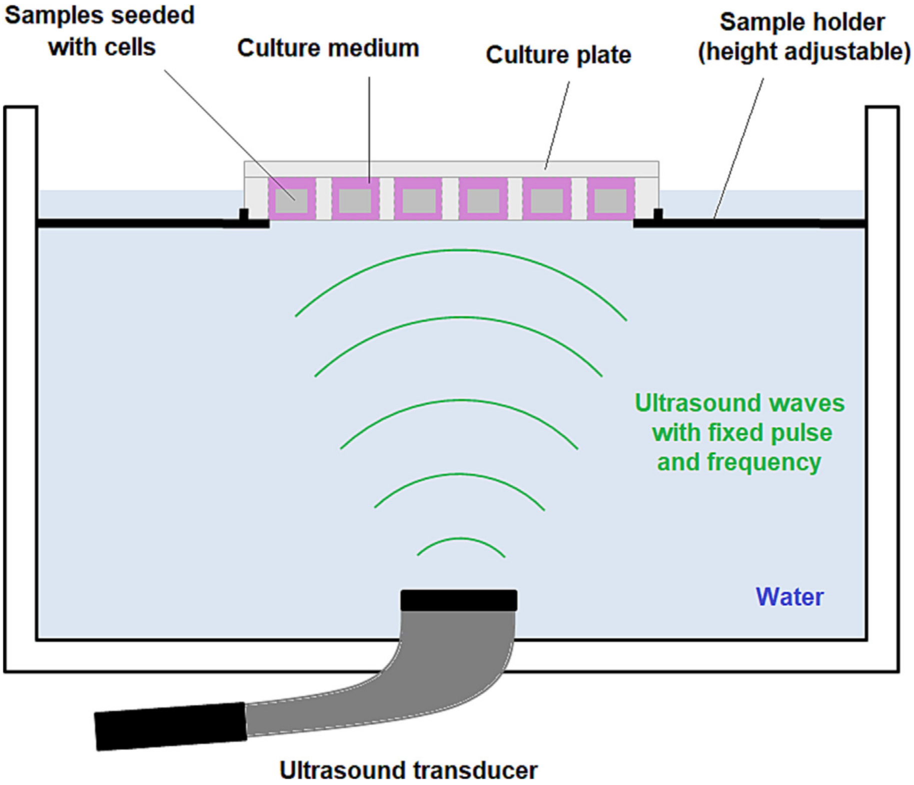

Fig. 5. Low Intensity Pulsed Ultrasound (LIPUS) indirect application method to activate the piezoelectric effect and stimulate cell growth and differentiation.

with treatment times ranging from a few seconds up to 20 min, being US applied once a day or more, and not always in every day of the test. Other parameters, and their common values for this application, include [75,76,79]: a sinusoidal ultrasound pulse of 1 ms, a repetition rate of 100 Hz and a spatial average - temporal average intensity (I_{SATA}) of 30 mW/cm^2.

5. Application as biosensors and actuators for real-time monitoring

If scaffolds are projected to serve as biosensors to monitor the mechanical loads supported during healing by daily activities, but also to assess the osseointegration status, larger piezoelectric coefficients are required. Note that the piezoelectric response of the intended scaffold is likely to decrease with the osseointegration level as the proteins adsorbed onto the material surfaces *in vivo* would neutralize the surface charges. Thus, while several studies have focused on the development of sensors for healthcare monitoring taking advantage of the piezoelectric effect [81], only a few and recent studies have assessed the combination of improved biofunctionality and self-monitoring capabilities in a single piezoelectric scaffold. In this scope, Adadi et al. [82] measured the voltage response to a mechanical pressure in electrospun fibrous PVDF-TrFE scaffolds, thus validating both capabilities. Polley et al. [83] also conducted these assessments in 3D printed $BaTiO_3$ and hydroxyapatite composite scaffolds, concluding that the piezoelectric values were appropriate for improved bone regeneration but limited for sensor or energy harvesting.

The functionality of biosensors aimed at monitoring bone regeneration is only required in the short term, until the newly formed bone grows enough to fill the original defect. In this sense, the implantation of biodegradable sensors would avoid the need for the patient to undergo a second surgery to remove the sensor. Biodegradable materials are therefore of the utmost interest for this application, as they (and their degradation by-products) can be excreted or reabsorbed into the patient's body with no side effects. Biodegradability is a characteristic possessed by many polymeric biomaterials used in TE applications, one important example being PLA, as its properties can be tuned so that the degradation rate can match the rate at which new bone is being formed. A biodegradable sensor based on PLA was developed by Curry et al. [84] for the monitoring of pressures into organs or tissues in which it can be integrated. According to the authors, and apart from the biocompatibility and biodegradability of the materials used, their device possesses other relevant advantages, including the possibility for miniaturization, easy of fabrication, precise measure of pressures up to 18 kPa and capacity to be implanted in any region of the body with minimal immune response.

High-sensitivity sensors based of piezoelectric nanogenerators have been developed by Wan et al. [16], showing great potential for their application in personalized healthcare for self-powered and sensitive human biomechanical sensing. Polydopamine-coated $BaTiO_3$ nanowires were integrated into an electrospun P (VDF-TrFE) matrix to form piezoelectric coaxial composite nanofibres with hierarchical architecture, high flexibility, adequate stability and improved sensitivity. The sensors developed showed capacity to detect low- and high-frequency signals towards external mechanical loads on the human body.

As another example of piezoelectric polymer-based actuator, Frias et al. [85] developed a PVDF device to stimulate the growth and proliferation of bone cells. Specifically, the actuator presented consisted of a PVDF thin film with silver electrodes on both sides and dip-coated with poly(methyl methacrylate) (PMMA) and a bone-like apatite layer. Cellular response of osteoblasts seeded on the polymeric surface was tested on both static and dynamic conditions, using in the later an alternating sinusoidal current

Table 3
Advantages and disadvantages of the application of lead-free materials with perovskite structure in the biomedical field.

Advantages	Drawbacks
Piezoelectric properties	Poor temperature stability
Relatively high piezoelectric coefficients	Low depolarization temperature
Biocompatibility	Low Curie temperature
Low toxicity	Large hysteresis
Adequate bioactivity with hard tissues	High leakage current

(voltage of 5 V, frequency at 1 and 3 Hz, during 15 min after 24 and 48 h post-seeding). The authors concluded that both static and dynamic configurations had an effect on cell viability and proliferation.

6. Future challenges

The introduction of ceramic piezoelectric materials in a thermoplastic matrix implies several challenges to be addressed in order to obtain functional and safe materials to be used in the BTE field. Different factors will be key in such a development of these innovative composite materials, including the volume fraction of the nanoparticles in the polymer matrix, the dispersion method or the interfacial interaction between the ceramics and the polymer phase through the addition of adequate surface modifiers [86]. Obtaining a more uniform distribution of the ceramic nanoparticles into the polymer matrix allows achieving a better piezoelectric performance [55]. Besides, it has been shown that if a non-piezoelectric polymer is used as base material, a relatively higher content of ceramic particles is required to endow the composite with sufficient piezoelectric response [87].

On the other hand, regarding the biofunctionality of the piezoelectric materials, the importance of the biocompatibility of the ceramic additive must be highlighted. In this paper, the substitution of lead has been stated as a way of improving the compatibility of the material. Research on this area will be essential, including the proposal of newly developed systems with perovskite-like structure. Lead-free piezoelectric ceramics has shown promise for their application as scaffolds for TE or as biosensors or actuators. However, there are still drawbacks that have to be counteracted to ensure a successful application of these perovskite materials [40], some of them summarized in Table 3.

The challenges to be addressed when exploiting the materials described in this review will be also connected to the manufacturing process intended to be used and the final properties of the structures (not only the raw composite material): How is the piezoelectricity of the 3D structures affected by the manufacturing process? If the matrix used for the formulation of the piezoelectric composite material is biodegradable, is piezoelectricity compromised by the degradation process? Could post-treatment of piezoelectric nanostructured composite materials improve the functionality of the final product? Even when the above mentioned gaps are filled, the clinical translation of any innovative biomaterial is always a long journey. In the particular case of piezoelectric materials, a specific requirement to be taken into account is the biomechanical profile of the area where the scaffold will be implanted and its associated electrical response, as these signals will be the initiator of the stimulus of the biomimetic scaffold.

7. Final remarks and conclusions

The development of nanostructured composite scaffolds with piezoelectric properties has shown promise as a strategy for improved bone tissue regeneration. A special mention should be given to the biomaterial formulations containing lead-free ceramic oxide nanoparticles with perovskite structure (high d_{33}) dispersed in a polymeric matrix, both with piezoelectric properties and biocompatible, which constitutes one of the systems with greatest potential for BTE applications.

It should be noted that, since the composition of the ceramic phases involved in the formulations are generally complex, the proposed synthesis method must be conveniently chosen and appropriate to obtain solid solutions. In most scientific works, piezoelectric materials have been synthesized by high-temperature solid state reaction or solution-based methods. These synthesis methods are generally complex and/or require a long time and high temperatures, which make them inefficient to obtain nanoparticles. The search for a new and facile synthetic route that would be simple, economical and environmentally safe is one of the most challenging issues related to the synthesis of functional complex oxides. In this regard, the application of mechanochemistry to the synthesis of ceramic materials is gaining great relevance and is becoming a very dynamic field, since it permits to obtain a great diversity of nanocrystalline materials with complex compositions in a simple way, using an easily scalable technique [88]. From the point of view of costs, mechanochemistry allows significant energy savings as no heat input is necessary, the processing method does not use any kind of solvent, and does not produce waste during preparation. This method have already been applied for the synthesis of solid solution oxides with perovskite structure, obtaining pure phases in really short times (between one and two hours) [89–91].

On the other hand, among the different inductive forces available to activate the piezoelectricity of the scaffolds, the most prominent are ultrasound stimulus and shear-stress stimulus (by perfusion flow). Using different types of mechanical stimuli have proven to be essential to try to understand the relationship in the mechanotransduction process and its effect on the final *in vitro* and *in vivo* biological response of the developed scaffolds. As described in this paper, the introduction of piezoelectric materials in the BTE field has promising applications that could lead to a significant breakthrough in the search of biomimetic tissue replacements.

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Ricardo Donate: Writing – original draft, Writing – review & editing, Visualization. **Rubén Paz:** Writing – review & editing, Visualization, Supervision, Project administration, Funding acquisition. **Rocío Moriche:** Writing – review & editing, Visualization. **María Jesús Sayagués:** Writing – review & editing, Visualization. **María Elena Alemán-Domínguez:** Writing – review & editing. **Mario Monzón:** Writing – review & editing, Supervision, Project administration, Funding acquisition.

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The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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