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Review article

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Piezoelectric materials as stimulatory biomedical materials and scaffolds for bone repair

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Abstract

The process of bone repair and regeneration requires multiple physiological cues including biochemical, electrical and mechanical - that act together to ensure functional recovery.

Myriad materials have been explored as bioactive scaffolds to deliver these cues locally to the damage site, amongst these piezoelectric materials have demonstrated significant potential for tissue engineering and regeneration, especially for bone repair. Piezoelectric materials have been widely explored for power generation and harvesting, structural health monitoring, and use in biomedical devices. They have the ability to deform with physiological movements and consequently deliver electrical stimulation to cells or damaged tissue without the need of an external power source. Bone itself is piezoelectric and the charges/potentials it generates in response to mechanical activity are capable of enhancing bone growth.

Piezoelectric materials are capable of stimulating the physiological electrical microenvironment, and can play a vital role to stimulate regeneration and repair.

This review gives an overview of the association of piezoelectric effect with bone repair, and focuses on state-of-the-art piezoelectric materials (polymers, ceramics and their composites), the fabrication routes to produce piezoelectric scaffolds, and their application in bone repair. Important characteristics of these materials from the perspective of bone tissue engineering are highlighted. Promising upcoming strategies and new piezoelectric materials for this application are highlighted.

Statement of significance

Electrical stimulation/electrical microenvironment are known effect the process of bone regeneration by altering the cellular response and are crucial in maintaining tissue functionality. Piezoelectric materials, owing to their capability of generating charges/potentials in response to mechanical deformations, have displayed great potential for fabricating smart stimulatory scaffolds for bone tissue engineering. The growing interest of the scientific community and compelling results of the published research articles has been the motivation of this reviewed article. This article summarizes the significant progress in the field with a focus on the fabrication aspects of piezoelectric materials. The review of both material and cellular aspects on this topic ensures that this paper appeals to both material scientists and tissue engineers.

Keywords

Bioactive; Bone Tissue Engineering; Scaffold fabrication; Characterization; Electrical stimulation; Nanofibres; 3D printing

Graphical abstract

1. Introduction

The biological performance of a material depends on the extent it is capable of mimicking the microenvironment and delivering cues to stimulate cellular response, a property demonstrated by some piezoelectric materials [1–4]. Bioelectrical signals [5,6], endogenous electrical fields [6,7] and external electrical stimulation [8–10] play crucial roles in modulating cellular behaviour and contribute to bone repair. Piezoelectric materials are capable of delivering these electrical cues without the need of an external stimulation device and able to enhance the physiological electric environment to stimulate repair [11–13]. These materials also show electromechanical behaviour (converse piezoelectric effect) and can be driven by physiological electrical changes to give rise to mechanical cues. There has been a rapid increase in the number of publications on the use of piezoelectric materials for bone tissue

engineering especially in the last few years (as shown in **Figure 1**) [12,14]. This state-of-the-art review covers the contemporary materials used, their fabrication techniques and resultant properties with a focus on orthopaedic applications.

Figure 1. The increasing trend in the number of publications within the last 5 years highlights the significance of piezoelectric materials and their potential for bone repair application.

2. Piezoelectricity: Background and association with bone

The discovery of piezoelectricity dates back to 1880 when the Curie brothers demonstrated that pressure applied to quartz crystals and Rochelle salt lead to generation of electrical charges on the surface of these materials [15,16]. The generation of surface charges is due to the distortion of internal dipoles arising from the applied mechanical force, as schematically depicted in **Figure 2 (a)**. The discovery of new piezoelectric materials such as lead zirconia titanate, barium titanate and poly (vinylidene fluoride) (PVDF) opened up more areas of application [17]. However, it was not until 1950s that the phenomenon was observed in various biological tissues [18,19]. The first attempt to use piezoelectric materials as implants

for bone was made in the 1980s [20]. Since the discovery of piezoelectric effects in bone by Fukada and Yasuda in 1957 [21], there have been extensive studies on electromechanical effects in bone and their role in modulating cellular behaviour to control growth and the remodelling processes [22–26]. Several theories suggested for the origin of stress generated potentials (electromechanical effect) in bone have been reviewed by Rajabi *et al* [14].

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(c) dependence of functional mode of operation on direction of implantation. Piezoelectric constants (d_{31} , d_{32} and d_{33}) are different in magnitude and lead to different magnitudes of electrical charges or potentials under the influence of similar mechanical stimuli.

A schematic of direct and converse piezoelectric effects, defined as voltage generation on mechanical deformation, and mechanical deformation on application of voltage, respectively is shown in **Figure 2 (a)** [27]. These effect are observed in materials where a mechanical deformation causes the formation of a net dipole moment and subsequent polarization of the material [17]. The phenomenon of piezoelectricity can also be observed after randomly arranged dipoles throughout the material are poled and/or aligned under the influence of high electric field at a specific temperature [28]. A schematic of the types of poling processes used for inducing or improving the piezoelectric behaviour in various materials is given in **Figure 2 (b)**. Corona poling is preferred over thermal (oil/fluid) poling to avoid sample contamination, especially for use in biological and cellular studies. Corona poling and thermal poling in air have both been applied to pole dense and porous materials, and can be used for inducing piezoelectric characteristics in complex scaffold structures [11,29,30]. The magnitudes of temperature and voltage used for poling depend on type of piezoelectric material and can be controlled to achieve quick, efficient and successful poling.

The piezoelectric charge constant (d_{ij} constant) is an expression of the amount of charge that the material generates in response to stress applied or alternatively, and represents the strain experienced by the material per unit electric field applied. The first subscript 'i' denotes the direction in which the polarization is generated (or applied electric field) and the second subscript 'j' denotes the direction of applied stress (or induced strain). The subscript values 1, 2 and 3 represent the orthogonal axes and 4, 5 and 6 denote rotation around their respective axes (as shown in **Figure 2 (c)**). The piezoelectric constants of materials used in the field of bone tissue engineering are summarized in **Table 1**.

Some piezoelectric materials are initially non polar (uncharged), with charges only generated when stressed; others are permanently polar, i.e. they carry a net dipole moment without the application of any force. The latter forms a sub-class of piezoelectric materials, termed ferroelectrics [31]. Ferroelectricity can alter the surface charge (zeta-potential (ζ -potential)) of materials in the wet environment by altering the interactions of ions and salts present in the fluid around it, particularly relevant for the physiological environment. This can also affect the adhesion of proteins on material surfaces, influencing the behaviour of cells, and consequently the process of tissue regeneration.

Table 1 Piezoelectric materials for bone regeneration, and their piezoelectric constants [^{*} d_{32} and d_{31} may be same or different depending on whether the films are bi axially or mono axially stretched; ^{**} piezoelectric materials which have gathered significant interest in the last decade; [±] the piezoelectric constants reported are significantly smaller than bulk ceramics but are comparable to bone; [†] [32] can be referred to for details on negative sign of constants]

	Material	Piezoelectric constant (pico Coulombs/N)	Ref.
Polymers	Poly(L-lactide) (PLLA)	$d_{14} = -9.82$	[14,33]
	Poly(vinylidene fluoride) (PVDF)	$d_{33} = -32^{\dagger}$, $d_{31} = 6.7^{\dagger}$	[34–37]
	Poly(vinylidene fluoride-trifluoro ethylene) (PVDF-TrFE)	$d_{33} = -25.2$ to -38°	[38–40]
	Poly hydroxy butyrate (PHB)	$d_{14} = 1-2$	[12,41]
Ceramics	Hydroxyapatite (HA)	$d_{33} = 1.5 - 2.4$	[42]
	Barium titanate (BT)	$d_{33} = 191$	[14,43]
	Lithium sodium potassium niobate (LNKN)	$d_{33} = 98$	[44]
	Lithium niobate (LN)	$d_{33} = 23$	[45]
	Boron nitride nano tubes (BNNT) ^{**}	$d_{33} = 0.3$	[14,46]
	Potassium sodium niobate (KNN)	$d_{33} = 93$	[47]
	Diphenylalanine (FF) ^{**}	$d_{33} = 18$, $d_{31} = 4$	[48]
Natural materials	Collagen ^{**}	$d_{15} \sim 2$	[14,18, 49]
	Bone	$d_{15} = 0.1-0.7$	[50,51]
	Silk ^{**}	$d_{14} = -1.5$	[52]
Composites	HA/BT	$d_{33} = 0.6$ to $2.8^{\pm}$	[30,53]
	LNKN/HA	$d_{33} = 2^{\pm}$	[54]

3. Characteristics of piezoelectric materials for biomedical use

3.1. Surface character

It is well known that the surface morphology/topography and chemistry modulate protein adhesion and consequently affect cellular behaviour [55,56]. These characteristics also affect the wettability and surface charge (ζ -potential) of the scaffolds governing interactions at material-cell interface [57,58]. Surface properties can alter the protein adhesion and give rise to varying cell response accordingly. Different fabrication techniques provide control over morphology of the scaffolds and further functionalization of the materials can be applied to immobilize biochemical agents [59,60]. The piezoelectric constants and ferroelectricity of the piezoelectric materials can be optimized and the topographical characteristics tailored according to requirement.

Control over morphology of scaffolds is important to correctly and independently assess the contribution of surface charge towards cellular behaviour. A study on hydroxyapatite/barium titanate (HA/BT) composites involved polishing of composite materials to obtain similar topography and roughness [61]. This post processing ensured that the observed differences in cellular behaviour were correctly correlated with the polarization of piezoelectric samples. Interestingly, it was observed that the polarization of scaffolds had little effect on cellular activities at day 7 [61].

Aside polarization, the morphology and roughness of piezoelectric scaffolds affects cellular behaviour. PVDF films and fibrous scaffolds have been shown to alter cell proliferation and alignment, suggesting that cellular behaviour can be tailored accordingly [57,62]. It has been reported that the roughness of coated polymer (PVDF films in this case, [35]) contributes to changes in cellular behaviour along with surface charge. Nano/micro roughness of the surface

has been known to cause changes to surface energy and wettability which has a direct effect on cellular behaviour [35,56,63].

3.2. Ferroelectricity

Recently, the charge distribution around the surface of certain ferroelectric materials immersed in fluid have been characterized by ζ -potential measurements [11,45]. These generated potentials of -30 to -80 mV have been associated with improved cellular response on these materials [11,45]. It has been suggested that enhanced interfacial polarization in ferroelectric composites is the main contributing factor towards increasing ζ -potential [11]. However, due to the limited number of studies where ζ -potential of bulk ferroelectric materials has been measured, the correlation between the polarization and ζ -potential of ferroelectric materials cannot be generalized.

The association of the nature of surface charge (positive or negative) of electroactive materials to cellular responses is hard to establish and ζ -potential measurements provide better insights to the correlation [45,64]. Surface charges of piezoelectric materials are altered in response to different mechanical deformations, and recording these minute ζ -potential variations is important to control cellular response. A small variation in surface potentials can lead to a change in local electro kinetics and directly affect cellular behaviour. In case of HA, it was observed that positive or negative surfaces had little or no difference in contact angle [65], while significant differences were noted in case polarized PVDF films [66]. The values observed for negative and positive HA surfaces differed by 2° while in case of PVDF, this difference was noted to be 20° which could be due to the altered surface chemistry as a result of corona poling [65,66]. Two different studies by Dubey *et al* demonstrated that polarizability and nature of surface charge of potassium sodium niobate (KNN) and its laminated composites play a key role modulating cellular behaviour and bioactivity of the

composites, respectively [54,67]. They also suggested that conductivity of KNN might also be responsible for increased proliferation of human osteoblast-like Saos2 cells [67].

Kelvin probe force microscopy (KPFM) has been utilized for nanoscale imaging of the surface potential of different types of materials including metals [68], semi-conductors [68,69], ferroelectrics [70,71], piezoelectrics [70], chemically functionalized, cell compatible scaffold surfaces [72,73] and biological materials [70,72,74–78]. However, there are conflicting reports correlating KPFM observations with surface potential; Rohm *et al* conducted KPFM measurements on perovskite thin films of methyl ammonium lead iodide and observed no correlation between ferroelectric domains and surface potential while results obtained in time resolved KPFM experiments performed by Strelcov *et al* on lithium niobate surfaces confirmed that ferroelectric domains had an influence on surface potential [79,80]. In another study, PVDF-TrFE thin films displayed a correlation in ferroelectric, piezoelectric and surface potential characteristics [70]. However, due to the presence of screening charges (charges/adsorbates which compensate the surface electrical properties), it is difficult to map the surface potentials using this technique and establish a correlation between ferroelectric domains and surface potentials [81,82].

3.3. Magnitude of voltage/charge generated

The most commonly used constant to describe the piezoelectricity of materials used for bone tissue engineering is the piezoelectric strain constant. Piezoelectric constant has been measured and shown to be comparable or greater than the values observed for bone.

Piezoelectric constant of up to 0.7 pC/N has been observed for bone in the shear mode [51].

Values reported for scaffolds are significantly higher in many instances and the charges generated can be higher than bone when dynamic mechanical stimulus is applied. However, if these charges are not found to be high enough to elicit a beneficial cellular response, there will be a need to improve piezoelectric characteristics of the scaffolds. In a study published in

2017, Wang *et al* fabricated core-shell structured composite sub-micron fibres of PVDF and graphene oxide and reported an increase in piezoelectric constant of 426% in comparison to neat PVDF fibres [83]. Incorporation of different fillers can help in tuning the piezoelectric behaviour for bone repair applications [84–86]. The electrical potential output generated by the materials can also be enhanced by physical structural modification post-fabrication [87]. It is also important to note that piezoelectric property of a scaffold has a role to play only under controlled dynamic conditions. Piezoelectric scaffolds must be exposed to similar levels of mechanical deformations as experienced by the cells *in vivo* to analyse the effect of charge generated as a consequence of piezoelectric effect; higher values of strain can lead to cell death [88]. These deformations are in the range 0-0.4% under normal physiological conditions, whereas on fracture, values up to 15% have been hypothesized [89–91]. A vertical vibrating plate was used to mechanically stimulate the materials in two different studies involving dynamic stimulation. However, the amount of mechanical deformation and consequently, the amount of charge experienced by the cells and the deformation induced in the scaffolds was not quantified [35,92].

The mode in which a piezoelectric scaffold is stimulated, and the value of piezoelectric constant are important and interlinked. The importance of directionality (or functional mode) was highlighted by Feng *et al* in a study using HA/BT composites *in vivo* [93]. The functional mode of operation of the material might differ depending on the direction of implantation of the piezoelectric scaffold *in vivo*, as depicted in **Figure 2 (c)**.

Figure 3 PFM is a useful tool to study nanoscale interactions by mapping piezoelectric response of different materials. a) Surface topography, b) PFM phase and c) PFM amplitude of a PVDF/GO nanofibre; d) AFM topography e) PFM amplitude and f) PFM phase images of FF nanotubes, and g) a vector PFM map representing the local electromechanical response, where the colour indicates the molecular orientation direction and the intensity indicates the magnitude of electromechanical response. Printed with permission from [83,94,95].

3D porous structures can be poled using the existing methods such as corona poling, however the challenge is to characterize the piezoelectric properties of these structures. Porous ceramic composites have been characterized using a piezo constant meter. A d33 or d31

meter which measures the amount of charge generated in the direction of poling when the force is applied in a parallel direction (d_{33} mode) or perpendicular (d_{31}) to it. Piezoresponse force microscopy (PFM) is a useful AFM-based tool to study the piezoelectric properties of a variety of synthetic and biological materials at the nano scale, as reviewed by Bystrov *et al* and Rodriguez *et al* [96,97]. PFM has been applied to polymeric fibres and self-assembling peptide nanotubes (summarized in **Figure 3**), and can provide insight to understand the piezoelectric property distribution and its effect on cellular interactions. However, the limitations of this technique should be considered to ensure that artefacts are not misinterpreted as true signals [98–100]. A comparison of the piezoelectric properties is only possible if both polymeric and ceramic materials are characterized similarly, facilitating understanding of the biological response of these materials.

3.4. Mechanical and physical characteristics

The physical and mechanical properties of scaffolds, including porosity, degradation and Young's modulus have key roles in governing the biological performance of piezoelectric materials. The porosity of the scaffolds need to be controlled to ensure cellular migration, response and optimize mechanical and piezoelectric properties [30,101]. Piezoelectric properties of different materials are influenced differentially by porous structure. The study of porous HA/BT composite scaffolds and their piezoelectric properties displayed an inverse relation between porosity and piezoelectric constant [30]. The enhancement of piezoelectric property with increase in density of scaffolds was attributed to the better inter-connectivity of the active piezoelectric phase of the ceramics [30]. In a different study of porous arrays of PVDF, higher piezoelectric potential and current values than bulk PVDF films were demonstrated [101]. These studies suggest towards the possibility of fabrication porous piezoelectric materials with tuneable porosity and piezoelectric characteristics. The mechanical properties of piezoelectric scaffolds are important to restore the functionality of

the substituted tissue *in vivo*, in particular material stiffness has a key role to play in cell-material interactions [102,103]. Boyan *et al* demonstrated that substrate stiffness of polymeric surface can modulate the response of mesenchymal stem cells [103]. Further, it is important to assess the stability of electrical properties such as ferroelectricity, the nature of the surface charge and piezoelectric constants to ensure that these properties are stimulatory over the time needed for regeneration, and that any shielding of their properties is tuned. Shielding can occur due to the cells/tissue growing around the scaffold and render the scaffold inactive. A major limitation of most studied piezoelectric materials, such as PVDF and BT, is their non-degradability. Polyhydroxyalkanoates, semi-crystalline poly(α -hydroxy acids) such as poly(L-lactide) (PLLA) and natural polymers such as silk are gaining traction as degradable matrices for piezoelectric scaffolds [104–106].

4. Synthesis and processing of piezoelectric materials

4.1. Piezoelectric polymers

The origin of piezoelectricity in polymers is attributed to the inherent crystal or chemical structure of the polymer material, which induces a net dipole/charge on mechanical deformation. Piezoelectric polymers have mainly been fabricated in three different morphologies; films, rods or tubes and fibres [12]. The main piezoelectric polymers investigated for their osteogenic capacity are PVDF, its copolymers (poly (vinylidene fluoride-trifluoro ethylene)(PVDF-TrFE) and poly (vinylidene fluoride-hexafluoropropylene)(PVDF-HFP), and PLLA [14]. A direct comparison of the ‘d’ constant of PLLA and PVDF (shown in **Table 1**) is hard to establish as they are observed in different activation modes (subscripts ‘i’ and ‘j’ denote activation modes). The ferroelectric behaviour of PLLA has only be observed at high temperatures and is known to decay fairly quickly at room temperature, whereas for PVDF it is stable and observable at room temperature

[33,107–109]. The principal methods used for fabricating piezoelectric polymers for bone tissue engineering are solvent casting, compression moulding (most commonly for films) and fibre spinning (e.g. electrospinning).

4.1.1. PVDF and its copolymers

PVDF is a semi crystalline polymer known to present five different crystalline phases: α (alternating trans-gauche conformation (TGTG')), β (all trans conformation (TTTT)), γ (alternating (triple trans)-gauche (T₃GT₃G')), δ (alternating trans-gauche (TGTG')) and ϵ (alternating triple trans-gauche (T₃GT₃G')) [110–114]. The different conformations determine the polar/non-polar nature of the polymer chain as shown **Figure 4**. The polar β , γ and δ phases are formed when the dipoles are arranged parallel to each other and perpendicular to the polymer chain, while the non-polar α and ϵ phases are formed when the net dipole moment cancels due to anti parallel arrangement [115,116]. The polar β - phase is of interest as it presents high piezoelectric characteristics to the material [115]. Poling of β -PVDF further improves its piezoelectric characteristics. PVDF-TrFE exists in all trans conformation (β - phase) and possesses a high electromechanical and piezoelectric coefficient for specific monomer concentrations [117–120]. PVDF-TrFE does not require special processing techniques to obtain β - phase crystalline structure and can easily be formed into complex/porous structures desirable for tissue engineering applications. PVDF-HFP has a lower crystallinity when compared with PVDF, yet presents good ferroelectric and piezoelectric behaviour [116,121].

Figure 4 Chain conformations of different phases of PVDF and PVDF-TrFE. Dipole moment cancels out in α - phase which is not the case for piezoelectric in PVDF-TrFE and β - phase and γ - phase of PVDF.

Various ways by which PVDF and its copolymers can be obtained in β - phase and subsequently be made piezoelectric by poling are shown in **Figure 5**. These methods have been reviewed in detail by Lanceros-Mendez *et al* [116]. Two recent works by H. Horibe *et al* have highlighted the control of phases using different solvents and anti-solvents during synthesis procedure [122,123]. It was observed that a solvent with higher dipole moment resulted in formation of polar β - phase which was also supported by slower solvent evaporation rate [122,123].

The techniques that involve stretching, such as electrospinning, can result in higher β - phase than α - phase [35,66,92,124,125]. In a series of studies by Ribeiro *et al*, PVDF films were produced with desired piezoelectric properties and tested for their osteogenic capability [35,66,92,125]. The films were solvent cast into rectangular samples, annealed and mechanically stretched and corona poled to maximize the piezoelectric performance.

Stretching only induces the formation of β - phase and the films need to be poled to align and lock the dipoles to observe increased piezoelectric response [126,127]. The temperature for mechanical stretching and the draw ratio was optimized in previous studies by the same group [34,128]. Protein adsorption, proliferation of pre-osteoblastic cells and the differentiation of human adipose derived stem cells into osteogenic lineage were found to be positively affected by these piezoelectric substrates [35,66,92,125]. Piezoelectric PVDF films and fibres have also been assessed *in vivo* and their suitability well demonstrated as bone substitutes [129].

Figure 5 Fabrication methods to obtain PVDF and its copolymers (PVDF-TrFE, PVDF-HFP) in β - phase.

Piezoelectric polymers have also been utilized as suitable coatings for existing implant materials for tissue engineering applications [130,131]. In a recent study, PVDF films were

deposited on titanium sheets and poled using corona discharge to enhance the bioactivity of the surface of Ti sheets [131]. A solution of PVDF in *N, N*-dimethyl formamide (DMF) and acetone was prepared and cast over a Ti surface. Poling of the samples was carried out using a corona discharge at 100°C and the effect of polarization on bone marrow mesenchymal stem cell behaviour assessed. Polarized samples exhibited enhanced cell proliferation (at days 4 and 7) and osteogenic differentiation (higher alkaline phosphatase (ALP) activity at day 21) of the cells as compared to non-polarized ones.

The converse piezoelectric effect has also been explored and mechanical stimuli delivered through PVDF actuators has been shown to be effective in stimulating bone growth [132,133]. This aspect is important to study as patients are immobilized in the early stages following an injury/fracture. Immobilization leads to an absence of mechanical stimulation (through physiological movements) to piezoelectric materials for electrical cue generation. The converse effect can therefore be used in the early stages to improve the biological performance of the scaffolds. This effect may be driven by external electrical stimulation devices, implanted batteries or varying physiological electrical environment.

Electrospinning of randomly aligned PVDF fibres/fibre mats have been shown to eliminate the need of stretching and poling the polymer constructs [134–136]. PVDF copolymer nanofibre mats obtained by electrospinning are also reported to be piezoelectric and can have possible application in tissue engineering [137,138]. Electrospinning requires optimization of various solution and processing parameters to control the diameter and morphology of the fibres [139]. Arinzeh *et al* used electrospinning for the fabrication of fibrous PVDF scaffolds at different voltages applied during the spinning and investigated the effects on β - phase formation and differentiation of human mesenchymal stem cells in osteogenic lineage [124]. The formation of electroactive phase was found to be effected by the magnitude of voltages used. The study demonstrated that electrospun scaffolds showed higher ALP activity and

matrix mineralization suggesting promoted differentiation of cells into osteogenic lineage. In a recent study, electrospun scaffolds of PVDF-TrFE were dynamically stimulated to guide human mesenchymal stem cells into osteogenic lineage [140]. It was found that streaming potentials of $\sim 61.1 \mu\text{V}$ were generated on dynamic stimulation of annealed scaffolds. These scaffolds were observed to show increased mineralization and osteogenic gene expression at day 28 compared to as spun PVDF-TrFE and PCL scaffolds [140].

Films and electro spun mats of PVDF-TrFE have been observed to have different levels of crystallinity and β - phase content which can play a key role in modulating cellular behaviour under static and dynamic conditions [62,141]. Porous films of PVDF-TrFE and PVDF-HFP have also been used and compared with PVDF for suitability in bone tissue engineering [142]. This study shows that PVDF-TrFE and PVDF-HFP present lower amount of β - phase than PVDF under similar processing conditions, however, it has been suggested that PVDF-TrFE is not suitable for bone tissue engineering applications as these scaffolds promoted an elongated cellular morphology [142]. PVDF microspheres have also been fabricated using the electrospray technique and their suitability for bone tissue engineering shown using MC3T3E1 cells and human mesenchymal stem cells [143,144]. However, the role of piezoelectricity was not detailed in these studies.

4.1.2. PLLA

Semi-crystalline PLLA does not require any additional poling treatment to induce piezoelectric effect. This has been attributed to the displacement of the C=O bond in PLLA in response to mechanical stress leading to generation of a net dipole moment and charge [145–147]. PLLA has been used to fabricate films, fibres and rods with piezoelectric behaviour [148–151] and it has been observed that crystallinity and polymer orientation play key role in piezoelectric characteristic [152]. It presents an additional advantage of fabricating degradable scaffolds [153–155]. Biocompatible PLLA microspheres have displayed good

potential for tissue engineering applications [156–158], however, the piezoelectric properties of those microspheres synthesized have not been studied to the author's knowledge. Different morphologies in which piezoelectric materials have been synthesized are shown in **Figure 6 (a)**.

PLLA-based scaffolds have been synthesized in various forms and their suitability as bone substitutes evaluated extensively; however, the role of piezoelectric characteristics in controlling cellular behaviour requires further attention. Fabrication techniques used for manufacturing PLLA based scaffolds have been reviewed by Laurencin *et al* [159]. In one of the first attempts on use of PLLA as bone substitute, Ikada *et al* fabricated PLLA films and rods which were drawn to fabricate piezoelectric scaffolds to be implanted in cat tibiae [160]. Callus formation around implants was reported to be dependent on the draw ratio of these piezoelectric scaffolds [160]. Such behaviour was attributed to the currents generated by strains in piezoelectric scaffolds due to the movements generated by animal while walking.

A selection of pertinent studies in which piezoelectric polymers have been used to test their feasibility for orthopaedic applications is given in **Table 2**. It can be seen that piezoelectric polymers have been used to manufacture 3D scaffolds with only one study exploring the role of piezoelectricity under dynamic stimulation. Further to this, the effect of protein adhesion on piezoelectric properties remains to be explored. The existing techniques in combination to the emerging approaches discussed in section 7 have great potential for tissue engineering applications and should be used to fabricate 3D constructs to leverage control over cellular behaviour.

Table 2 Fabrication techniques of piezoelectric polymers and their assessment using different cell lines for orthopaedic application

Material	Fabrication/ processing technique	Cell type	Loading regime	Key findings	Ref.
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PVDF	Films coated on titanium sheets via solvent casting and poled using corona discharge	Bone marrow mesenchymal stem cells	No mechanical loading (Static)	Increased proliferation, ALP activity and osteogenic gene expression	[131]
	Mechanically drawn and electrically poled solvent cast (Drying at 120°C) films	Human adipose stem cells	Dynamic analysis was carried out using a vertical vibration module	Increase in ALP activity in piezoelectric samples under dynamic stimulation	[92]
			Static. Comparison of poled and non-poled samples	No significant difference observed in osteogenic differentiation of cells	[66]
		MC3T3-E1 murine pre-osteoblasts	Dynamic	Increased proliferation on piezoelectric samples under dynamic stimuli	[35]
			Static. Comparison between poled β , unpoled β and α -phase samples	Higher cell viability and similar cell density in all samples at day 7	[125]
	Electrospun fibres	Human mesenchymal stem cells	Static. Comparison of fibres spun at different voltages	Increased ALP activity Increased mineralization	[124]
PVDF, PVDF-HFP and PVDF-TrFE	Porous solvent cast (Room temperature drying) membranes	MC3T3-E1 murine pre-osteoblasts	Static	Cell proliferation and morphology were influenced by porosity and microstructure of membrane surface	[142]
PVDF-TrFE	3D electrospun scaffolds	Human mesenchymal stem cells	Dynamic analysis using sinusoidal compression	Enhanced osteogenic differentiation on stimulated scaffolds	[140]
PLLA	Piezoelectric films and rods	<i>In vivo</i> analysis		Increased callus formation around piezoelectric implants	[160]

4.1.3. Polymer matrix composites and their application as bone substitutes

Polymer matrix composites (PMCs) allow for combination of manufacturing flexibility and the processing of otherwise brittle ceramics with higher piezoelectric constants to complex forms. PLLA based composites have extensively been studied for application in the field of bone tissue engineering [161,162], as reviewed in [159]. Though piezoelectricity has been observed in electrospun fibre mats based on PLLA [148,163–165], the focus has been to improve the suitability of the scaffolds for desired application under static environment

without the mention of the contribution of piezoelectric properties of fillers or PLLA itself [166,167].

Table 3 Filler loading percentages used in different studies (number in bracket represents filler % used for biological studies).

Base material	Filler type (particles)	Loading percentages	Refs
PVDF-TrFE	BT	0-5% v/v (5%)	[11]
		10% v/v	[168]
	BNNTs	1% w/w	[169]
HA	BT	0-80% w/w (40%)	[53]
		90% v/v	[61]
		40%	[170]
		20-80% v/v (80%)	[171]
		50-90% v/v	[30]
		90% v/v	[172]
		80% and 90% w/w	[173]

Polymer matrix composites can be produced, taking advantage of the flexibility of polymer processing techniques, and impart piezo-electrical and bioactive behaviours [174].

Membranes of PVDF-TrFE/BT have been reported to support bone formation in several studies [11,168,175,176]. A recent study published in 2016, Chen *et al* used poled solvent cast membranes of PVDF-TrFE/BT to explore the suitability of membranes for repairing bone defects [11]. BT nano particles were surface modified with polydopamine (PDA) to improve their dispersion in the polymer matrix, and reduce their tendency to agglomerate or sediment during processing [11]. Interestingly, instead of mentioning the piezoelectric properties, the authors highlighted that ferroelectric property of these membranes have a crucial role to play and help in mimicking the physiological electrical micro-environment (steady state bioelectric potential). The surface potential of the samples was found to be dependent on the increased polarization of the samples due to the presence of nano sized BT [11]. The study shows that the composite membranes have promising potential for clinical application owing to the improved osteogenic capability demonstrated *in vitro* and *in vivo*. Improved osteogenic behaviour of bone marrow derived mesenchymal stem cells was

observed *in vitro* and enhanced healing of a parietal bone defect in rats was observed in *in vivo* experiments [11]. PMCs used for orthopaedic applications, along with the results obtained are listed in **Table 4**.

Table 4 Summary of key findings of studies involving use of PMCs. (*Static represents the assumption that treating defects in skull did not initiate a piezoelectric response.)

Material	Fabrication/ processing technique	Cell type	Loading regime	Key findings	Ref.
PVDF- TrFE-BT	Solvent casting followed by poling	Bone marrow Mesenchymal stem cells and <i>In vivo</i>	Static*	Improved cellular activity and favourable osteogenic differentiation. Rapid bone regeneration <i>in vivo</i>	[11]
		<i>In vivo</i>	Static*	Higher gene expression of key osteoblast markers was observed around composite membranes	[168]
	Solvent extraction using water followed by hot pressing	<i>In vivo</i>	Static*	Similar or better morphometric parameters than Polytetrafluoroethylene (PTFE) membranes. A mixed profile of gene expression was observed	[175]

Unlike this study which involved no external mechanical stimulation, Genchi *et al* used ultrasound to mechanically stimulate piezoelectric PVDF-TrFE/BNNT composite films and evaluated the osteogenic differentiation capacity of Saos-2 osteoblast-like cells [169]. Cell culture on films was performed in presence and absence of ultrasound to analyze the contribution of piezoelectric behaviour of the films. Composite films were manufactured by dispersing BNNTs into the solvent and mixing them with the polymer solution. It is important to note that methylethylketone (MEK) was used as the solvent in this study as solvents like DMF and DMAc. It was found through simulation that the electrical potentials generated as a consequence of piezoelectric effect were in the range suitable for cellular stimulation [169]. Solvent free routes such as dry powder mixing and compression moulding and addition of bioactive fillers have also been utilized to fabricate piezoelectric scaffolds [177]. The composites prepared displayed enhanced bioactivity through increased apatite

formation over sample surfaces. The percentages of fillers used in selected piezoelectric composite materials for bone repair are summarized in **Table 3**.

4.2. Ceramics

Barium titanate (BT), lithium niobate (LN), potassium sodium niobate (KNN), hydroxyapatite (HA) lithium sodium potassium niobate (LNKN) and zinc oxide (ZO) are lead-free piezoelectric ceramics that provide a better alternative to lead-based systems which present toxicological risks [14,178–181]. Park *et al* conducted studies in which specialized cylindrical samples of BT were fabricated and implanted in dog femora in an attempt to augment the process of bone repair [20,182]. The piezoelectric samples were fabricated by slip casting, firing and subsequent poling of BT powder. The poling of samples was carried out in a fluid filled chamber with a constant electric field. The results of the study were appealing in terms of compatibility and interfacial strength of the material with surrounding tissue. However, no significant differences were observed for piezoelectric and non-piezoelectric samples.

Figure 6 (a) Different morphologies of piezoelectric polymers or polymer matrix composites explored for bone repair application, (b) Ceramics have been used in a variety of scaffold morphologies which have been found suitable for bone tissue engineering application [30,54,183–186] (c) Fabrication techniques for piezoelectric ceramic (or ceramic matrix composite) based scaffolds. Images adopted from [30,54].

Cold isotactic pressing was used by Wang *et al* to fabricate porous LNKN scaffolds and evaluated their suitability as bone substitutes [187]. Poly vinyl alcohol (PVA) was used as a binder and ammonium oxalate monohydrate (AOM) as the porogen. PVA, AOM and LNKN were ball milled, die pressed into desired shape and sintered to obtain the samples. Samples were poled in air by applying a direct current (dc) electric field. Cytotoxicity analysis of the samples revealed that osteoblasts cells were more active on negatively polarized (negatively and positively polarized surfaces are defined depending on the type of electrode (during

poling) the surface of the material is exposed to) surfaces than on non-polarized samples. LN was used in two different studies which confirmed the compatibility of charged LN surfaces with two different osteoblast cell lines [45,188]. However, these studies did not focus on fabrication of scaffolds, and ceramic samples in the form of wafers or plates were purchased and processed accordingly for cellular studies. The results confirmed that charged LN surfaces showed better attachment and proliferation of cells. These recent studies suggest a new way of characterising piezoelectric scaffolds highlighting the fact that ferroelectricity, surface charge and surface potential might have a crucial role to play in modulating cellular behaviour.

HA-based ceramics have gathered significant interest of the scientific community for designing scaffolds for bone substitutes owing to their bioactivity and osteogenic capability [189–191]. Polarization of sintered HA samples has been reported to lead to surface charges generation, playing a crucial role in modulating cellular behaviour [190,192]. The piezoelectric effect in HA samples was not observed until a study by Gandhi *et al* in 2014. HA pellets were fabricated by sintering of HA powders using spark plasma sintering technique which displayed piezoelectric properties [42,193]. Spark plasma sintering process is a modification of the traditional sintering process that ensures that the process of sintering can be completed at lower temperatures and in smaller time durations [194]. Sintered dense tablets of HA were polarized in a heated furnace using a dc electric field the piezoelectric characteristics were measured using a piezo constant meter [42].

Polarization of HA samples has been reported to increase the wettability and surface energy; and the nature of polarization (positive or negatively charged surface) is linked to variable cellular behaviour [65]. A heated furnace with platinum electrodes on samples was utilized to perform polarization and mineralization and proliferation of human foetal osteoblast cells were significantly higher on negatively charged surfaces [65]. It was suggested that the

negatively charged surface allowed absorption of calcium ions which facilitated the process of mineralization on these surfaces and adsorption of the cell adhesive proteins improved the cellular response [65]. The proposed mechanism suggests piezoelectric materials that are capable of generating negative charges on the surface have great potential for tissue engineering application.

Use of modified sintering environments for HA based scaffolds improve the performance of scaffolds and presents an enhanced cellular response. Kumar *et al* fabricated HA scaffolds using different sintering conditions (dry air or water vapour) and analyzed the response of MC3T3 bone cells on the polarized samples [64]. Sintering in water vapour atmosphere ensures that dehydration of hydroxide ions, which are believed to be the carrier ions participating in the process of polarization, does not occur and the samples consequently present an increased surface energy which contributes to improved cellular behaviour [65,190,195]. The results of this study have been corroborated by a more recent study by Nakamura *et al* which stated that fabrication of HA ceramics by sintering in saturated water vapour atmosphere increased the polarization capacity of the samples [195]. Subsequently, these samples had better wettability and showed improved cell adhesion and spreading. Poled HA ceramics have been found to play a key role in controlling *in vivo* and *in vitro* cellular response, however, different fabrication techniques are used across studies which makes comparison challenging [29,196–199]. The effect of different sintering atmospheres on the piezoelectric characteristics has not been discussed in any of the studies above.

The different approaches used to fabricate piezoelectric scaffolds using ceramics or ceramic-based composites are given in **Figure 6 (c)**. The key findings of different studies concerning piezoelectric ceramics used for orthopaedic applications have been summarized in **Table 5**.

Table 5 Studies of scaffolds using piezoelectric ceramics and their effect on cellular behaviour.

Material	Fabrication/ processing technique	Cell type	Loading regime	Key findings	Ref.
LiNbO ₃	Commercially obtained single crystal substrates	Human osteoblast-like Saos-2 cells	Static	Increased cell number on charged surfaces at day 7. Increased ALP on negatively charged surface when compared to positive one	[45]
	Commercially obtained wafers	MC3T3-E1 murine pre-osteoblasts	Static	Increased cell proliferation rate and mineralization	[188]
HA	Dense samples synthesized using different sintering conditions (wet and dry)	Mouse osteocyte-like.	Static	Improved cellular adhesion through increased surface energy and wettability	[195]
		MC3T3-E1 murine pre-osteoblasts	Static	Improved cell proliferation and metabolic activity	[64]
	Dense samples using conventional sintering	Human foetal osteoblasts	Static	Increased early stage proliferation and matrix mineralization	[65]
	Porous cylindrical samples using conventional sintering and polarization	MC3T3-E1 murine pre-osteoblasts	Static	Increased cell number and matrix mineralization on negatively charged surface	[29]
BT	Dense cylindrical sample using slip casting and conventional sintering	<i>In vivo</i>		Good biocompatibility and strong interfacial bond with the bone	[20,182]
KNN	Disc samples prepared by solid state reaction followed by sintering and polarization	MC3T3-E1 murine pre-osteoblasts	Static	Enhanced protein adsorption and cell proliferation over polarized surfaces	[47]

4.2.1. Ceramic matrix composites and their application as bone substitutes

Ceramic matrix composites (CMCs) have great potential as bone tissue engineering materials, the relatively high piezoelectric properties of certain ceramics can be combined with bioactive fillers to obtain favourable cellular responses. HA/BT based ceramics are the most explored composite materials for fabrication of piezoelectric scaffolds for bone regeneration [200,201]. For example, Feng *et al* implanted HA/BT scaffolds in jawbones of dog and observed that HA/BT promoted osteogenesis significantly in comparison to HA ceramics [93]. Isotactic pressing was used to fabricate green bodies of the samples, which were

subsequently sintered and poled. In their work, it was noted that the osteogenic response was direction dependent suggesting that piezoelectric effects have play a role. Several other studies reported fabrication of piezoelectric composite scaffolds of HA/BT with low porosity using spark plasma sintering or conventional sintering processes [53,61,170,171]. HA/BT rod-like nanocomposites have recently been fabricated using a hydrothermal process [202]. The piezoelectric charge constant of these composites were reported (1.54 pC/N), close to that of bone, however, no cell studies were conducted [202].

Unlike these studies, in a more osteomimetic approach, Zhang *et al* fabricated HA/BT scaffolds with aligned porous structures and observed significantly higher cell densities and ALP activity in comparison to dense scaffolds [30,172]. Green bodies of the composite scaffolds synthesized via ice templating were sintered and poled to make piezoelectric samples [30,172]. The piezoelectric coefficient observed for these samples was reported to be higher than that of the bone. However, no difference was noted among polarized and non-polarized groups of samples due to the absence of dynamic loading. A recent study by Yang *et al* (2017) used an *in vitro* dynamic loading device to apply cyclic loading to HA/BT piezoelectric composites [173]. It was observed that biocompatibility and bone-inducing activity of the composite samples was greater than that of HA under cyclic loading. The force used for cyclic loading of the samples was fixed, however, the amount of strain being transferred to the composites was not quantified. The indenter used to press the samples did not cover the whole surface of the sample which might have led to non-homogenous distribution force through the sample [173]. In a recent study, Shokrollahi *et al* fabricated porous freeze cast composites of BT/akermanite ($\text{Ca}_2\text{MgSi}_2\text{O}_7$) with piezoelectric characteristics higher than that of bone [203]. Cytotoxic evaluations with human bone marrow mesenchymal stem cells confirmed that the composited were biocompatible, yet osteogenic capacity and the role of piezoelectricity were not studied. Different structures,

such as porous scaffolds [30,172], nanoparticles [183–186], layered structures [54] and dense discs, that have fabricated and used for tissue engineering application are shown

schematically in **Figure 6 (b)**. Different studies in which PMCs and CMCs have been

assessed for their osteogenic capability have been summarized in **Table 6**.

Table 6 Summary of key findings of studies involving use of CMCs. (#Dynamic represents that piezoelectric composites were stimulated electrically.)

Material	Fabrication/ processing technique	Cell type	Loading regime	Key findings	Ref.
HA-BT	Dense disc shaped samples using conventional sintering	Osteoblast cells <i>In vitro</i>	Dynamic	Higher number of cells were observed on 10/90(HA/BT) composites under dynamic loading	[173]
	Porous disc samples were obtained by ice templating followed by sintering and polarization	MG63 cells	Static	Increased proliferation, differentiation and adhesion of cells. No difference between piezoelectric and non-piezoelectric samples	[30,172]
	Dense disc shaped samples obtained by SPS followed by poling	Human osteoblast like cell line	Dynamic #	Increase in cell growth and proliferation	[53,170]
LNKN-HA	Laminated composite materials using tape casting followed by sintering and poling	-	Static	Higher <i>in vitro</i> bioactivity was observed for composite samples (in comparison with poled HA)	[54]
NKN-HA	Layered composites were formed by compaction, followed by SPS and poling	Human osteoblast like Saos2 cells	Static	Increased cellular proliferation with culture duration.	[67]

5. Biological performance of piezoelectric materials

Piezoelectric materials are capable of modulating cellular behaviour through surface charges generated in response to deformation occurring through cellular interaction, vibration

stimulus or both. Steady state bioelectric potentials, which appear in the bone in a non-stressed state, and injury potentials of the bone arising due to a fracture play a key role in modulation of bone growth, remodelling and regeneration [204–210]. These electrical signals have an effect on cellular processes which can also be controlled through external stimulation devices [205,211–213]. Endogenous potentials up to 100 mV have been observed in non-stressed bone while, under stress, streaming potential values in the range of 0.5 to 3 $\mu\text{V}/\mu\epsilon$ were observed depending on the frequency of load applied [204,214]. PVDF and its copolymers have been displayed to mimic these potentials and show an improved osteogenic response both *in-vitro* and *in-vivo* [11,140]. The electric potentials in fractured bone have been shown to be ~200-250 mV which suggests that cellular response can be further improved if piezoelectric scaffolds can generate similar potentials under physiological levels of deformations [208].

Piezoelectricity of the scaffolds is only activated under dynamic conditions. Different studies in which the effect of mechanical stimulation on cell lines for osteogenic application have been studied are reviewed in [215,216]. A frequency of 0-5 Hz and deformation of up to 25% has been studied and shown to effect proliferation and differentiation capacities of different cell types [215,216]. Several mechanisms have been associated with mechanical stimulation of cells depending on the cell type and the type of regime used [215–217], however, a direct comparison is hard to establish due to variability in experimental parameters. It has recently been shown that cellular behaviour is also modulated by the activation of piezo receptors on mechanical deformation [218,219].

The use of piezoelectric and electrically conductive materials for delivering electrical stimulation to desired regions ensure controlled and efficient delivery of these important cues [220–222]. The use of conductive polymers for this purpose requires an external stimulatory device, which is not the case with piezoelectric materials. Electrical stimulation to control

cellular behaviour also requires optimization of various parameters including the frequency, amplitude, duration and nature (alternating or direct) of the signal [8,223,224]. To the author's knowledge, there has been no mention in previous studies as to how or if these parameters can be controlled through the mechanical stimulation of piezoelectric materials. Direct electrical stimulation has been shown to affect cells by regulating the voltage-gated calcium channels [8,222]. A similar mechanism has been suggested for piezoelectric scaffolds which promoted osteogenic differentiation of mesenchymal stem cells [140].

Figure 7 a) and b) show images of piezoelectric scaffolds used in this study and immunohistochemical staining for collagen type I after 28 days of osteogenic differentiation in dynamic conditions respectively [140]. Additionally, piezoelectric materials can alter the adhesion of proteins which has a direct effect on cellular response [66,92,140]. The cellular behaviour can be altered due to the varying charge distribution (electrical double layer) around the surface of scaffolds, or due to electric fields and currents which are set up in a localized manner [45]. It has also been shown that in case of neuronal, cells calcium ionic pathways play key role in controlling the differentiation of cells exposed to piezoelectric stimulation [225]. To gain a better understanding of the interactions and provide more quantitative assessment of the processes involved, it has also been highlighted that modelling and simulation of piezoelectric materials can be a helpful tool [226].

Figure 7 a) Image of piezoelectric scaffolds, approximately 3 mm thick. Scale bar = 6 mm (i). Scanning electron microscope (SEM) images of as-spun PVDF-TrFE (ii), annealed PVDF-TrFE (iii) and PCL (iv) at 2000 $\times$ magnification; b) Representative gross images and histological evaluation of scaffolds after 28 days undergoing osteogenic differentiation in dynamic conditions. Images of as-spun PVDF-TrFE (i), annealed PVDF-TrFE (ii) and PCL (iii) scaffolds. Immunohistochemical staining for collagen type I of as-spun PVDF-TrFE (vi), annealed PVDF-TrFE (v) and PCL (vi) scaffolds. (Scale bars: A-C, 6 mm; D-F, 100 μ m); c) 3D printed piezoelectric microstructures including a dot array (i), square arrays (ii and iii) and honeycomb array (iv). Printed with permission from [140,227]

. Using piezoelectric materials with controlled deformation offers another advantage as the cells are not only under the influence of an electrical stimulus but also experience different levels of mechanical strain the scaffold is subjected to and therefore co-stimulation of cells can be achieved and optimized for synergistic performance. However, this research is still in an early phase and there is a need to conduct deeper investigation to understand the mechanisms associated with biological performance of these materials.

6. Emerging piezoelectric materials and strategies

Piezoelectric materials have emerged as a class of smart materials which have a myriad of applications ranging from tissue engineering, drug delivery to sensors [218]. The emergence of these materials and their wide usage has motivated researchers to adopt new and

innovative approaches such as solution blow spinning (SBS), and 3D printing to make suitable structures with desired properties. These have been discussed in the sections below.

The magnetoelectric effect has recently gained interest of the tissue engineering community as it follows the same principle as piezoelectricity, the stimulating force being a magnetic field [228]. The magnetic field induces deformation which leads to generation of electric polarization which has been shown to effect cellular proliferation of MC3T3E1 cells [228]. These materials have been shown to be effective in different applications such as drug delivery, tissue engineering and targeted cell manipulation [228–236].

6.1. 3D printing of piezoelectric materials

3D printed BT [237–239], modified KNN [240], PVDF [241,242] and PLLA [243] have recently been fabricated for piezoelectric sensors and functional scaffolds which have limitedly been researched for tissue engineering applications. There are however challenges in processing and formulation of the raw material for printing and inducing piezoelectricity. Use of binders and flow additives is essential to optimize the rheological properties for printing. These parameters greatly depend on the type of material combinations desired and control over shape and size of scaffolds is difficult to establish. Schult *et al* fabricated 3D printed cylindrical green parts (11.7 mm diameter and 3.51 mm height) for making BT scaffolds [238]. It was observed that the green bodies formed were unstable and sintering lead to substantial shrinkage. In another study concerning piezoelectric ceramics, Yayun Li fabricated KNN based 3D ceramic structures using direct ink writing method and observed piezoelectric and ferroelectric behaviour in the complex structured samples [240]. The solid content of the ink was adjusted to optimize viscosity for processing. The method involved preparation of a suitable ink/suspension used with a direct ink writing equipment to fabricate structures that were subsequently sintered and poled using thermal poling (in silicone oil) [240]. This method was suggested as an alternative to using dies or lithographic masks to

make complex structures. Lithography can be used to fabricate dies, masks and masters with complex structures over which the desired material can be deposited and moulded [244]. In an approach based on soft lithography, micro structured PVDF scaffolds were fabricated and tested for cytocompatibility using human osteosarcoma cells [244]. Preliminary examination of piezoelectric effects showed their potential as mechanically active tissue engineering scaffolds and bio sensors [244]. Two photon lithography has also been utilized to fabricate 3D nanocomposite scaffolds which promoted differentiation of human osteoblast like cell line [245]. The piezoelectric charge constant was measured using PFM to a value of ~ 0.57 pm/V which is similar to that of bone [245]. Although there are challenges in formulation and processing there is huge scope for 3D printed piezoelectric materials and patient specific scaffolds. Piezoelectric microstructures printed using modified BT particles are shown in **Figure 7 b)** [227].

6.2. Piezoelectric nanofibre fabrication

Nano fibrous materials have gained interest of the tissue engineering community as they provide a 3D network with high surface area for cells to adhere and interact with. SBS has recently emerged as a technique to fabricate fibrous scaffolds for this application [246]. SBS does not require electric fields and therefore electrically active materials can be formed without risk of shorting, it also requires simple equipment and is amenable to scaling [247,248]. In a recent study, Bolbasov *et al* highlighted the importance of controlling the amount of piezoelectric β - phase in PVDF-TeFE in order to obtain a better cellular response when compared to electrospun scaffolds [248]. Electrospinning (ES) and SBS have been used to fabricate ceramic or composite fibre using piezoelectric materials [249–253]. Isakov *et al* fabricated aligned BT nanofibres using ES and characterized their ferroelectric and piezoelectric properties [253]. A sol- gel precursor solution was the precursor used in the ES

setup and the fibres obtained were calcined. Raman spectroscopy and PFM was used to identify the presence of ferroelectric phases [253]. In another study, SBS was used by Cena *et al* to fabricate lithium niobate (LN) microfibres from sol-gel precursors [251]. The solution used for spinning microfibres was a mixture of poly (vinyl pyrrolidone) (PVP), isopropyl alcohol, water and LN solution (prepared by dissolving lithium carbonate and ammonium niobium oxalate in deionized water) [251]. Different electrical measurements such as impedance, modulus and conductivity were carried out after making pellets from fibres. However, no piezoelectric or ferroelectric measurements were carried out in this study [251]. A recent study by Holopainen *et al* discusses the fabrication HA fibres using electroblowing which involves spinning of fibres by gas assisted ES, a combination of SBS and ES, which can enable smaller diameter fibres to be produced than SBS at faster rates than ES [254]. The fibres soaked in simulated body fluid displayed rapid and homogenous formation of apatite layer suggesting high bioactivity of the fibres synthesized [254].

Apart from the materials discussed above, piezoelectric materials such as diphenylalanine based peptide nanotubes (FFPNTs), liquid crystal elastomers (LCEs) and boron nitride nanotubes (BNNTs) have been explored little for tissue engineering applications [95,255–261]. Silk [52,262,263], PHB [41] and its copolymers have also been manufactured to display piezoelectric properties, yet their piezoelectric behaviour has not been explored in context of bone tissue engineering to the author's knowledge [264]. BNNTs have already shown promising results for application in the field of bone tissue engineering. Danti *et al* incorporated BNNTs in primary human osteoblast cells and explored the effect of piezoelectricity by stimulating the samples using low frequency ultrasound [265]. It was observed that mineralization and expression TGF- β 1 became significant in the cells with BNNTs that were stimulated by ultrasound. The mechanism responsible was assumed to be the electrical cues that were generated by BNNTs due to the piezoelectric effect. Xia li *et al*

culture rat bone marrow derived mesenchymal stem cells on BNNTs layer to assess the compatibility and osteogenic differentiation capacity of the material [256]. The experiments were conducted in static environment and the effect of piezoelectricity was not discussed. BNNTs were shown to enhance proliferation and alkaline phosphatase activity of the cells. The results suggest that BNNTs might prove as useful fillers for fabricating piezoelectric scaffolds for bone regeneration. Rodriguez *et al* fabricated self-assembling peptide hydrogels by dissolving monomer of diphenylalanine (FF) modified fluorenyl-methoxycarbonyl (Fmoc) (Fmoc-FF) in dimethyl sulfoxide (DMSO), these self-assembled 3D nano structures were shown to have piezoelectric properties at nano scale [95]. PFM was used to characterize the piezoelectric response of the hydrogels [95]. The results concerning piezoelectricity of these structures are promising as piezoelectric hydrogels can find application in the field of tissue engineering but the accumulation of degradation products of these hydrogels can cause toxic effects [48,259,266].

Liquid crystal elastomers are an emerging group of materials that are of interest in responsive, morphing and piezoelectric systems; however, they can involve complex synthesis and the materials required for the same are currently expensive [260].

Furthermore, composites based on the piezoelectric component of bone, collagen (fibrillar bovine Coll type I), have also been used to fabricate materials for bone substitute [267,268].

Zanfir *et al* demonstrated that use of BT in combination of collagen and hydroxyapatite ensured that the composite scaffolds exhibit osteoconductive properties [268]. The composite scaffolds were fabricated by mixing collagen gel with mineral phase (HA/HA+BT) followed by chemically crosslinking, freezing and freeze-drying. The improved osteoconductivity was attributed to the incorporation BT nanopowder in the composites [268]. However, the study did not include any polarization treatment of scaffolds to induce piezoelectricity, which might further add to the functionality of the scaffolds and improve cellular response.

A major limitation of the existing studies is the absence of controlled mechanical stimulation applied to piezoelectric scaffolds. Different systems such as four point bending, flexcell (a device used for applying mechanical deformation to materials in a controlled manner), ultrasound have been utilized over the years to study the effect of mechanical stimulation on different cell types [216,269–271]. These systems can be adopted to stimulate cells on piezoelectric substrates to ensure that mechanical as well as electrical cues are delivered to the cells. However, various parameters associated with the system, such as magnitude, nature (e.g. cyclic loading, sine wave), and rate of strain might have to be controlled to obtain a synergistic response. An extensive analysis is still required to test the feasibility of piezoelectric materials as ideal substitutes for clinical application. Piezoelectric materials promise great future potential for tissue engineering applications.

7. Concluding remarks

Piezoelectric materials are gaining significant interest in the tissue engineering community owing to their capability to mimic *in vivo* micro-environment under static as well as dynamic conditions. The 3D printing and fibre spinning of piezoelectric materials/composites and their scaffolds are promising areas that have been little explored for bone tissue engineering applications. Promising results obtained with ceramics are far from a clinical application because of the absence of compelling explanations to the variation in responses observed. It is of utmost importance to apply consistent characterization tools/techniques to these materials.

The existing research in the field focusses on fabricating piezoelectric scaffolds with optimized value of piezoelectric ‘d’ constant and assessing cellular responses on polarized samples. It is very important to analyse the effect of the transfer of mechanical deformations of the bone to the scaffolds and subsequent generation of electrical cues on different cell

types. Piezoelectric films, fibres and rods have shown promising results, however, their clinical potential is yet to be exploited, a major limitation being the non-degradability of most of the piezoelectric materials explored to date. The use of emerging strategies like 3D printing of piezoelectric materials to build complex structures can prove very useful to design piezoelectric bioactive implants with clinical applicability. Piezoelectric characteristics should be quantified using PFM followed by cellular studies on mechanically stimulated scaffolds. Use of PFM will ensure that the mechanism associated with cell material interactions can be better understood at the nanoscale. The study of piezoelectricity in (protein based hydrogel)/hydrogel based systems has opened a new and promising area of research. Another aspect considering clinical application of these materials is the absence of mechanical deformation in the early stages following implantation of the scaffolds. The results of *in vitro* and *in vivo* studies are appealing and clearly suggest that piezoelectric materials have the potential for the fabrication of new and exciting smart scaffolds for bone tissue engineering, as well as other tissues.

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