

Piezoelectric Polymers as Biomaterials for Tissue Engineering Applications

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Abstract

Tissue engineering often rely on scaffolds for supporting cell differentiation and growth. Novel paradigms for tissue engineering include the need of active or smart scaffolds in order to properly regenerate specific tissues. In particular, as electrical and electromechanical clues are among the most relevant ones in determining tissue functionality in tissues such as muscle and bone, among others, electroactive materials and, in particular, piezoelectric ones, show strong potential for novel tissue engineering strategies, in particular taking also into account the existence of these phenomena within some specific tissues, indicating their requirement also during tissue regeneration.

This referee reports on piezoelectric materials used for tissue engineering applications. The most used materials for tissue engineering strategies are reported together with the main achievements, challenges and future needs for research and actual therapies. This review provides thus a compilation of the most relevant results and strategies and a start point for novel research pathways in the most relevant and challenging open questions.

Keywords: tissue engineering; piezoelectric; scaffold; smart materials; polymers

1. Introduction

Metals, alloys and ceramic materials are being replaced by polymers in different application areas including aerospace and automotive industries, electronics, sensors, actuators and tissue and biomedical engineering. Different processing techniques have been developed for the production of polymers with tailored properties, including electrical, mechanical, thermal, chemical and surface properties, among others, addressing specific applications demands [1-2].

Polymers present attractive properties when compared to inorganic materials. They are light weight, inexpensive, mechanically and electrically tough, they show excellent compatibility with other organic and inorganic materials for the development of multifunctional hybrid systems, and some of them are biodegradable and/or biocompatible [3-5].

The increasing advances in materials science and engineering is allowing the improvement and optimization of the so-called *smart materials* and, in particular, smart polymer materials, for a larger number of application areas [6-10].

Smart materials are materials with reproducible, significant and stable variations of at least one property when subjected to external stimuli. *Smart materials* are typically classified according to the output response and include piezoelectric materials, materials that develop a voltage when a mechanical stress is applied or vice-versa; shape memory materials, in which a large deformation can be induced and recovered by temperature or stress variations; temperature responsive polymers, magnetostrictive materials, pH sensitive materials, self-healing materials, thermoelectric materials and conductive polymers, among others [11-13]. These materials are also generally known as *active materials*.

Particularly interesting for sensor and actuator applications, are materials that undergo deformation under a specific stimuli or than provide a specific stimuli under mechanical force and/or deformation. Depending on the transduction mechanism, they can be broadly classified

as non-electrically deformable polymers (actuated by non-electric stimulus such as pH, light and temperature, for example) and electroactive polymers (EAP) when the transduction mechanism involves electro-mechanical coupling. The later are further classified as dielectric EAP, which electromechanical response is dominated by electrostatic forces and ionic EAP which actuation mechanism involves the diffusion of ions [14-15]. Electrically conductive polymers are another class of electrically active materials that is attracting increased attention as they show simultaneously high conductivity and the physico-chemical properties of polymers [16-18].

In the last decades, a variety of natural and synthetic materials with various molecular designs emerged as potential biomaterials for tissue and biomedical engineering [19]. Natural materials are attractive for biomedical and related applications as they are obtained from natural sources, exhibiting similar properties to the tissue they are replacing, many of them containing specific cues for cell adhesion and proliferation and allowing cell infiltration [20]. On the other hand, polymers from natural origin are often difficult to process and show poor mechanical and electrical properties [21]. In this way, a variety of synthetic polymers such as poly(lactic acid) (PLA) [22-23], poly(glycolic acid) (PGA) [24-25], poly(lactic-co-glycolic acid) (PLGA) [26-27], poly(ethylene glycol) (PEG) [28-29], and polycaprolactone (PCL) [30] have been widely used to produce materials/scaffolds for tissue engineering [31].

Although an extensive list of polymer has been studied regarding tissue engineering applications, most of the developed scaffolds have been used in a passive way, just as support for the cells and tissues [32]. Nevertheless, it was verified that for some specific cells and tissues, the active behavior of the material used for the scaffold development can be taken to advantage, providing even the necessary stimuli for proper tissue regeneration. This fact gave rise to the strong increase of the development of smart materials for tissue engineering applications [33].

Being electrical signals one of the main physical stimuli present in the human body and, in particular, the electromechanical signals, this review is devoted to summarize the research efforts, main conclusions, main challenges and needs as well as the strong potential of developing electroactive scaffolds based on piezoelectric polymers for specific tissue engineering applications.

In a piezoelectric material (**Figure 1**), an electrical response due to mechanical excitation or *vice versa* can be observed. In these types of materials a certain anisotropy in its structure is required. In synthetic polymers that are in noncrystalline or semicrystalline form and are originally isotropic, they are typically subjected to a poling procedure (such as corona) to meet this requirement [34]. The most common way to describe the piezoelectric effect is by the so-called direct effect, where the piezoelectric d_{ij} coefficient is given by (**Equation 1**).

$$d_{ij} = \left(\frac{\partial D_i}{\partial X_j} \right)^E = \left(\frac{\partial x_i}{\partial E_j} \right)^X \quad (1)$$

where D is the electric induction; E is the electric field strength; X is the mechanical stress; and x is the strain [35]. In this sense, it is possible observe that the piezoelectricity is the relation between the electrical variables (D and E) and the mechanical parameters (X and x).

With respect to the so-called inverse piezoelectric effect, the e_{ij} coefficient is obtained by (**Equation 2**).

$$e_{ij} = \left(\frac{\partial D_i}{\partial x_j} \right)^E = - \left(\frac{\partial X_i}{\partial E_j} \right)^x \quad (2)$$

The direct piezoelectric effect (d_{ij}) concerns the conversion of the mechanical energy into electrical energy while the inverse piezoelectric effect (e_{ij}) describes the conversion of electrical energy into the mechanical energy.

2. Electrical cues in human body

Many of the major functions in cells and organs of the human body are controlled by electrical signals. As early as in the 18th century it is described the use of electrostatic charge

for skin lesion treatment [36] and in 1983, electrical potentials ranging between 10 and 60 mV depending on the human body location were measured [37].

Electric fields and potentials induce distinct effects on cells and it has been proven that small applied electric fields can guide a variety of different cell types to move and migrate directionally such as corneal, epidermal and epithelial cells [38-41]; can modulate the phenotypes of vascular endothelial cells [42]; can regenerate nerve fibers [43] and are widely used in orthopedic practices, showing the improvement of ligament healing *in vivo* [44].

2.1. Piezoelectricity in human body

Extensive and classic studies of the piezoelectric properties of bone and other biological materials have been also reported. The piezoelectricity can be referred as an extended property of living tissue, playing a significant role in several physiological phenomena [45]. Piezoelectricity can be thus found in different parts of the human body (**Figure 2**) such as bone, tendon, ligaments, cartilage, skin, dentin, collagen, deoxyribonucleic acids (DNA) and conceivably, in cell membranes [45-50].

2.1.1. Bone

Bone is a dynamic tissue in constant adaptation and remodeling through complex feedback mechanisms, involving electromechanical processes, due to its piezoelectric characteristics. Due to its piezoelectric nature, bone is the paradigm for piezoelectric electromechanical effect in human tissue [51].

The first study reporting the piezoelectric properties of the bone was in 1955 [52]. Few years later, electric currents in bone and the generation of electric potentials when the bone is mechanically stressed were verified [53-54]. This phenomenon, recognized as piezoelectricity, is independent of the cell viability. The mechanical stress produces electrical signals and these signals represent the stimulus that promotes bone growth and remodeling according to the

Wolff's law [55]. The biomechanical properties of bone, in particular its piezoelectric activity, have been addressed microscopically [56] and macroscopically, with models using finite element analysis [57]. Further, it has been also hypothesized a mechanism by which the piezoelectric signals can regulate the bone growth [58]. At the cellular level, the bone cell type that plays an important role in the bone structure development and appears to be involved in bone mechanotransduction, the osteocytes, was identified [59]. Consequently, for bone regeneration, these cells communicate with other bone cells, such as osteoblasts and osteoclasts. The influence of electrical stimulation on bone healing has been studied *in vitro* [60-66] and *in vivo* [67-72] and it has been demonstrated that the application of these stimulus can enhance and stimulate osteogenic activities. In this way, the osteoblasts are affected by electromechanical signals to apposite bone tissue [73-74], the piezoelectric nature of bone, leading to natural conversion of the mechanical stimuli into electrical ones.

2.1.2. Collagen and other piezoelectric tissues

Due to their collagenous structure, tendons and ligaments also exhibit piezoelectricity, giving rise, therefore, to an electrical potential variation when a mechanical stress is applied [75-76]. The piezoelectricity of dry tendons was measured [77], as well as the electrical potentials generated in hydrated tendon [78-79], the piezoelectric coefficient decreasing with increasing hydration [80].

Piezoelectric effect has been also observed in different soft tissues, such as skin, callus, cartilage and tendons, as well as in hard ones, such as bone, and appears to be associated with the presence of oriented fibrous proteins [45, 81]. All connective tissue contains one or more types of fibrous molecules such as collagen, keratin, fibrin, elastin, reticulum or cellulose structure, showing also piezoelectric properties [45].

It seems evident from the literature that the piezoelectric effect can be attributed to the main organic constituent of tissue, which is collagen in the case of the bone and tendons [82-83].

Thus, it has been shown that the crystalline unit of collagen is polar hexagonal (C_6) [56], showing piezoelectric properties. Further, as previously indicated, it has been shown that dry bone is piezoelectric, i.e., a mechanical stress induces a polarization (direct effect) and an application of an electric field produces a change in the material geometry or strain (converse effect). It was reported that for dry fibers, the polarization results from the displacement of the hydrogen bonds formed in the polypeptide chains of the collagen crystals. Other studies confirmed such findings. Thus, in [84] the piezoelectric and pyroelectric behavior of collagen were measured independently from the bone, confirming that the electroactive properties arise from the structure of collagen molecules. It was suggested that the crystalline structure of collagen changed under wet conditions and that the bound water promotes a change its crystal symmetry to the point where no piezoelectric properties were observed [78, 85]. A certain minimum amount of water concentration, which increases the crystal symmetry, is nevertheless required to maintain the overall structural integrity. Further, it was also suggested that, due to the variability of the electroactive behavior of collagen in wet and dry states, wet bone shows different piezoelectric symmetry relation [78, 85]. More recently, studies of the piezoelectric response of human bone using a piezoresponse force microscope, in order to measure this effect at nanometer scale resolution directly in the collagen matrix, resulted in the quantification of the piezoelectric response in $7 - 8 \text{ pC.N}^{-1}$ [86].

With respect to other biological tissues, the electrical polarization variations were also verified in hair when subjected to stress [87] as well as in DNA [75]. Finally, investigations in the calcifications commonly found in human pineal gland tissues resulted in the determination that the pineal gland contains non centrosymmetric material which, according to crystallographic symmetry considerations, is also piezoelectric [88].

3. Electrically active materials for tissue engineering

Advances in the understanding of electrical properties of tissues and cells are increasingly attracting attention to this area of research. Living cells show many properties of electrical systems, *i.e.*, they generate electromotive force, regulate the potential difference whenever needed, use varying resistances in series or in parallel, switch *on* and *off*, control and rectify current flow and store charge [89-90]. An electric voltage exists across the plasma membrane, while the inside of the cell remains more negative than the outside. By convention, the potential outside the cell is called zero; therefore, the typical value of the membrane potential is in the range of -60 to -100 mV [91].

Thus, conductive polymers have been applied in tissue engineering applications. One of the most studied conductive polymers for tissue and biomedical engineering is polypyrrole (PPy) that has been proven to be a promising substrate for cell growth and proliferation, in particular for axon growth *in vitro* and *in vivo* experiments [90, 92]. Studies reveal that the application of an external electrical stimulus to the material, and consequently to the cell, enhances axons outgrowth to levels beyond the ones obtained for cultures on non-conducting polymers [92-93]. One of the major drawbacks of the used conductive polymers for *in vivo* applications is their inherent inability to biodegradation, which may induce chronic inflammation and require surgical removal [94]. In order to solve this issue, attempts to blend them with suitable biodegradable polymers have been carried out. Thus, nerve guidance channels (NGCs) were fabricated from an electrically conductive, biodegradable polymer of PPy and poly(D,L-lactide-co-epsilon-caprolactone) (PDLLA/CL) [92]. Further, the influence of the applied current intensity in the neurite outgrowth was evaluated and it was found that a current intensity of 1.7–8.4 $\mu\text{A}/\text{cm}$ leads to the largest enhancement of neurite outgrowth on conductive PDLLA/CL and PPy surfaces.

Polyaniline (PANI) is the oxidative product of aniline under acidic conditions and is commonly known as aniline black [95] and the ability of PANI and PANI variants to support cell growth has been evidenced [96], independently of the oxidation state [97]. In this way,

adhesion and proliferation of cardiac myoblasts (H9c2) on conductive PANI substrates have been reported [98]. Both non-conductive emeraldine base and conductive salts forms of PANI were found to be biocompatible and to support cell attachment and proliferation, which attracted much attention of this material for tissue and biomedical applications. Further, there are other electrically conductive polymers that are being studied for tissue and biomedical engineering such as poly(3, 4-ethylenedioxythiophene) (PEDOT) for cochlear implants, vision prosthesis, neural regeneration devices and neural recording electrodes [99].

Carbon nanotubes (CNTs) are other group of conducting fillers incorporated into non-conductive polymers to provide structural reinforcement and electrical conductivity into the scaffolds and to direct cell growth [100]. Some studies indicated that CNTs are cytotoxic, while others revealed that carbon nanotubes are excellent substrates for cellular growth [100]. As a result of these studies, it is claimed that when used in suspension, CNTs seems to be toxic to cells, while they appear to be non-toxic if immobilized into a specific polymer matrix or culture dish [101].

Table 1 summarizes some relevant experimental conditions and cells used for electrical stimulation based on electrically conductive polymers. These materials have been particularly explored for neural development and it was verified that the application of electric fields influence the rate and orientation [43] and also the extension and direction [102] of the neurite outgrowth of cultured neurons *in vitro*.

Common to the aforementioned materials is the need of an external power supply in order to induce electrical signals and thus to promote electrical stimulus to the cells. This is a strong drawback for in-vivo applications and thus, it is important to develop a generation of biomaterials including combinations of biological, chemical, mechanical and electrical stimulatory cues, being the last ones without external power supply and therefore wires.

In this scope, piezoelectric polymers appear as a possibility for applying electrical signals to the cells by mechano-electrical transduction. Piezoelectric materials generate transient surface

charge variations and therefore electrical potential variations when mechanical solicitation are applied to the material and, therefore, no need for additional energy sources or electrodes [103] are requires.

4. Piezoelectric soft biomaterials and structures

General for all materials used as scaffolds, the design of these bioactive biomaterials is another important parameter to consider and a suitable morphology, in combination to the piezoelectric characteristics, has to be optimized for proper cell response.

Many processing methods have been developed to process biomaterials into scaffolds with different dimensionalities and morphologies [104-105]. Different structures of biomaterials including microspheres, fibers, porous membranes, hydrogels and sponges have been designed and used in tissue engineering [19]. However, effects of internal biomaterials structures remain largely unexplored and the comparison of cell response in the different structures types remains elusive. In particular, just a few scaffold morphologies have been used for piezoelectric tissue engineering including films, membranes and fibers, among others. Some structures of β -poly(vinylidene fluoride) (PVDF) are shown in **Figure 3**.

Porous scaffolds have been obtained by solvent casting/salt leaching, phase separation, gas foaming, gel casting, precipitation and emulsion freeze-drying [104-105]. The main drawbacks of these methods are associated with the possibility to obtain scaffolds with an inaccurate and limited interconnectivity pore morphology that is disadvantageous for uniform cell seeding and tissue growth [31, 106]. This major drawbacks can be overcome by fibrous scaffolds produced by electrospinning, a method that offers the ability of control the pore interconnectivity and moreover the internal and external morphology of fibers by controlling processing parameters such as applied voltage, solution viscosity and conductivity, among others [106]. Moreover, electrospinning allows the production of scaffolds with small pore size, density and high surface area [105-106]. The pores of the scaffolds should be large

enough to allow cell migration, where they eventually become bound to the ligands within the scaffold. Therefore, for any scaffold, a critical range of pore sizes exists, which may vary depending on the cell type and tissue being engineered [107]. The fiber diameter of the scaffolds produced by electrospinning can range from 5 nm to a few microns [106]. A similar method to electrospinning is electrospray, which allows the preparation of polymeric micro- and nanoparticles that can also be used as support for cell expansion and differentiation [108-109]. Other methods can be used for particle formation however this method might overcome some of the drawbacks associated with conventional microparticle-producing methods, such as solvent casting, single and double emulsion, spray-drying, porous glass membrane emulsification and coacervation [108].

More recently, rapid prototyping (RP) technologies also known as solid free-form fabrication (SFF) allows translating computer data files such as computer-aided design (CAD), computed tomography (CT), magnetic resonance imaging (MRI) and convert the digital information through layered manufacturing SFF machines into a 3D scaffold [105-106, 110]. Three-dimensional printing (3DP), fused deposition modeling (FDM), stereolithography apparatus (SLA) and selective laser sintering (SLS) are widely been applied in the fabrication of materials with unique geometries with controllable pore architecture which could not obtained by conventional methods [106, 108]. Various biomaterials are commonly used in RP technologies such as PEG, PLGA, PCL, collagen, starch, HA and TCP [104, 111-112]. However, to the best of our knowledge few studies report the production of piezoelectric scaffolds by these methods, being stereolithography the most commonly used method for the fabrication of piezoelectric scaffolds based on PLLA [112].

These scaffold structures have to be achieved with the few natural and synthetic materials exhibiting piezoelectric properties, the most relevant ones, with the respective piezoelectric properties being reported in **Table 2** and **3**, respectively.

However, to demonstrate the suitability and effect of the piezoelectric properties for tissue engineering applications, synthetic polymers have been mostly chosen instead the natural ones for their use as scaffolds. These have known compositions and can be designed to minimize immune response. They can be tailored to produce a wide range of scaffold geometries and hybrid structures by combining polymers with other organic or inorganic hybrid structures.

Among all polymers, PVDF [113] and vinylidene fluoride (VDF) [114] copolymers, are the synthetic, semi-crystalline polymers with the highest electroactive properties, including piezoelectric, pyroelectric and ferroelectric properties [114].

As previously mentioned, it is possible to find electrical activity and even piezoelectricity in many parts of the human body. For that reason, it seems be advantageous to employ biomaterials based on piezoelectric properties for active tissue engineering of specific tissues.

5. Tissue engineering based on piezoelectric polymers

Studies of the use of piezoelectric polymers for tissue engineering applications are mostly devoted to bone, neural and muscle regeneration.

Table 4 summarizes the main works using piezoelectric polymers, the intended applications and scaffold morphology, together with the cultivated cells.

Dynamic assays were performed in the studies marked with * contrary to the others where only static assays were carried out. It is to notice that when no dynamic conditions are used, the suitability of the piezoelectric effect is not proven, but just the suitability of the material and the relevance of the (positive or negative) surface charge, when the material is poled.

It is to notice that the most used polymer is PVDF and its co-polymers as, due to its larger piezoelectric response, serve as an ideal material platform for proving the concept of mechano-electrical transductions for tissue engineering. Also several sample morphologies have been used, such as films, fibers, porous membranes and 3D porous scaffolds for different

applications in tissue engineering, mainly for bone, muscle and nerve regeneration. With the challenge to mimic the architecture of these tissues, the fibers have proved to be one of the favorite choices and for most of the studies mesenchymal stem cells have been chosen. For bone tissue engineering applications, PVDF fibers were produced and its effect on biological function was studied with hMSCs [128]. It was verified that the cells attach to the PVDF fibers and present a larger alkaline phosphatase activity and early mineralization when compared with the control, showing the potential for the use of PVDF scaffolds for bone tissue engineering applications. The same cells were also used with PLLA fibers to study their biocompatibility and suitability for bone differentiation and the same results were obtained [132]. Regarding nerve regeneration, fibers were also used and it was verified that the cells attach and the neurites extend radially on the random aligned fibers, whereas the aligned fibers directed the neurite outgrowth, demonstrating their potential for neural tissue engineering [150-151].

On the other hand, despite the demonstrated potential, there is still just a few conclusive works addressing the effect of the electrical stimulus promoted by the piezoelectric response of the materials, as for these studies, specific dynamical mechanical stimulus should be applied during cell culture, for example, by the use of mechanical bioreactors. The applied mechanical stimulus can be vibration, compression or stretching of the piezoelectric scaffold. In this scope, piezoelectric materials based on PVDF films, have been used to study the effect of mechanical stimulation of bone cells, by converse piezoelectric effect. On a substrates submitted to dynamic mechanical conditions, the stimulation was achieved with an alternating sinusoidal current (AC) of 5 V at 1 and 3 Hz for 15 min at each frequency. It was verified that mechanical stimulation of bone induces new bone formation *in vivo* and increases the metabolic activity and gene expression of osteoblasts in culture [122]. The influence of the same piezoelectric substrate, PVDF film, on the bone response cultivated under static and dynamic conditions was also investigated [124]. The dynamic culture was performed on a

home-made bioreactor system with mechanical stimulation by placing the culture plate on a vertical vibration module at a frequency of 1 Hz with amplitude of ~1 mm. The results showed that the surface charge under mechanical stimulation improves the osteoblast growth and consequently, that electroactive membranes and scaffolds can provide the necessary electrical stimuli for the growth and proliferation of electrically responsive tissue and in particular of tissues which also show piezoelectric response, such as bone. The same dynamic culture was used to enhanced osteogenic differentiation of human adipose stem cells, proving that dynamic mechanical stimulus in combination with suitable osteogenic differentiation media can offer tools to better mimick the conditions found *in vivo* [127]. Moreover, piezoelectric (PVDF) has been used as actuator in *in vivo* assays for orthopedic applications [123]. After one month implantation it was verified that the converse piezoelectric effect can be used to stimulate the bone growth at the bone implant interface.

Concerning nerve regeneration, neurons were cultured directly on electrically charged PVDF polymer growth substrates to determine if local electrical charges enhance nerve fiber outgrowth *in vitro* [43, 145]. Piezoelectric PVDF substrates generated 2-3 mV at 1200 Hz when placed on standard incubator shelves and it was concluded that the enhanced outgrowth process was induced effectively by the piezoelectric output of the films.

The study of the piezoelectric effect of polyurethane/PVDF (PU/PVDF) fibers was also investigated for wound healing applications [166]. For this, the scaffolds were subjected to intermittent deformation of 8% at a frequency of 0.5 Hz for 24 h and the results indicated that piezoelectric-excited scaffolds showed enhanced migration, adhesion and secretion, leading to more rapid wound healing than those on the control scaffolds. *In vivo* assays were also performed with the implantation of these scaffolds in rats and a higher fibrosis level was verified due to the piezoelectric stimulation, which was caused by random animal movements leading to the mechanical deformation of the scaffolds.

So, it is possible to conclude that piezoelectric materials would be useful in many tissue engineering applications. In *in vitro* assay, the mechanical deformation can be made by the use of bioreactors and in *in vivo* assay, the stimulation was made by movements of the patient which will cause the mechanical deformation of the scaffolds (Figure 1). Further, since the piezoelectric effect can be found in several human body tissues, as previously mentioned, it is natural to assume that piezoelectric scaffolds will be needed, at least, for the proper regeneration of those tissues.

Up to now, the most commonly used polymer to study the piezoelectric effect in tissue engineering applications was the PVDF, due to its larger piezoelectric response. This polymer is non-degradable, which can be a limitation for applications including scaffold implantation. On the other hand, it can be successfully used as scaffold for cells stimulation before implantation, due to its large piezoelectric response and physico-chemical stability.

6. Final remarks, conclusions and main challenges

Tissue engineering has emerged as an alternative to conventional methods for tissue repair and regeneration, but different strategies can be chosen; as represented in **Figure 4, left**. Basically, it consists in choosing appropriate cells, materials and biochemical and physical signals to repair, maintain or regenerate the tissue function. The cells can be harvested directly from the patient or stem cells can be used to be combined with a biomaterial scaffold to be grown *in vitro* without (route B of figure 4, left) or with (route C of figure 4 left) signals and then implanted. It should be also noted that the bioreactor use in tissue engineering is an attempt to simulate an *in vivo* physiological environment. The scaffolds can also be implanted directly to facilitate cell regeneration *in vivo* (route A of figure 4, left).

Within this general strategy, it seems evident the need of a combination of physical and biochemical stimuli giving rise to the suitable environment for tissue regeneration. In particular, one of the most interesting effects to be applied in a next generation of materials

for scaffold development is the possibility of electrical stimulation, in order to promote electrical stimulus to the cells, which is essential to improve functionality of specific tissues during tissue regeneration.

A biomimetic approach also shows the specific need of piezoelectric scaffolds and supports for tissue engineering applications, related to the existence of this phenomena in the living tissue.

In particular, bone, as the paradigm of piezoelectric tissue, can undergo increased regeneration success rate by applying piezoelectric related tissue engineering strategies. Thus, **Figure 4, right** shows a promising strategy for the repair or regeneration of damaged bone. This tissue engineering therapy involves harvesting healthy cells (adult or stem cells) culturing in an appropriate scaffold for growth *in vitro* in a bioreactor which will provide the proper biochemical and physical stimulus and then implanted. The main purpose of this strategy is recreating the bone tissue environment, including biochemical and electromechanical stimulus.

It can be concluded that piezoelectric materials can be used to further explore and implement novel tissue engineering strategies. Polymer materials with suitable piezoelectric response can be tailored in terms of physicochemical properties and microstructure, leading to suitable scaffolds designs. On the other hand, their full potential has not been achieved and novel bioreactor concepts should be developed mimicking in-vivo mechanical conditions and allowing thus to explore, mediate through the mechanical solicitation of the materials, the electrical stimulation of the cells.

A new generation of studies involving bioreactors is needed in order to achieve a deep knowledge of the mechano-electro transduction effects on specific cells.

Once this is achieved, two strategies can be followed based on piezoelectric stimulation (figure 4):

- a) Bioreactor culture for pre-differentiation and cell implantation

b) Scaffold implantation

For the later, tailored piezoelectric materials with controlled biodegradation will be also needed. In any case, it can be stated already that tissue engineering strategies based on electroactive materials open a novel and rich field of research with strong potential for actual innovation to this field.

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Tables and Table Captions

Table 1: Relevant works on electrical stimulation conditions applied in tissue engineering strategies based on conductive polymers (adapted from [90]).

Conductive Scaffolds	dc/ac current; potential	Duration	Cells
PANI/PCL/gelatin	dc: 100 mV	1 h	Mouse neuronal cerebellum stem cells (C17.2)
PANI/poly(L-lactide-co- ϵ -caprolactone) (PANI/PLACL)	dc: 0 - 200 mA	48 h	NIH-3T3 fibroblasts
PPy	dc: 100 mV	2 h	Rat neuronal pheochromocytoma (PC12)
PLGA coated with PPy	10 mV cm ⁻¹	2 h	PC12
PPy/poly(L-lactic acid) (PPy/PLLA)	dc: 100 mV mm ⁻¹	2, 24 h	Human skin fibroblasts
PDLLA/CL coated with PPy	dc: 0, 2, 8 e 20 μ A mm ⁻¹ mV ⁻¹	-	PC12
PPy	ac: 50 μ A at 0,05, 5 and 500 Hz	-	Vascular smooth muscle cells (VSMC)
PPy/PLLA	dc: 50 mV mm ⁻¹	24h	Human cutaneous fibroblasts
Indium–tin oxide (ITO)	ac: 100 mV (100 Hz)	30 min/day (3 days)	PC12
PLLA/CNT	ac: 10 mA (10 Hz)	6 h/day	Osteoblasts
PCL/PPy	dc: 10 V	4 h/day	Dorsal root ganglia (DRG)
PPy	Biphasic 100 μ s pulses of 1 mA.cm ⁻² at 250 Hz	8 h/day	Cochlear neural explants
copolymer of hydroxyl-capped PLA and carboxyl-capped aniline pentamer (AP) (PLAAP)	Electric potential of 0.1 V (1 Hz)	1 h/day	PC12
PPy/Chitosan	dc: 100 mV	4 h	Schwann Cells

Table 2 – Biodegradable polymers with natural origin and corresponding main piezoelectric response (adapted from [75]).

Natural Polymers			Piezoelectric coefficient -d ₁₄ (pC/N)
<i>Polysaccharides</i>	Cellulose	wood	0.10
		ramie	0.20
	Chitin	crab shell	0.20
		lobster apodeme	1.50
Amylose	starch	2.00	
<i>Proteins</i>	Collagen	bone	0.20
		tendon	2.00
		skin	0.20
	Keratin	wool	0.10
		horn	1.80
	Fibrin	elongated films of fibrinogen-thrombin clot	0.20
Deoxyribonucleic acids salmon DNA (at -100 °C)		0.07	

Table 3 – Piezoelectric natural and synthetic polymers.

Polymer	Dielectric constant (1 kHz; 25°C)	Piezoelectric Coefficient (pC/N)	Ref.
PLA	3.0 – 4.0	9.82	[115- 116]
Polyhydroxybutyrate (PHB)	2.0 – 3.5	1.6 – 2.0	[117- 118]
PVDF	6 - 12	24 - 34	[119- 120]
Poly(vinylidene fluoride- trifluoroethylene) (PVDF- TrFE)	18	38	[120]
Polyamide-11	5	4	[121]

Table 4 – Material type, scaffold design and cells used for different applications.

Applications	Material type	Scaffold design	Cells type used	Ref.
Bone regeneration or Bone tissue engineering	PVDF and copolymer	[Films	MC3T3-E1 Goat marrow stromal cells into osteoblast Human adipose stem cells	[122-124] [125]* [126 - 127*]
		Fibers	Human mesenchymal stem cells (MSCs)	[128]
		Blends membranes (porous)	NIH3T3 mouse fibroblast	[129]
	PLLA	Films	Implementation on male cats	[130]
		Fibers	Human fetal osteoblasts (hFOB) Human mandible–derived mesenchymal stem cells (hMSCs)	[131] [132-133]
	PHB and copolymers	Films	Bone marrow cells	[134]
		Fibers	Human osteoblasts (HOB) Bone marrow cells	[135] [134]
		3D Blends membranes (porous)	MC3T3-E1	[136]
		Fibers - hydrogel D-periodic	Rat tail tendon	[137]

	Collagen		type I collagen fibrils		
			3D matrices	Human fetal osteoblastic cells (hFOB 1.19) and Bovine osteoblasts	[138]
				Human adipose-derived stem cells (ASCs)	[139]
	Composites	PVDF/starch/natural rubber (NR)	Blends membranes (porous)	NIH3T3 mouse fibroblast	[129]
		PVDF-TrFE/starch/NR	Blends membranes (porous)	NIH3T3 mouse fibroblast	[129]
		PVDF-TrFE/Barium titanate (BT)	Membranes	<i>in vivo</i> evaluation of rats	[140]
				Human alveolar bone-derived cells (Osteoblastic cells)	[141]
				Osteoblastic cells from human alveolar bone fragments	[142]
			Fibroblasts from human periodontal ligament (hPDLF) and keratinocytes (SCC9)	[143]	
	PLA/demineralized bone powders (DBP)	Fibers	hMSCs	[132]	
PLLA covered with	3D Porous Scaffold	Saos-2 osteoblast-like cells	[144]		

		bonelike apatite			
		Apatite/collagen	3D Porous Scaffold	Saos-2 osteoblast-like cells	[144]
Nerve or neural regeneration	PVDF	Films		Mouse neuroblastoma cells (Nb2a)	[43, 145]*
				Spinal cord neurons	[146]
		Blends membranes (porous)		Dense and microporous membranes: neuronal cells	[147]
	Channels/Tubes			Nerve guidance channels: <i>in vivo</i> assay: mouse sciatic nerve model.	[148]
				Tube containing nerve growth factor (NGF) and Collagen gel: <i>in vivo</i> assay: Wistar rats.	[149]
	PVDF-TrFE	Films		Poietics Normal Human Neural Progenitors	[150]
				Nb2a	[43]*
		Fibers		Dorsal root ganglion Poietics normal human neural progenitors	[151] [150]
	Tubes		In vivo implementation: rat sciatic nerves	[152]	
PLLA		3D Porous scaffold	<i>In vivo</i> implementation: Sprague	[153]	

				Dawley rats	
	Collagen		Fibers	Schwann cells	[154]
			3D gel matrices	Embryonic rat cerebral cortices	[155]
Muscle regeneration	PVDF		Films	C2C12 myoblast	[156]
			Fibers	C2C12 myoblast	[156]
			Meshes	In vivo study in rabbits	[157]
			Fibers	In vivo study in rabbits	[158]
	Composites	Au-PLLA	Fibers	primary rat muscle cells	[159]
Others applications	Cartilage	PHB	3D scaffolds	Human adipose-derived stem cells (hASCs)	[160]
	Abdominal hernia repair	PVDF	Meshes	Implanted subcutaneously in rats	[161]
					[162]
	Endothelialization	PVDF	Films	Human cell line, EA.hy 926	[42]
	Vascular surgery	PVDF	Monofilament sutures	In vivo study Adult female chinchilla rabbits	[163]
					[164]
	Spinal cord injury regeneration	PHB-co-3-hydroxyvalerate (PHB-HV)	3D scaffold by freeze-drying technique	primary culture of neurons and astrocytes from the hippocampus of P4 Wistar rats	[165]
Wound healing	PU/PVDF	Fibers	NIH 3T3	[166]	
	PPy/PLLA	Membranes	Human Skin Fibroblast	[167]	

		PVDF-TrFE	Electrospun fibers	Human skin fibroblasts	[168]
	Tissue sensors	PVDF	Microstructures	Human osteosarcoma (HOS)	[169]

Figures and Figure Captions

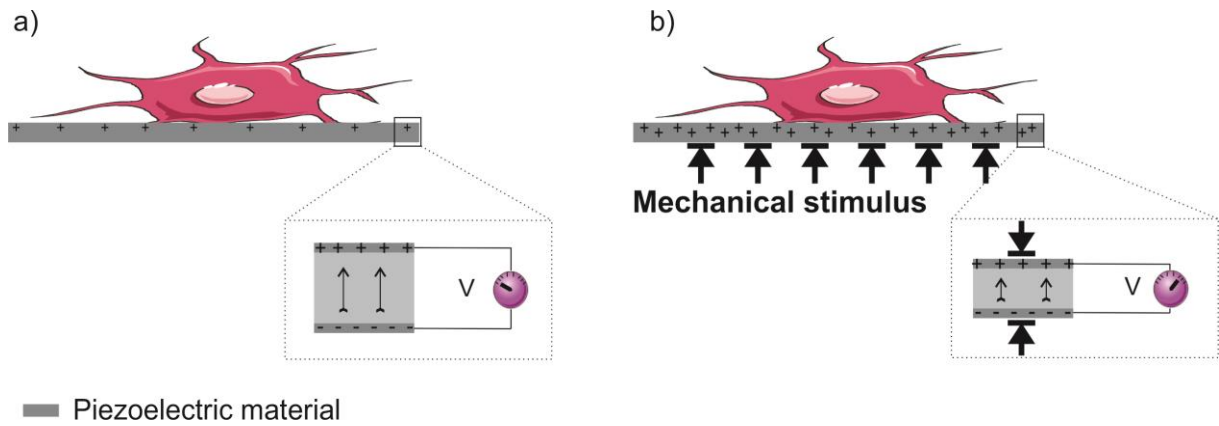


Figure 1 – Schematic representation of the piezoelectric effect (piezoelectric material representation at the bottom of the image) and corresponding cell culture on piezoelectric supports a) without and b) with mechanical stimulus, the later leading to an electrical potential variation of the materials which is, in turn, influences cell response.

Electric

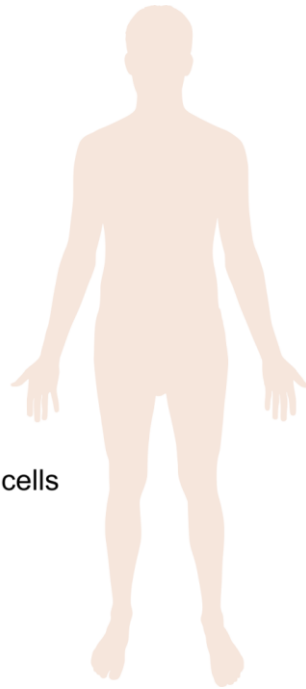
Corneal cells

Epidermal cells

Nervous system

Retinal cells

Vascular endothelial cells



Piezoelectric

Bone

Cartilage

DNA

Ligaments

Skin

Tendons

Figure 2 – Representative human body location in which electrical and piezoelectric signals are relevant.

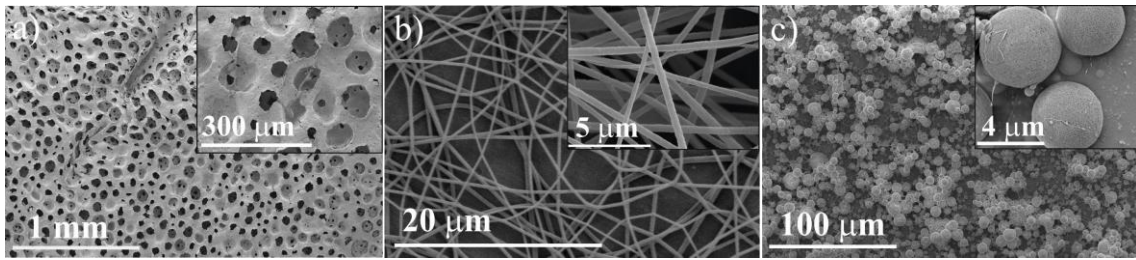


Figure 3 – β -PVDF obtained in different morphologies: a) porous membranes, b) electrospun fibers and c) microparticles.

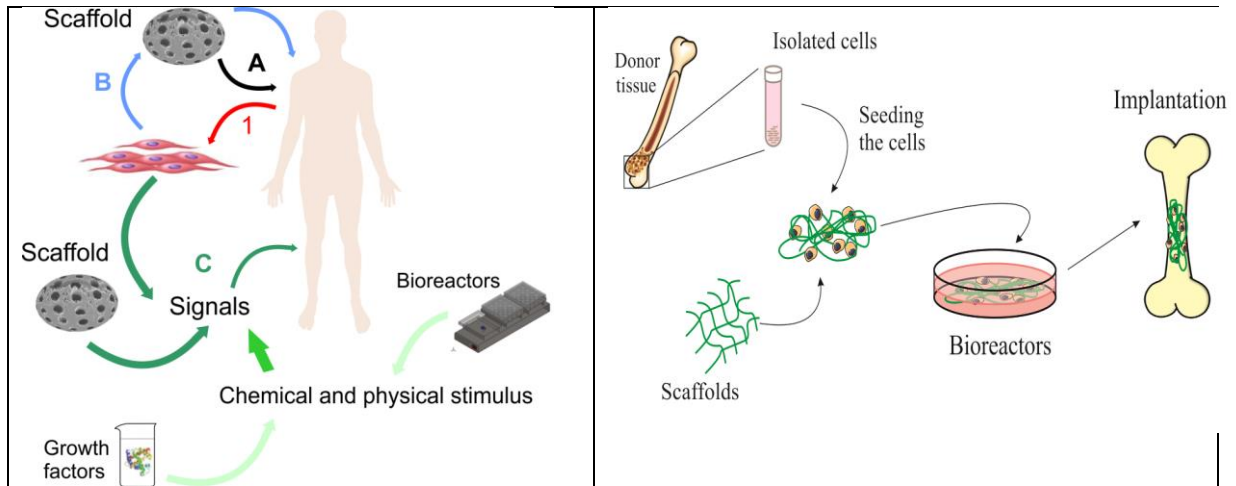


Figure 4 – Left) Schematic representation of the different strategies of the tissue engineering field: 1 - The cells can be harvested directly of the patient; A - Scaffold implanted directly; B - Cells cultured in scaffold and then implanted; C - Cells cultured in scaffold with appropriate signal, namely chemical (such as growth factors) and physical (such as mechanical using a bioreactor) and then implanted. Right) Tissue engineering strategies for bone regeneration.