Enhanced piezoresponse and surface electric potential of hybrid biodegradable polyhydroxybutyrate scaffolds functionalized with reduced graphene oxide for tissue engineering

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A B S T R A C T

Piezoelectricity is considered to be one of the key functionalities in biomaterials to boost bone tissue regeneration, however, integrating biocompatibility, biodegradability and 3D structure with pronounced piezoresponse remains a material challenge. Herein, novel hybrid biocompatible 3D scaffolds based on biodegradable poly(3-hydroxybutyrate) (PHB) and reduced graphene oxide (rGO) flakes have been developed. Nanoscale insights revealed a more homogenous distribution and superior surface potential values of PHB fibers (33 ± 29 mV) with increasing rGO content up to 1.0 wt% (314 ± 31 mV). The maximum effective piezoresponse was detected at 0.7 wt% rGO content, demonstrating 2.5 and 1.7 times higher out-of-plane and in-plane values, respectively, than that for pure PHB fibers. The rGO addition led to enhanced zigzag chain formation between paired lamellae in PHB fibers. In contrast, a further increase in rGO content reduced the α-crystal size and prevented zigzag chain conformation. A corresponding model explaining structural and molecular changes caused by rGO addition in electrospun PHB fibers is proposed. In addition, finite element analysis revealed a negligible vertical piezoresponse compared to lateral piezoresponse in uniaxially oriented PHB fibers based on α-phase (P212121 space group). Thus, the present study demonstrates promising results for the development of biodegradable hybrid 3D scaffolds with an enhanced piezoresponse for various tissue engineering applications.

1. Introduction

Bioelectricity is an integral part of living systems in which endogenous electric fields affect cellular processes such as chemotaxis, migration, proliferation, differentiation, and ion transport [1]. Living tissues, such as bone, can produce bioelectrical cues and endogenous electric fields due to their piezoelectric nature [2,3]. Piezoelectric materials convert mechanical deformation (direct piezoelectric effect) to electrical signals and vice versa (inverse piezoelectric effect) [4]. The presence of the piezoelectric nature in the bones has been associated with non-centrosymmetric collagen molecules [2,3,5]. For instance, the human tibia can produce ~300 μV of the piezopotential during walking [1]. Such piezoelectricity plays an important role in bone reconstruction by affecting cell migration, proliferation and differentiation [6]. Therefore, piezoelectric stimulation is one of the key factors in efficient bone regeneration [7], which has not been studied in detail thus far.

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Bone fractures are some of the most common injuries that can result from high-energy, such as vehicle or sports accidents, or low-energy trauma owing to osteoporosis [8,9]. For instance, over 200 million people in the world suffer from osteoporosis [10], moreover, every year, more than 8.9 million (one person every 3 s) of those people have osteoporosis fracture [11]. Therefore, the need for the development of new biomaterials and implants remains due to several factors. First, all introduced artificial materials and implants to the human body for bone regeneration should be biocompatible, osteoconductive, and osteoinductive [12]. Second, these materials and implants should have a 3D structure mimicking the extracellular matrix, which provides mechanical support of fractured bone and cell penetration, adhesion, proliferation, differentiation, and nutrition/waste diffusion [13]. For these purposes, the development of tissue engineering has introduced 3D polymer scaffolds as a promising way to repair bone defects [14]. In addition, the presence of piezoelectric properties in 3D polymer scaffolds is highly desirable [7]. Recently, the successful application of piezoelectric scaffolds based on polyvinylidene fluoride (PVDF) for bone tissue regeneration was demonstrated in vivo [15]. PVDF is a nonbiodegradable polymer [16], thereby requiring a second surgery associated with the additional risk of infections and inflammation [17,18]. To avoid the second surgery associated with the removal of implants, materials should be biodegradable for subsequent bone regeneration [19]. However, the design of biocompatible 3D polymer scaffolds that are simultaneously biodegradable and piezoelectric represents a significant challenge.

Polyhydroxyalkanoates (PHAs) are a class of natural biocompatible and biodegradable storage polymers formed by various microorganisms [20]. Among PHAs, polyhydroxybutyrate (PHB) and its copolymers are widely known due to their versatility and combination of acceptable mechanical properties with high biocompatibility, minimal tissue toxicity, and the ability to degrade [21–23]. The constant pH during biodegradation distinguishes PHB from other PHAs, such as poly(lactic acid) (PLA) or poly(e-caprolactone) (PCL) [24]. Moreover, porous 3D PHB-based scaffolds can be prepared using the cost-effective, simple electrospinning (ES) technique [18,25,26]. Furthermore, PHB is a piezoactive polymer, making it very attractive for bone tissue engineering. In addition, the enhanced deposition of calcium carbonate, which is a bone filling material [27], on the surface of PHB fibers in 3D scaffolds under dynamic mechanical conditions was reported [25]. Nonetheless, the structure of PHB and its piezoelectric properties have not been sufficiently studied. Fukada et al. first demonstrated the shear piezoelectric response in PHB due to the presence of the orthorhombic α-crystalline phase (the chiral helix) [28,29]. Later, Ort et al. suggested the presence of another crystalline structure (β-phase) with the zigzag chain conformation between the α-crystalline lamellae phase in stretched PHB films [30]. Recently, S. Phongtamrug et al. comprehensively studied stretched PHB films, including modeling, and proposed zigzag chain conformation packing (β-phase) in a hexagonal unit cell of a = b = 9.22 Å, c (chain axis) = 4.66 Å, and γ = 120° with the space group P32+21 for the β-phase in PHB stretched films [31]. Such crystalline packing is similar to quartz, thereby possessing a normal and bending piezoelectric response [32]. However, instead of the crystalline β-phase, the formation of the non-crystalline mesophase Pnc (partially reversible to the α-phase) between stretched lamellae in PHB has been proposed [33,34].

Despite a fundamental need to characterize the PHB structure and piezoresponse, the design of biomaterials with appropriate piezoelectric properties for tissue engineering applications remains quite challenging. Varying the composition of nonbiodegradable PVDF, the effect of piezoelectric properties on cell migration was shown [35]. Meanwhile, biodegradable PHB has a significantly lower piezoelectric response than nonbiodegradable PVDF and its copolymers [36]. Therefore, the improvement of the piezoelectric properties of PHB can be required for tissue repair. Designing hybrid materials is beneficial for exploiting the advantages of both organic and inorganic components [37]. In this regard, the addition of nonpiezoelectric nanofillers, such as reduced graphene oxide (rGO), demonstrated their efficiency in improving the piezoelectric response of polymers [38]. For instance, the effective piezoelectric response from the entire 3D PHB-based scaffold surface was shown to be significantly improved using conductive nanofillers such as 1D carbon nanotubes [39]. However, 1D carbon nanotubes exhibit toxicity in mammalian cells [40,41]. As an alternative, rGO has been proposed as a 2D biocompatible material for diverse applications in medicine due to its versatile properties [40,41]. For instance, an enhancement of piezoresponse in scaffolds based on other biodegradable poly(3-hydroxybutyrate-co-3-hydroxyvalerate) polymer via the addition of GO has been recently demonstrated [42]. In that study, piezoresponse was quantitatively estimated at the macroscale. While only molecular structure and crystallinity were characterized there without a comparison with the crystalline structure in hybrid polymer scaffolds, the characterization of piezoresponse in hybrid organic materials requires a comprehensive approach with the focus on the analysis of the crystalline structure, molecular composition, and crystallinity. Moreover, electrostatic parasitic contribution preventing piezoresponse estimation at the nanoscale remains challenging [43,44], especially for organic materials possessing significantly lower piezoresponse compared to ceramics.

Therefore, PHB-rGO composites are very attractive candidates as biodegradable materials with a sufficient piezoelectric response for tissue repair. However, hybrid PHB-rGO composites with improved piezoelectric response have not been developed thus far. Moreover, the mechanism of the influence of nanofillers on the piezoelectric properties of PHB fibers still requires additional research endeavors. In addition, a comprehensive study of the structure and piezoelectric properties of biocompatible PHB-rGO composites at the domain level using advanced nanoscale techniques has not yet been reported to the best of our knowledge.

Thus, in the present work, we have designed novel hybrid biocompatible PHB-rGO scaffolds with an enhanced piezoelectric response and performed a comprehensive study of the influence of rGO addition on scaffold morphology, molecular and phase composition, crystalline structure, surface potential, and piezoresponse at the nanoscale. To the best of our knowledge, scanning probe microscopy (SPM) is applied for the first time to visualize the surface potential and piezoelectric domain distribution in pure PHB and hybrid PHB-rGO fibers with taking into account the electrostatic contribution. Quantitative analysis of SPM revealed a significant increase in the piezoresponse and surface potential in the developed PHB-rGO scaffolds, which is explained by the changes in their crystalline structure and molecular composition. The results of modeling the elastic properties of the crystalline cell unit of the chiral α-helical PHB were used to simulate the piezoelectric response in pure PHB fibers.

2. Materials and methods

2.1. Materials

Poly[(R)3-hydroxybutyrate] (PHB, natural origin), indium tin oxide-coated polyethylene terephthalate (ITO-PET) films (60 Ω/sq), sodium azide, and porcine pancreatic lipase were purchased from Sigma-Aldrich (USA). Chloroform was acquired from Ekos (Russia). Graphene oxide (GO) was obtained by the improved Hummers method [45]. In this regard, a mixture of graphite flakes (3.0 g) (particle size distribution + 100 Mesh, Sigma Aldrich, Steinheim, Germany) and KMnO4 (18.0 g) (purity ≥99%, Sigma Aldrich, Steinheim, Germany) was added into a mixture of H2SO4/H3PO4 (9:1) (purity 99.999%, Sigma Aldrich, Steinheim, Germany). Then, the solution heated to 50 °C was stirred for 12 h. The obtained product was cooled down to room temperature and spilled onto ice (ca. 400 mL). Afterwards, a color of the product was changed by the addition of 30% H2O2 (3 mL) (30 wt% in H2O, Sigma Aldrich, Steinheim, Germany). The obtained product with the brown color was centrifuged (4500 rpm, 15 min) and subsequently washed in deionized water. The obtained GO was freeze-dried and redispersed in water using a ultrasonic processor (70%). The obtained solution was used as a precursor of rGO percolated hybrid PHB-rGO scaffolds.
water, 30% HCl (30 wt% in H₂O, Merck KgaA, Darmstadt, Germany), and ethanol (≥ 99.8%, Sigma Aldrich, Steinheim, Germany). The solid product, collected from the filter, was dried at room temperature in vacuum overnight. To form rGO, the obtained GO powder was thermally reduced in a porcelain boat using a tube furnace at 800°C for 3 h in an Ar/H₂ atmosphere (95/5) with a ramping rate of 10°C/min. The obtained rGO flakes had the lateral size of 0.5–1.5 μm and layer numbers of 11–13.

2.2. Fabrication of the samples

The nonwoven fibrous scaffolds were fabricated by ES. As the first step, PHB powder was dissolved in chloroform at a concentration of 5 wt%. Prior to polymer dissolution, in the case of hybrid scaffolds, rGO was added to PHB powder at one of the following concentrations: 0.2, 0.7, and 1.0 wt%. Therefore, all the samples were named PHB, PHB-0.2rGO, PHB-0.7rGO, and PHB-1rGO, and these abbreviations are used throughout the manuscript. To dissolve PHB and to achieve the homogenous dispersity of rGO in the chloroform/PHB solution, all solutions were subjected to ultrasound for 4 h. After solution, the polymer solution was loaded in a plastic syringe, which was connected to a syringe pump used to feed solutions through an extension tube connected through 27-gauge needles (Fisnar, USA). Then, a voltage of 4–6 kV was deposited on the samples before scanning electron microscopy (SEM) analysis. The diameter distribution of PHB and PHB-rGO composites was visualized using VESTA 3.

2.3. Samples characterization

The morphology of the scaffolds was investigated by scanning electron microscopy (FEI Quanta 200 3D instrument) operated in a high vacuum; the energy of an electron beam was 10 keV. A silver coating was deposited on the samples before scanning electron microscopy (SEM) analysis. The diameter distribution of PHB and PHB-rGO composite fibers was obtained by measuring 100 fibers from each sample using ImageJ software [46].

The phase composition and structure of the prepared scaffolds were investigated by X-ray diffraction using an X-ray diffraction instrument (XRD 6000 Shimadzu (Japan)) with CuKα radiation (λ = 0.154 nm) in the 2θ range from 5° to 60° at a step size of 0.01°/2θ at 40 kV and 30 mA. The PDF4+ database was used to analyze the obtained diffractograms. The crystallite size for the selected Bragg reflections in the fabricated PHB-rGO scaffolds was estimated using the Scherrer Eq. (1):

\[
D = \frac{\lambda}{β\cosθ},
\]

where \( k \) is a shape factor; \( λ \) is the X-ray wavelength; \( β \) is the full width at half maximum intensity; and \( θ \) is the Bragg angle.

Differential scanning calorimetry (DSC) was performed to evaluate the crystallinity changes in PHB scaffolds doped with rGO using TA Instruments SDT Q600 V20.9. DSC analysis of scaffolds with a mass of ~5 mg was carried out using an aluminium pan, and the temperature varied from room temperature to 650°C with a heating rate of 10°C/min in air. The degree of crystallinity of the fabricated hybrid scaffolds was calculated using Eq. (2):

\[
XC = \frac{\Delta H_m}{\Delta H_{m0}} \times 100%,
\]

where \( \Delta H_m \) – the heat of fusion (J/g) and \( \Delta H_{m0} \) – the heat of fusion of 100% crystalline PHB, which equals 146 J/g [47].

The molecular structure of the hybrid scaffolds was studied by Raman spectroscopy using a WITec Alpha300R microscope equipped with a diode-pumped 785-nm NIR laser (TOPTICA, Germany) and a UHTS-300 spectrometer with a CCD camera cooled to ~70°C (iDus 401 BR-DD, ANDOR, Great Britain). Upon measurements, the CaF2 glass (as a substrate for scaffolds), a 40x/0.5 NA Nikon objective (Japan) were used. A laser power of ~5 mW was used during the measurements.

The surface electric potential and piezoelectric response of individual polymer fibers at the nanoscale were investigated with Kelvin-probe force microscopy (KPFM) and piezoresponse force microscopy (PFM), respectively, using a Ntegra Aura Atomic Force Microscope (NT-MDT, Russia) scanning probe microscope equipped with an external HF2LI Lock-in Amplifier (Zurich Instruments, Switzerland). For KPFM measurements, conductive Cr/Pt-coated ElectriMulti75-G cantilevers (Budget sensors, Bulgaria) with a spring constant of 3 N/m and resonance frequency of 75 kHz were used. The surface potential of the fibers was determined under an AC driving voltage of 0.5 V amplitude with a frequency of 11 kHz in a non-contact regime. For the minimization of a parasitic electrostatic contribution in the PFM measurements [43,44], hard conductive Cr/Pt-coated ElectriTap 190-G cantilevers (Budget sensors, Bulgaria) with a high spring constant of 48 m and resonance frequency of 190 kHz were used as well as an external DC voltage was applied to compensate the surface charge. Piezoelectric responses of the fibers were recorded in contact mode at a frequency of 21 kHz and AC excitation voltages of 3, 6, and 9 V.

The surface chemical composition of scaffolds was studied via X-ray photoelectron spectroscopy (XPS) using a Thermo Fisher Scientific XPS NEXA spectrometer with a monochromatic Al Kα, X-ray source operating at 1486.6 eV. The flood gun was used for the charge compensation. Survey XPS spectra were collected at the pass energy of 200 eV and energy resolution of 1 eV, whereas high-resolution XPS spectra were recorded with the pass energy of 50 eV and energy step of 0.1 eV. 3 different surface areas per scaffolds with the size of 200 μm² were analyzed by XPS. Prior to XPS measurements for avoiding a contribution of the contamination, all scaffolds were cleaned in deionized water using ultrasound and subsequently dried in a vacuum chamber.

2.4. Computer computations

2.4.1. Structure modeling

In this study, we employed the density functional theory to calculate the elastic properties of the PHB α-phase at the generalized gradient approximation (GGA) level of theory, using the functional by Perdew, Burke, and Ernzerhof (PBE-GGA) [48]. All calculations are carried out using the VASP (Vienna ab initio simulation package) [49–51] software with the plane-wave basis sets and the projector-augmented wave method (PAW) [52].

The included valence electrons are 1s² for hydrogen, 2s²2p² for carbon, and 2s²2p⁴ for oxygen. A plane-wave kinetic energy cut-off of 520 eV was used for all calculations. Brillouin zone is sampled using a 4 × 4 × 8 Monkhorst-Pack k-point mesh for the α-phase of PHB [53]. The total energy is converged to within 0.01 eV. The atomic structures are visualized using VESTA 3.

After the full relaxation of the studied conventional unit cells, the stress-strain relationship approach is applied to calculate the elastic constants [54], as implemented in VASP. The elastic constants are calculated by setting six finite distortions of the lattice at a step size of ±0.03 Å and deriving the elastic constants from the strain-stress relationship. Furthermore, it should be noted that the total elastic constants include not only the contributions from the distortions with rigid ions but also the contributions from the ionic relaxations [55].

In addition, the bulk modulus (B), shear modulus (G), and Young’s modulus (E) are determined with DFT-calculated elastic constants through the Voigt-Reuss-Hill (VRH) method [56]. For an orthorhombic system, the calculation formulas were reported by Razumovskiy et al. [57].
2.4.2. Electromechanical response simulation

Using stiffness and compliance matrices determined by DFT, the direct and converse piezoelectric response of PHB fibers were calculated using the finite element method (FEM) via Comsol® Multiphysics software (v.5.6). A coupling between the structural and electrical domains of the PHB α-phase was used in a ‘strain-charge’ form [58], which is written as:

\[ S = s_{\varepsilon}T + d^T E, \quad (3) \]
\[ D = dT + e_{\varepsilon}T E, \quad (4) \]

where \( S \) – the strain, \( T \) – the stress, \( E \) – the electric field, \( D \) – the electric displacement field, \( s_{\varepsilon} \) – the material compliance, \( d \) – the material coupling properties (piezoelectric tensors) \( e_{\varepsilon} \) – the relative permittivity at the constant stress, and \( e_0 \) – the permittivity of free space. The density and relative permittivity were taken from the derived values reported in the literature [59,60]. To evaluate the piezoelectric response, a 3D model of PHB was created for fibers with a radius of 1.5 \( \mu m \) and length of 10 \( \mu m \) (Supporting information, Fig. S1A, C), mimicking the morphology and size of pure PHB and PHB-rGO fibers deposited on ITO-PET in the present study. In the case of the converse piezoelectric response, a voltage of 9 V was applied to the top of a fiber along the longitudinal axis with a length of 50 nm (Fig. S1A), simulating contact of the PFM probe with the fiber. The distribution of the electric field homogeneously reduced in the fiber volume from the area of the applied voltage to the opposite (bottom) fibers surface is also shown in Fig. S1B. To simulate the direct piezoelectric response, a uniform pressure of 1 kPa was applied along the curved surface (Fig. S1D). For both the converse and direct piezoelectric effect simulations, the bottom side of the fibers was rigidly fixed and electrically grounded.

2.5. In vitro assays

For the biodegradation study in vitro, all scaffolds were cut with the size of 3 \( \times \) 1 cm\(^2\). The pancreatic lipase was chosen as the enzyme for degradation. Lipase is a well-known non-specific esterase, which is used in numerous studies for polymer degradation [20,61,62]. PHB and PHB-rGO scaffolds were immersed in 0.25 mg/mL lipase-containing phosphate buffered saline (PBS) at pH = 7.4 and 37 \( ^\circ \)C for 30 days. It is reported that the biodegradation of PHB-based films become stronger at the lipase concentration of 0.25 mg/mL in PBS [20]. Sodium azide with a concentration of 1 mg/mL was used to inhibit the growth of microorganisms in the solution, which was renewed every 2 days. Meanwhile, pH of the solution was controlled by a pH-meter (3510 model, Jenway, UK). Prior to measuring the relative mass changes of scaffolds during biodegradation, the scaffolds were washed and dried after the immersion in lipase-containing PBS. Relative changes in scaffolds mass caused by biodegradation were evaluated gravimetrically using analytical balance Quintix35–1S (d = 0.01 mg, Sartorius, Germany).

3. Results and discussion

The concept of the study is shown in Fig. 1. Using a cost-effective electrospinning technique, 3D fibrous hybrid PHB scaffolds were fabricated to reveal the influence of rGO addition on the fibers morphology and molecular and crystalline structures. To evaluate the effect of rGO addition on the piezoresponse and surface potential at the nanoscale, single PHB-rGO fibers were deposited on conductive ITO-PET substrates. The analysis of the obtained results and observed interrelationships between the molecular/crystalline structure and piezoelectric response/surface potential of hybrid fibrous PHB-rGO scaffolds are presented below, and the results of the modeling of the elastic properties of crystalline α-helical PHB and its piezoresponse.

3.1. Morphology and structure of the hybrid PHB-rGO scaffolds

Fig. 2A–D represents SEM images of the PHB scaffolds before and after rGO doping. Defect-free hybrid PHB-rGO fibers with rGO contents from 0.2 wt% to 1.0 wt% were formed by electrospinning. The average diameter of fibers decreased from 2.1 ± 0.4 \( \mu m \) to 1.5 ± 0.3 \( \mu m \) after rGO doping for pure PHB and PHB-rGO scaffolds with 1.0 wt% rGO, respectively. The decrease in the fiber diameter of hybrid PHB-rGO fibers can be caused by the increased solution conductivity affecting the Taylor cone size and charge accumulation on the rGO flakes in a solution jet thereby leading to their electrostatic repulsion during
In addition to the observed reduction in fiber diameter, the crystalline and molecular structure of PHB-rGO scaffolds also demonstrated changes. Despite the increased rGO content in the PHB scaffolds, XRD analysis revealed all typical Bragg reflections of orthorhombic crystal structure (PDF4+ #068–1411), which are assigned to the α-phase of PHB (Fig. 2E). However, the addition of rGO up to 1 wt% revealed a decrease in the crystallite size ($D$) of PHB in the (0 2 0) and (1 1 0) planes from 26 nm to 23 nm and from 24 nm to 19 nm (inset Fig. 2E). At the same time, 0.7 wt% rGO demonstrated an intensity increase of a Bragg reflection at approximately 20°, which is assigned to the zigzag conformation of PHB by many authors [30,31,33,34]. However, the crystalline structure of this conformation is still under discussion. One of the first, Orts et al. proposed the zigzag conformation (β-form) between stretched PHB lamellae (α-form) [30]. Recently, Phongtamrug et al. demonstrated a model of the hexagonal packing of zigzag chains with the space group $P3\overline{2}21$ [31], where a Bragg reflection of 20° is assigned to the (110) planes of the PHB β-phase (Fig. 2E). In contrast to the paracrystalline PHB β-phase, Edith Perret et al. rather suggested the packing of zigzag chains in a highly oriented noncrystalline $P_{\text{nC}}$ mesophase, which is partially reversible to the α-phase under cyclic tension loading [33,34].

Therefore, the observed decrease in $D_{(020)}$ and $D_{(110)}$ spacing can be explained by the compression of α-crystals due to embedding of rGO flakes. In turn, it induces the formation of a highly oriented zigzag conformation, which results in the increased intensity of the Bragg reflection at 20°. Similar changes in the crystalline structure of a stretched PHB fiber were also reported in the literature [33,34]. Notably, the observation of Bragg reflection at 20° in the scaffold without rGO addition indicates the zigzag chain conformation in electrospun PHB fibers. This can be attested to the stretching of the fibers by the centrifugal force at the contact of the polymer jet with a rotating cylindrical collector when the solvent evaporates quickly, and crystallization occurs.

Fig. 2F represents the Raman spectra in the 200–1800 cm$^{-1}$ region due to the presence of all typical peaks of rGO and PHB. The analysis of the Raman spectrum of rGO powder showed two peaks located at ~1350 cm$^{-1}$ and ~1585 cm$^{-1}$, which correspond to the D and G bands of sp$^2$-hybridized carbonaceous materials [65]. In addition, a bump was found at 2900 cm$^{-1}$ (Supporting Information, Fig. S2), which is assigned to a 2D band of graphene with a high disorder degree [66]. All known Raman reflection lines from 222 cm$^{-1}$ (CH$_3$ torsion) to 1725 cm$^{-1}$ (C=O stretching of crystalline phase) of PHB were detected for scaffolds without rGO [67]. PHB-0.2rGO scaffolds presented the same peaks as pure PHB scaffolds, which can be explained by the low rGO content. In turn, the increase in the rGO content in the PHB fibers to 0.7 wt% and 1.0 wt% resulted in the appearance of the D and G bands, thereby confirming the coexistence of the PHB-rGO complex.

The D band corresponds to the vibrations of aromatic domains and is present in the case of disorder/defects [68,69], while the G band relates to the E$_2g$ phonon of sp$^2$ carbon pairs [70]. The D/G intensity ratio ($I_D/I_G$) serves as a diagnostic tool to account for disorder/defects in electrospinning [63,64].
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graphene-based materials. A decrease in the $I_D/I_G$ ratio indicates domain size growth and removal of defects [65]. Notably, this ratio was $\sim 1.36$ for the fabricated rGO powder, whereas the hybrid PHB-0.7rGO and PHB-1rGO scaffolds demonstrated slightly reduced values of $I_D/I_G$ ratio up to $\sim 1.22$. Li Dan et al. explained similar decrease $I_D/I_G$ ratio values for PHB-rGO composites by forming the covalent or noncovalent bonds of the polymer to basal and edge planes of the rGO [71]. Zhijiang et al. also showed the possibility of the formation of a covalent bond between PHB and multivalled carbon nanotubes [72]. However, the overlapping of the D band peak of rGO and PHB peaks can contribute to $I_D$ values and $I_D/I_G$ ratios as well.

In addition to the observed changes in the SEM, XRD, and Raman results of the hybrid scaffolds, DSC revealed no effect of rGO doping on the melting temperature of PHB scaffolds (Table 1). However, rGO addition resulted in a reduction in the melting enthalpy and, thereby, the degree of crystallinity of PHB scaffolds calculated by Eq. (2).

According to the above-presented results, a slightly reduced degree of the crystallinity of hybrid scaffolds can be caused by embedding rGO flakes into regular lamellar stacking of PHB fibers (Fig. 3A (i)). The integration of rGO flakes and their electrostatic interaction during ES would result in the compression of α-crystals and their partial dissolution [33,34], since XRD analysis resulted in reduced $D_{1000}$ and $D_{1100}$ spacing (inset, Fig. 2E). In turn, due to the deformation of α-crystals (Fig. 3A (iii)), a locally generated stress in the short tie chain parts between lamellae causes their transformation to the zigzag conformation (crystalline β-phase or noncrystalline mesophase $P_m$) [30,31,33,34]. Increasing the rGO content can increase the stress even further, thereby preventing the formation of the zigzag chain conformation between lamellae (‘high’ rGO content, Fig. 3A (iii)). PHB-0.7rGO scaffolds showed an increase in the Bragg reflection at 20° associated with the zigzag chain conformation (Fig. 2E), whereas a further ‘high’ increase in the rGO content in PHB scaffolds up to 1.0 wt% demonstrated similar intensity as pure PHB scaffolds. In turn, the broken chain segments in PHB can generate radicals [31]. As a result, (Fig. 3B), a covalent bond of the COOH group can appear between PHB and rGO [71,72]. Raman spectroscopy revealed slightly decreased $I_D/I_G$ ratio values for the PHB-0.7rGO and PHB-1.0 scaffolds.

In addition to the characterization of the molecular composition, crystalline structure and crystallinity changes, the enzymatic biodegradation of the developed hybrid PHB-rGO scaffolds was studied. For this purpose, pure PHB and hybrid scaffolds was immersed in a lipase-containing PBS solution for 30 days. As a result, the relative mass loss after 30 days of biodegradation varied approximately from 2.5% to 4% for all scaffolds, i.e. there was no statistical difference between the pure PHB and hybrid scaffolds with different rGO content up 1.0 wt%. The obtained results are in a good agreement with the literature, where films based on PHB and its derivatives were studied [62,73]. It is worth to mention that the initial molecular weight is one of a key parameter affecting biodegradation rate of PHB-based samples [73].

As mentioned above, the development of simultaneously biodegradable and piezoelectric materials allowing to avoid second surgery and providing electrical stimuli for bone tissue repair is still a big challenge [7,19]. Concerning biodegradation, the absence of rGO (with content up to 1 wt%) impact on the PHB degradation rate was observed. Therefore, designed hybrid PHB-rGO scaffolds are very promising for bone tissue regeneration. In turn, the molecular and crystalline structures determine the surface charge and electromechanical properties of materials [47,74,75]. The effect of the observed structural changes on the surface charge and piezoresponse in the hybrid PHB-rGO fibers was evaluated and discussed below.

### 3.2. rGO effect on the PHB fibers surface potential and surface composition

Biopiezoelectricity exists in native tissue [2,76,77], thereby making artificial biocompatible piezoelectric materials very promising for the electrical stimulation of tissue regeneration [18]. Nonetheless, biochemical processes in the human body are still scarcely studied. Moreover, in some intended biomedical cases (e.g., disabled person or patient immediately after surgery), there is no possibility to provide long-term or constant piezostimulation. Therefore, investigation of the surface electric potential of the materials under static mechanical conditions (without piezostimulation) is required.

Fig. 4 represents 4D images of pure PHB and PHB-rGO separate fibers on a conductive ITO-PET substrate. The images consist of 3D plots of the fiber topography overlapped by the surface potential distribution, which is presented in more details in the Supporting Information (Fig. S4). According to the topography profiles (black curve, inset to Fig. 4), homogeneous ellipsoid microfibers were observed for all the composites. In the case of pure PHB scaffolds, fibers have irregular potential distribution with higher values at the edges compared to the center part (top of the height). In turn, the addition of rGO flakes and an increase in their content lead to a homogeneous potential distribution on the fiber surface. The PHB-1rGO composite demonstrated similar voltage values at the edges and top of the fibers.

Despite the distribution, the average values of the fiber surface potential also significantly increased with increasing rGO content (Fig. 5A) from 33 ± 29 mV to 314 ± 31 mV for pure PHB and PHB-1rGO, respectively. Moreover, a linear dependence of the average surface potential values of PHB fibers from rGO content varying up to 1.0 wt% was observed (inset to Fig. 5). This can be explained by the presence of the rGO flakes, which have charged oxygen groups on the surface (Supporting Information, Fig. S3A) [78,79]. The surface charge of the reduced graphene oxide and its derivatives varies in a wide range from mV to several volts depending on the oxygen groups content [80,81]. Based on the obtained results for the potential distribution and its average values, we can hypothesize that the rGO flakes are electrostatically attracted to the fiber surface in a high electric field upon ES, as shown in Fig. 5A.

The increase in the surface potential values of hybrid scaffolds with rGO doping has been confirmed by the changes in surface composition of scaffolds using XPS. These measurements were performed in the extreme conditions, i.e. for pure PHB with the lowest surface potential values and PHB-1rGO with the highest surface potential values. Survey XPS spectra revealed the presence of only carbon and oxygen elements on the surface of scaffolds, indicating the absence of contamination (Supporting Information, Fig. S3B).

The analysis of high-resolution XPS spectra for pure PHB and PHB-1rGO scaffolds demonstrated a significant difference in the C 1s and O 1s regions as shown in Fig. 5B and C, respectively. The C 1s region can be fitted with three typical peaks for PHB and rGO as follows [82,83]: at 286 eV corresponding to C=C bonds, at 286.7 eV assigned to C–O bonds and at 289 eV related to C=O bonds (Fig. 5B). Also, π–π* satellite was observed at the highest binding energy. Pure PHB scaffolds for the C–C, C–O, and C=O peaks demonstrated 1.17:1.08:0.75 ratio, respectively, which is close to the theoretical ratio for pure PHB (1:1:1) [82]. Since the contribution of C=O peak to C1s region significantly increased from 36% for pure PHB scaffolds to 53% for PHB-1rGO scaffolds (Fig. 5B), the addition of 1 wt% of rGO resulted in a shifted ratio to 1.59:0.63:0.78 values. This is explained by the contribution of sp² hybridized carbon atoms from rGO flakes [83], which are located close to the surface of electrospun fibers, since XPS sensitivity in the polymer depth varies up to 10 nm [84]. Despite the observed low intensity peak corresponding to

### Table 1

DSC results of pure PHB and hybrid PHB-rGO scaffolds.

<table>
<thead>
<tr>
<th>Sample</th>
<th>$T_m$ [°C]</th>
<th>$H_m$ [J/g]</th>
<th>$X_c$ [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>PHB</td>
<td>175</td>
<td>91</td>
<td>62</td>
</tr>
<tr>
<td>PHB-0.2rGO</td>
<td>175</td>
<td>91</td>
<td>62</td>
</tr>
<tr>
<td>PHB-0.7rGO</td>
<td>175</td>
<td>88</td>
<td>60</td>
</tr>
<tr>
<td>PHB-1.0rGO</td>
<td>175</td>
<td>86</td>
<td>59</td>
</tr>
</tbody>
</table>
the remaining water after scaffold cleaning (Fig. 5C), the O1s region can be deconvoluted on two typical peaks for PHB and rGO as follows [82, 83]: at 532 eV and 533.4 eV corresponding to C–O and C=O bonds, respectively. As it is seen, the addition of 1 wt% of GO caused changes in the shape of O 1s region due to the increased contribution of C–O bonds from 31% to 53% (Fig. 5C). In turn, it is well known that C–O functional groups are polar, thereby contributing in the increased surface potential of hybrid scaffolds compared to pure PHB (Fig. 5A). The increased number of C–O functional groups can originate from: (i) the surface of rGO; (ii) the carboxyl covalent bond between PHB and rGO flakes as shown in Fig. 3B. However, FTIR spectroscopy did reveal a pronounced peak of C=O for rGO flakes (Supporting Information, Fig. S3A). Meanwhile, Raman spectroscopy results demonstrated a possibility of the chemical interaction between PHB and rGO.

Thus, based on the KPFM and XPS results, a significantly increased surface potential of hybrid PHB-rGO fibers in comparison with pure PHB one is explained by the dispersity of rGO close to the surface of electrospun PHB fibers. The electrostatic attraction of rGO flakes to the surface of electrospun fibers can be expected upon ES when a high electric field is applied. In turn, the rGO addition resulted in the increased content of polar functional groups (C=O) at the surface of fibers, thereby increasing the surface potential of hybrid fibers. According to Raman and XPS spectroscopy, the increased number of C=O bonds can also indicate the formation of COOH covalent bond between rGO and PHB.

3.3. Piezoelectric response of PHB-rGO fibers at the nanoscale

The PFM technique allows studying the piezoelectric response of the materials at nanoscale insight into the polarization distribution within individual domains by detecting their mechanical vibrations in response to the applied AC bias [85]. The topography of the fiber used for PFM measurements was similar to the results presented in KPFM (images are presented in Supporting Information (Fig. SS)). To obtain detailed information about the spatial arrangement of polarization, combined vertical (VPFM) and lateral (LPFM) PFM measurements of separate
PHB-rGO fibers were performed. Bright and dark contrasts in phase VPFM (VPFM-p) images display downward and upward polarization directions, respectively. Fig. 6 shows separate PFM images of the amplitude (VPFM-a/LPFM-a) and phase (VPFM-p/LPFM-p) for pure PHB and PHB-rGO scaffolds. The contrast variations in the amplitude, shown in PFM images, are due to the presence of a polydomain state in the pure PHB and PHB-rGO fibers with domain sizes varying on the order of tens to hundreds of nanometers (inset, Fig. 6 A-D). Moreover, a bright-dark contrast in the phase images of vertical and lateral PFM signals resulted in neighboring domains with almost opposite polarization directions (Fig. 6). Whereas, the contrast in the phase images of the LPFM signal was visible but less pronounced than that of VPFM. The analysis of the relative phase distribution confirmed the presence of two opposite polarized states of piezoeactive domains (Fig. 6E-F). A similar contrast (polydomain state) of VPFM and LPFM was shown in the case of poly(vinylidene fluoride)-trifluoroethylene nanomaces [85,86].

Polar inclusions (domains) with the in-plane piezoresponse in PHB and PHB-rGO fibers can be expected due to the confirmed presence of the orthorhombic crystalline \( \alpha \)-phase with a space group \( P2_12_12_1 \) (Fig. 2E), which has only shear piezoelectric components \( d_{14}, d_{25}, d_{36} \). The investigation of the shear tensors in single crystals by PFM is a complicated task requiring special sample arrangements [74]. In turn, as shown in the present study, polymer fibers have an amorphous phase and polycrystalline structure with an uncontrollable orientation of the domains (crystals). Therefore, some of the domains induce torsion of the cantilever, leading to an LPFM response. Therefore, this torsion can also be detected in VPFM due to the buckling effect [87]; in addition, the
The orientation of α-crystals results in the response of some domains in VPFM. Nonetheless, the observed presence of the zigzag confirmation between lamellae in the fibers can also have an out-of-plane piezoresponse. According to Phongtamrug et al. [31], this zigzag confirmation is packed into a hexagonal unit cell of the $P3_21$ space group (similar to quartz), which has not only shear ($d_{14}$, $d_{25}$, $d_{26}$), but also bending ($d_{12}$) and normal piezoelectric tensors ($d_{11}$) [32].

The average values of the vertical (out-of-plane) and lateral (in-plane) effective PFM responses from the whole surface of the fibers are presented in Fig. 7. Pure PHB fibers exhibited an effective out-of-plane piezoresponse of $0.18 \pm 0.04$ pm/V. It was significantly improved by the addition of rGO flakes with contents from 0.2 wt% to 1.0 wt% due to the changes in the structure of hybrid scaffolds (Section 3.1). The highest average value ($0.45 \pm 0.08$ pm/V) was demonstrated by PHB-0.7rGO fibers. At the same time, only PHB-0.7rGO composite fibers had a significantly higher effective in-plane response ($0.56 \pm 0.12$ pm/V) compared to pure PHB fibers ($0.34 \pm 0.03$ pm/V).

These changes in the effective piezoelectric response of PHB fibers with rGO doping are given as relative distributions of the out-of-plane and in-plane fiber responses (amplitudes), as shown in Fig. 7B and C, respectively. In the designed system, there are two competing processes: (1) the addition of some amount of rGO leads to the formation of zigzag chains, resulting in a higher piezoresponse; (2) a higher rGO content can stimulate deformation of α-crystals, canceling zigzag chain formation with an associated lower crystallinity degree.

The rGO nanoflake addition led to an increased surface area of the fibers with VPFM-a > 1.62 pm (Fig. 7B), thereby proving a significant increase in the effective vertical piezoresponse in hybrid PHB-rGO fibers compared to that for pure PHB ($0.18 \pm 0.04$ pm/V). At the same time, the surface area of pure PHB fibers with LPFM-a > 3.1 pm ($0.34 \pm 0.03$ pm/V) was between those of PHB-0.2rGO and PHB-1rGO (Fig. 7C), therefore confirming an insignificant difference in the effective lateral piezoelectric response between pure and hybrid PHB fibers with 0.2 and 1.0 wt% rGO. Interestingly, PHB-0.2rGO fibers had a larger surface area with amplitudes higher than 5 pm (0.56 pm/V) and 6 pm (0.67 pm/V) for the vertical and lateral PFM responses, respectively, compared to pure PHB and PHB-1rGO fibers. Meanwhile, for both VPFM-a and LPFM-a (Fig. 7B and C), PHB-0.7rGO fibers always demonstrated a larger surface area with amplitude compared to all other PHB composite fibers. Therefore, PHB-0.7rGO fibers had the highest effective piezoelectric response due to a larger number of domains with stronger vertical and lateral piezoresponses compared to that of pure PHB fibers (Fig. 7A). For instance, the relative distribution of VPFM-a for pure PHB fibers with an amplitude higher than 5 pm (0.56 pm/V) was less than 1%, whereas PHB-0.7rGO had ~30% (insets to Fig. 7B). A similar tendency was observed in LPFM-a for PHB and PHB-0.7rGO.
Fig. 6. Vertical (VPFM) and lateral (LPFM) PFM images of the amplitude (VPFM-a/LPFM-a) and phase (VPFM-p/LPFM-p) of the fibers: (A) PHB; (B) PHB-0.2rGO; (C) PHB-0.7rGO; (D) PHB-1.0rGO. Insets demonstrate corresponding PFM images at higher magnification (scale bar is 1 µm). The relative distribution of (E) VPFM-p and (F) LPFM-p for pure PHB and hybrid PHB-rGO fibers.
These results are in good agreement with the proposed model of zigzag chain formation (Fig. 3A), which is more pronounced in the electrospun PHB-rGO fibers than in the PHB fibers but reduced at “high” 1.0 wt% rGO content with a subsequent decrease in α-crystal size due to deformation.

Thus, according to the XRD results (Fig. 2E), the excellent out-of-plane and in-plane piezoelectric activity of PHB-0.7rGO fibers at the nanoscale can be explained by a higher amount of zigzag chains, whereas a higher rGO content (PHB-1rGO) prevents their formation and reduces the α-crystal size (Fig. 3A). Moreover, KPFM analysis revealed a close distribution of rGO flakes at the fiber surface. This can explain why even a low rGO content of 0.2 wt% demonstrates a higher effective out-of-plane response compared to pure PHB fibers. In addition, Raman spectra suggest that PHB and rGO molecular interactions (covalent or noncovalent bonds) may provide specific dipoles contributing to the fiber piezoresponse [4]. In addition, the impact of a potential piezoelectricity of rGO on the piezoresponse in PHB-rGO fibers is not expected. Recently, a low piezoelectric response of 0.24 pm/V for GO was simulated and explained by the deformation of the oxygen doping regions [88]. However, the piezoresponse in GO is lower than that in pure PHB fibers as shown in the present study. Moreover, it is well-known that rGO has a significantly lower number of the oxygen groups as compared to that for GO.

3.4. Modeling of the PHB crystalline structure and piezoelectric response simulation

As discussed above, the proposed structures (Fig. 3A) for the zigzag chain conformation, such as the β-phase with a hexagonal unit cell of the P321 space group [31] or noncrystalline Pnc mesophase [33,34], still need additional research. Meanwhile, the presence of a dominant orthorhombic α-phase in PHB is unquestionable. Therefore, the piezoelectric response of the well-studied PHB α-phase can be quantified and visualized using computer simulation using FEM. Such simulations are useful for interpreting the results and developing matrixes based on the α-phase of PHB.

For piezoresponse simulation of an orthorhombic unit cell with a space group P212121 for the PHB α-phase (Fig. 8A), the elastic properties were determined for an optimized structure by DFT released in VASP. The calculated stiffness matrices \( C_{ij} \) (in GPa) and the corresponding compliance \( S_{ij} \) (in GPa\(^{-1}\)) matrices for the PHB α-phase are presented in the Supporting Information (S1 and S2). A comparison of the obtained values of the PHB α-phase shows good agreement with the values calculated by the first principles approach [89]. Based on the standard of mechanical stability for orthorhombic crystals [90], the studied PHB
α-phase systems were mechanically stable under compression. Notably, the calculated bulk modulus ($B = 6.35$ GPa), shear modulus ($G = 3.07$ GPa), and Young's modulus ($E = 7.92$ GPa) in the present study demonstrated comparable values to the other DFT study ($B = 5.79$ GPa, $G = 3.07$, $G = 2.82$ GPa, $E = 7.27$ GPa) [89].

Despite the elastic properties, the coupling matrix of tensors is also required for piezoelectric simulation. PHB is a chiral polymer consisting of a left-handed (2/1) helix arranged along the c-axis in the orthorhombic unit cell (α-phase): $P2_1 2_1 2_1$ – $D_{2}$ [28]. This crystalline symmetry $P2_1 2_1 2_1$ has only shear piezoelectric tensors, such as $d_{14}$, $d_{25}$, and $d_{36}$ [32]. Since each hydroxybutyrate monomer has a chiral carbon atom in the D(-)configuration [75], the sign of $d_{14}$ is negative for the PHB α-phase. Macropiezoelectricity dependence on the drawing of the chiral polymer. Assuming a uniaxial orientation of the PHB fiber and a perpendicularly applied electric field to the fiber surface (Fig. S1), $d_{36}$ disappears, and other piezoelectric coefficients of the α-phase can be set to $d_{14} = d_{25}$ [91,92].

Using the first value of $d_{14} (-1.4$ pC/N) reported by Fukada et al. [29], the abovementioned assumptions and geometry mimicking PFM experiments (Fig. S1A-B), displacement at 9 V was observed in α-phase PHB fibers, as shown in Fig. 8B. Due to the color contrast, it is seen that the displacement is located near the area of applied voltage. Red arrows indicate the direction of displacement. The displacement was mainly observed in the lateral direction (XY-axis) of the fiber due to the shear piezoelectric nature of the α-phase. The vector map of the XY-plane for the highest curved point along the longitudinal axis of the entire fiber demonstrates shear displacement with opposite directions (Fig. 8C). However, there was a resulting vector of the displacement at the point of applied voltage (PFM probe), which could result in the lateral PFM response (inset to Fig. 8C). It should be noted that the arrow sizes only qualitatively visualize the changes in the amplitude and do not correspond to the displacement values.

To investigate this lateral displacement at the area of applied voltage (9 V), the $d_{14}$ values were varied from 0 to 8 pC/N (Fig. 8D). It is seen that a $d_{14}$ value ($-1.4$ pC/N) reported by Fukada et al. [29] would result in a significantly lower lateral displacement value (1.1 pm) compared to the observed effective lateral PFM response of pure PHB fibers (Fig. 7C). In turn, such a lateral displacement of 3 pm at the area of simulated contact with the PFM probe according to the elaborated model can be estimated as $d_{14} \approx 3.8$ pC/N. In addition, the domains with an effective lateral piezoresponse higher than 6 pm, corresponding to the observed 25% of the surface area of PHB-0.7rGO fibers (Fig. 7C), would relate to $d_{14} \approx -7.6$ pC/N according to the elaborated model (Fig. 8D). Meanwhile, in the performed piezoelectric simulation of the...
PHB fiber with orthorhombic symmetry (P2₁2₁2₂), the values of vertical displacement in the area of applied voltage (PFM probe) at d₁₄ of 0–8 pC/N range are an order of magnitude lower compared to lateral one (Fig. 8E). However, the average values of the effective vertical piezoresponse of hybrid PHB-rGO fibers varied from ~1.6 pm (for pure PHB) and ~4.0 pm (for PHB-0.7rGO), and some surface areas of fibers exhibited even higher the vertical piezoresponse than 5 pm. Thus, assuming the presence of only the α-phase in PHB fibers and comparing its model (Fig. 8) with experimental PFM results (Fig. 7), it is seen that the effective piezoresponse in the developed PHB and PHB-rGO fibers is sufficiently higher than the first measured value of d₁₄ ~ ~1.4 pC/N in thin films by Fukada et al. [29].

Hypothesizing the presence of the α-phase with d₁₄ ~ ~1.4 pC/N, reported for PHB fibers [29], induces a maximum of 7.9 μV between the opposite ends of the fiber, whereas polarity changes in the transverse direction. Whereas, the d₁₄ ~ ~3.8 pC/N with the displacement corresponding to the observed effective lateral PFM response in pure PHB fibers generate a 2.7 times higher surface potential (21.6 μV). In the case of d₁₄ ~ ~7.6 pC/N, 43.3 μV could be expected as a maximum from the surface of the PHB fiber with orthorhombic symmetry. However, the polydomain structure was observed in the electrospun pure PHB hybrid PHB-rGO fibers (Fig. 6), allowing us to suggest a nonhomogeneous surface piezopotential distribution.

Lamellae on the surface of electrospun fibers are oriented along the curved fiber surface [93,94]. Thus, PHB fibers or films having lamellae (compatible with orthorhombic crystal symmetry in PHB) on their surface would exhibit mainly a shear piezoelectric response. Nonetheless, pure PHB fibers in the present work had a comparable effective vertical PFM response with respect to the lateral response (Fig. 7). Taking into account a negligible small vertical piezoresponse in PHB fibers with orthorhombic symmetry from the simulation (Fig. 8D), the coexistence of several local factors in electrospun PHB fibers can take place due to: (i) randomly oriented lamellae; (ii) the bucking effect from the lamellae oriented along the surface [87]; and (iii) the presence of a zigzag chain conformation, which may have a hexagonal unit cell [31], due to stretching upon electrospinning; (iv) specific dipoles contribution from PHB and rGO molecular interactions [4]. In contrast, hybrid PHB-rGO (at 0.7 wt%) fibers exhibit 2.5 and 1.7 times higher vertical and lateral piezoresponses than pure PHB fibers, respectively. This can be explained by the enhanced zigzag chain formation and deformation (inducing defects) of α-crystals as well as the specific molecular interaction between rGO and PHB (Figs. 2, 3). Thus, the developed PHB-rGO fibers with enhanced surface potential and piezoelectric activity are considered as promising materials for the fabrication of matrices for a variety of tissue engineering applications.

4. Conclusions

In summary, we have designed novel biocompatible electrospun 3D hybrid PHB-rGO-based scaffolds with an enhanced surface potential and piezoresponse. Extensive characterization revealed the changes in the molecular composition, degree of crystallinity, and crystalline structure induced by rGO addition to PHB scaffolds. A corresponding model of the observed zigzag chain formation between paired α-crystal lamellae and molecular interactions in hybrid electrospun PHB-rGO fibers is presented. Hybrid PHB-rGO fibers exhibit a more homogenous distribution of the surface potential and its average value is increased by ~9.5 times, from 33 ± 29 mv to 314 ± 31 mv for pure PHB and PHB-1rGO, respectively. Nanoscale insight using PFM showed the presence of piezoelectric domains in PHB fibers with both out-of-plane and in-plane responses. For hybrid PHB fibers (0.7 wt% rGO content), the effective out-of-plane and in-plane piezoresponses were significantly enhanced by 2.5 and 1.7 times, respectively. This is assigned to α-crystal deformation, increased zigzag chain formation, and presumably molecular interactions between the polymer and rGO. A further increase in the rGO content led to increasing α-crystal deformation and to preventing zigzag chain formation, thereby resulting in a reduced degree of crystallinity and piezoresponse of the PHB scaffolds. Using computer simulation, the calculated elastic properties of the orthorhombic unit cell with the P₂₁2₁2₂ space group allowed revealing a negligible small vertical piezoresponse compared to the lateral piezoresponse at uniaxially oriented PHB fibers and a perpendicularly applied voltage. These results allow boosting further biological studies of the developed piezoelectric 3D PHB-rGO scaffolds for usage in various tissue engineering applications.

CRediT authorship contribution statement


Declaration of Competing Interest

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

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the