Biomimetic Materials Based on Poly-3-Hydroxybutyrate and Chlorophyll Derivatives

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Article

Biomimetic Materials Based on Poly-3-hydroxybutyrate and Chlorophyll Derivatives

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Abstract: Biomimetic materials are substances that replicate natural structures. There are several methods for producing such materials with highly developed surfaces from a wide range of polymers, but electrospinning is of particular interest due to its simplicity of implementation and the ability to modify the produced fibrous materials resembling structures found in living organisms. This study explores new biomimetic materials based on polyhydroxyalkanoates, specifically poly-3-hydroxybutyrate, modified with chlorophyll derivatives. The research investigates the impact of chlorophyll derivatives on the morphology, supramolecular structure, and key properties of nonwoven materials. The obtained results are of interest for the development of new biomimetic environmentally friendly materials for biomedicine with special properties.

Keywords: poly-3-hydroxybutyrate; chlorophyll derivatives; electrospinning; antibacterial properties; supramolecular structure

1. Introduction

Tetrapyrroles and their derivates are the basis of essential physiological functions in most living organisms [1]. In this connection, tetrapyrroles and their derivatives are of a great interest for the creation of new materials and biomedical tools [2,3].

Scientists have achieved great success in the synthesis and modification of tetrapyrroles due to the directional design of photosensitive molecules with varying the nature of the metal, peripheral substituents or the length of the conjugated double bond system [4]. It is important to emphasize the successes in the organic synthesis of chlorophyll and its derivatives [5]. A great attention is drawn to chlorophyll derivatives due to a set of its unique properties.

Chlorophyll's functions include accumulation, transport of energy and driving charge separation reactions in reaction centers [6]. It possesses antioxidant, antigenotoxic and antimutagenic properties [7–9]. Its derivatives are bioactive, easily modified and their synthesis cost is low [5,10,11]. Industrial forms of chlorophyll are well known, and they are successfully used in food industry, in cosmetics and medicine [9,12].

A particular success should be considered the effective use of chlorophyll derivatives in photodynamic therapy [5]. Their effectiveness is due to combination of light, photosensitizer and cytotoxic agent [13]. In case of chlorophyll derivatives, the cytotoxic agent is the molecular oxygen which is produced only upon in situ irradiation [14]. So, it is possible to control the work of such remedies for therapeutic purposes effectively.

Nanoparticles based on chlorophyll derivatives for photodynamic therapy are well known due to their good photosensitizing properties caused by the energy transfer from the nanoparticle to the photosensitizer [15].

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In general, success in combining porphyrins and polymers as the carriers is observed in a large number of scientific papers. Systems with polymers such as chitosan [16]; polyethylene glycol [17]; poly(lactide-co-glycolide) [18,19] are well studied. But special attention should be paid to such a polymer as poly-3-hydroxybutyrate (PHB) [20,21]. PHB has many advantages over other polymers, among which the key ones are: high biocompatibility, stability of properties and controlled biodegradation in a wide temperature range [22,23].

In this work, it was decided to create biomimetic materials based on PHB and various chlorophyll derivatives for a detailed study of their suitability for the production of polymer-porphyrin systems for biomedicine. As a method of obtaining biomimetic systems electrospinning (ES) was chosen. ES allows to achieve maximum similarity of created materials to the structures produced by nature due to the formation of a highly developed hierarchical structure of fibrous material [24]. Biomimetic materials based on PHB-porphyrin electrospun composites are already known: polyhydroxybutyrate/5,10,15,20-tetrakis(4-hydroxy-phenyl)-21H,23H-porphine [25]; polystyrene/polyhydroxybutyrate/graphene/tetraphenylporphyrin [26], polyhydroxybutyrate/Hemin [27], polyhydroxybutyrate/tetraphenylporphyrin [28].

Thus, the aim of the work is to study biomimetic systems of PHB-chlorophyll derivatives for biomedicine.

2. Materials and Methods

2.1. Materials

For this study, was chosen biopolymer poly-3-hydroxybutyrate (PHB), obtained through microbiological synthesis (16F series, produced by BIOMER, Frankfurt, Germany). This polymer exhibited a crystallinity of 60%, a molecular weight of 206 kDa, a density of 1.248 g/cm^3, and a melt flow index of 10 g/10 min (180°C, 5 kg). Powdered derivatives of chlorophyll: mC$_2$N+, mC$_2$N, mC$_2$NH$_2$, mC$_3$OH and mC$_4$ (Figure 1) were utilized as functional additives. The synthesis of the amide derivatives of chlorin e$_6$ from pheophorbide a methyl ester was described in detail earlier in articles [29] for compound mC$_3$OH, [30] for compound mC$_4$, [31] for compound mC$_2$NH2. 13^1-N-(2-N′, N′-diethylaminoethyl) amide-15^2,17^3-dimethyl ester of chlorin e$_6$ (mC2N(Et)2) was synthesized and then quaternized by methyl iodide (mC$_2$N(Et)$_2$Me$^+$ I$^-$) according to the reported procedures in [32]. Both substances were dissolved in chloroform (Biolot, St. Petersburg, Russia) for the experiments.

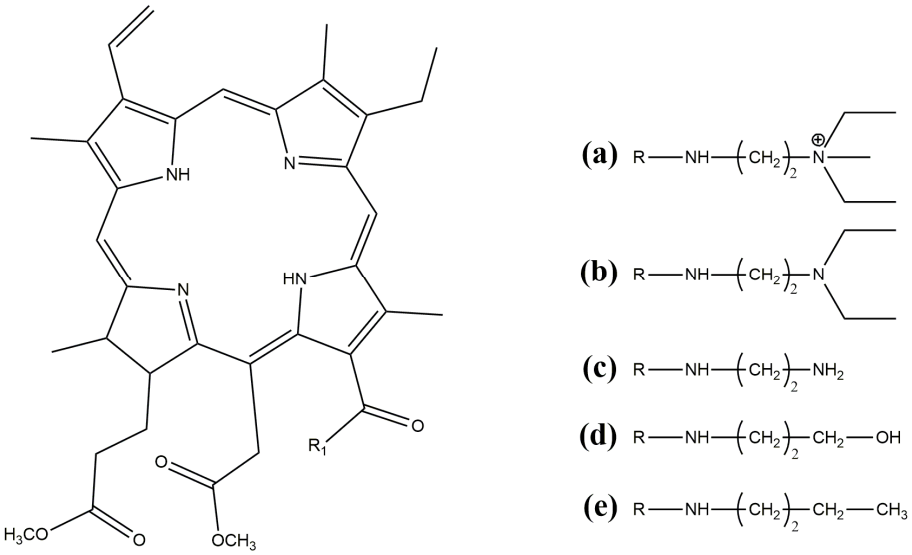

Figure 1. Structural formulas of chlorophyll derivatives: (**a**) mC$_2$N+; (**b**) mC$_2$N; (**c**) mC$_2$NH$_2$; (**d**) mC$_3$OH; (**e**) mC$_4$.

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2.2. Methods

2.2.1. Obtaining of nonwoven fibrous materials

Fibrous nonwoven materials were obtained using the electrospinning (ES) method utilizing a single-capillary setup (Figure 2). Homogeneous solutions were prepared, including a 7% concentration solution of PHB and chlorophyll derivatives dissolved in chloroform with an additive concentration of 0.03%. Each solution was used in a volume of 25 ml.

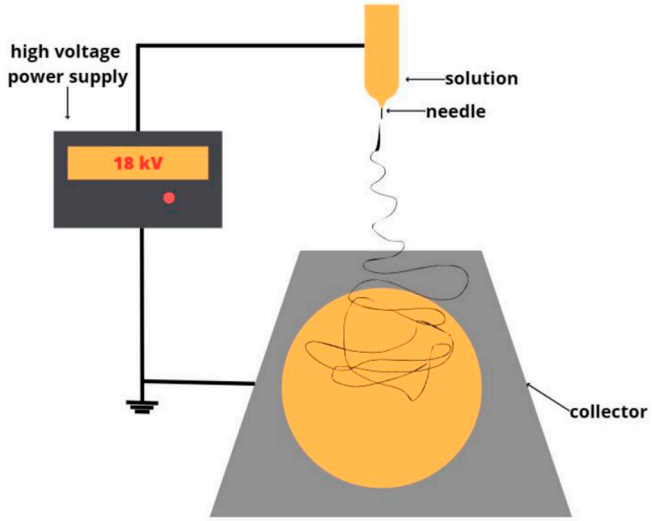

Figure 2. Single-capillary ES setup.

The forming process proceeded as follows: under the influence of electrostatic force, fibers were drawn from the solution in the cell and deposited on a substrate located on the lower electrode. During the forming process, the solvent evaporated, and the fiber solidified. The obtained nonwoven materials were subjected to drying to remove solvent residues and excess moisture for 48 hours at 24 °C.

2.2.2. Scanning Electron Microscopy (SEM)

Scanning electron microscopy (SEM) images of electrospun PHB-chlorophyll derivatives composites were obtained using the Tescan VEGA SBU II (Brno, Czech Republic) on samples coated with a platinum layer for 10 minutes. Structural changes, including variations in fiber diameter, defects and morphology, were analyzed using the Olympus BX43 microscope (Olympus, Tokyo, Japan). The primary morphological properties of the fibers were measured through micrography, facilitated by Olympus Stream Basic software.

2.2.3. Differential Scanning Calorimetry (DSC)

Differential Scanning Calorimetry (DSC) is an experimental method used to study the thermal properties of materials. In this study, the DSC 214 Polyma (Netzsch, Selb, Germany) was employed. The methodology involved two heating cycles (from 20 °C to 220 °C) and two cooling cycles (from 220 °C to 20 °C) with a temperature change rate of 10 K/min, samples were analyzed in an argon atmosphere. DSC measures the heat flow absorbed or released by a substance as its temperature changes, so this method allows for the determination of heat capacity, thermal transitions, crystalline and amorphous properties of the material.

2.2.4. Fourier-Transform Infrared Spectroscopy (FTIR)

The spectra of the samples were obtained using the Lumos BRUKER infrared spectrometer (Karlsruhe, Germany) employing the multiple attenuated total internal reflection method on a

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diamond crystal. The spectral resolution was set at 2 cm^{-1}. The analysis was conducted in the wavelength range from 400 to 4000 cm^{-1}. This method enabled a detailed examination of the material's interaction with infrared radiation, providing valuable insights into its chemical structure and composition.

2.2.5. Wettability

The surface wettability of PHB/Chlorophyll derivatives samples was measured using the contact angle (CA) method, which quantifies surface moisture. Water droplets (2 μL) were dispensed onto the material's surface using an automatic dispenser, and the contact angle formed between the droplet and the surface was measured. We utilized an optical microscope, M9 №. 63649, with an FMA050 lens (AmScope, Moscow, Russia), and analyzed the data using Altami Studio 3.4 software.

2.2.6. Air Permeability

The air permeability of membranes, represented by the Gurley number, was measured following the standard protocol [33]. The applied pressure was 1.22 MPa, the air volume was 100 mL, and the test area was 6.5 cm^2. The relative experimental error was ± 5%.

2.2.7. Antimicrobial Properties

Antimicrobial activity of the samples was studied using the strains of *Escherichia coli* O157:H7. The lysis zone was considered as a marker of antimicrobial activity. The Petri dishes were inoculated with a standardized inoculum of test microorganisms. The samples were placed into the Petri dishes. The strain was grown in BSA/agar at 37 °C and allowed to stay with the samples for 24 h.

2.2.8. Mechanical Properties

The mechanical properties of the material, such as strength (the maximum load the material can withstand) and elongation at break (how much the material can stretch before breaking) were measured using the testing machine DVT GP UG 5. The stretching speed was 25 mm per minute. Elongation at break was calculated as the difference between the final and initial length of the sample, expressed as a percentage of the initial length. The results were averaged based on no fewer than 5-7 measurements, with a measurement error of ±0.2%.

3. Results

3.1. Morphology of PHB/chlorophyll derivatives electrospun materials

Electrospun materials can be characterized as fibrous materials with highly developed surface area. Such materials have a high degree of similarity to living structures, that is, they are biomimetics. The key to such a highly developed complex hierarchy of structure is morphology. The morphology of the fibers largely depends on the conditions of ES. The key parameters of ES should include: voltage, properties and flow rate of the spinning solution, the distance between the electrodes, the pressure exerted on the solution [34]. Table 1 shows the most important parameters of fiber molding based on solutions of PHB/chlorophyll derivatives. The capillary configuration has a great influence on the formation of fibers of the set diameter, it remained constant and was 0.1 mm. Other important parameters were selected experimentally. Thus, the introduction of the additive had an effect on the viscosity of the molding solution, increasing it by an average of 20%.

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Table 1. Features of the ES process of PHB/chlorophyll derivatives electrospun materials.

Sample	Diameter of capillary, mm	Distance between the electrodes, mm	Gas pressure on the solution, kg(f)/cm²	Voltage, Kv	Viscosity, Pa s
PHB	0.1	210	10	18	1.0
PHB/mC2NH2		220	10	17-18	1.2
PHB/mC2N		220	10	17-18	1.2
PHB/mC2N+		220	10	17-18	1.2
PHB/mC4		220	10	17-18	1.2
PHB/mC3OH		220	10	17-18	1.2

The formation process of the PHB/mC2N solution was hindered; the fibers did not adhere properly to the substrate due to possible high humidity, leading to incorrect distribution and accumulation of fibers on the substrate.

In the current work, 5 chlorophyll derivatives (Figure 1) are considered, differing in the structure of the R_1 radical. The types of R_1 are shown in Figure 1. A number of studies have shown that additives containing polar groups even in very small concentrations (proportion of percents) have a significant effect on molding properties [35,36]. The chlorophyll compounds considered in this work are distinguished by the presence of such polar groups. They contribute to improving the electrical conductivity of the solution, and, as a result, the ability to form fibers at a higher voltage with a higher efficiency. A higher voltage contributes to the formation of thinner fibers; however, it may be accompanied by an increase in the flow velocity of the solution jet, resulting in fibers with a thicker diameter [37]. In this regard, it is important to ensure optimal viscosity of the solution, as well as the pressure of the air column on the spinning solution.

It is important to note that despite a very small percentage of the additive (0.03%), a significant effect on the material was mentioned. In all five cases, the formation of a highly developed material structure was observed. However, differences should be noted. Figure 3 shows SEM images of PHB based electrospun materials with different chlorophyll derivatives.

Nonwovens based on PHB are distinguished by the presence of specific defects – thickening (Figure 3a). Such defects are often found for pure PHB fibrous materials. In a number of works scientists use various methods of reducing thickenings: preparing PHB based blends with other polymers, for instance, PHB/PEG in works by Thanh et al. [38] or PHB/PCL in works by Borisova et al. [39]; using plasticizers like polyanilines in works by Ahmed et al. [40] or oligomeric lactic acid in works by Arrieta et al. [41]; using nanoparticles in works by Romero et al. [42] and others.

In the case of materials based on PHB/chlorophylls, it should be noted that PHB/mC4 (Figure 3b) and PHB/mC2N+ (Figure 3e) allowed to minimize and almost completely reduce the number of such defects respectively. In these cases, the number of thickenings is minimal, there are no gluing's completely, the fibers are well cured. While other chlorophylls did not show a satisfactory result, moreover, they even worsened the quality of the material. So PHB/mC3OH (Figure 3c) promotes the formation of highly porous fibers, which is usually associated either with a high rate of solvent evaporation [43] or with excessive friability of the amorphous phase due to the intermolecular interaction of the polymer and the additive [44]. While PHB/mC2N (Figure 3f) shows a significant increase in defects in the form of glues and smudges that make the material unusable, reducing its mechanical properties to a minimum.

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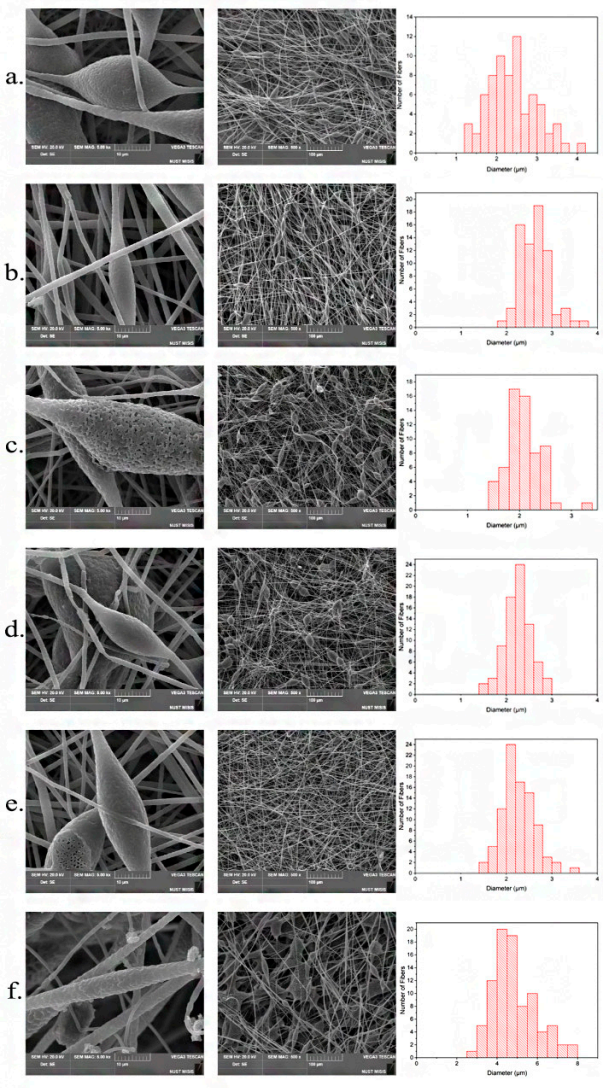

Figure 3. SEM images of electrospun materials based on PHB with different chlorophyll derivatives: (**a**) PHB; (**b**) PHB/mC$_4$; (**c**) PHB/mC$_3$OH; (**d**) PHB/mC$_2$NH$_2$; (**e**) PHB/mC$_2$N+; (**f**) PHB/mC$_2$N.

Table 2 shows the most significant parameters of the morphology of the fibrous material. It is clearly seen that in all cases the additives contributed to a decrease in average fiber diameters and only mC$_2$N had the opposite effect. In addition to the defects that made the material brittle and unusable, the diameter of the fibers increased by 1.4 times in case of PHB/mC$_2$N. For the rest of the additives, there was some variation in fiber distribution (Figure 3), but, in general, the average diameter of the fibers decreased by about 1.5 times. The smallest diameter was observed for the mC$_3$OH sample with the highest porosity. All additives led to a decrease in bulk density of the material by about 1.5 times, however, there was no obvious correlation between the average diameter of the fibers and the density of their laying, as can be seen from the results (Table 2).

Changes in the porosity of the material and the proportion of open pores were estimated using the Gurley method. It is clearly seen that the introduction of the additive significantly increased the proportion of open pores capable of passing a larger volume of air per unit of time (Table 2). This characteristic directly demonstrates the importance of the introduction of additives for the planning of high-pored materials. Thus, the highest values were shown by materials containing mC$_2$NH$_2$ and mC$_3$OH, where the smallest average diameters were detected. To the surprise, materials with the least number of defects PHB/mC$_4$ and mC$_2$N+ showed a less high increase in the proportion of open pores, which may be due to a greater degree of surface development and a smaller proportion of open space between the pores due to high fiber entanglement.

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Table 2. Morphology of PHB/chlorophyll derivatives electrospun materials.

Sample	Average diameter, μm	Bulk density, g/cm3	Air permeability, ml	Time by Guerly method, s
PHB	3.5	0.30	0.38	50.0
PHB/mC$_2$NH$_2$	2.24	0.18	3.75	26.7
PHB/mC$_2$N	4.86	-	-	-
PHB/mC$_2$N+	2.27	0.13	2.78	36.0
PHB/mC$_4$	2.63	0.16	2.35	42.6
PHB/mC$_3$OH	2.08	0.16	4.35	23.0

3.2. Chemical structure PHB/chlorophyll derivatives electrospun materials.

Figure 4 shows FTIR spectra of PHB/chlorophyll derivatives electrospun materials. It is clearly seen that the obtained spectra are identical to the spectrum of pure PHB. The observed effect is due to a very low concentration of additives (0.03%) in the material and a low probability of its localization on the fiber surface.

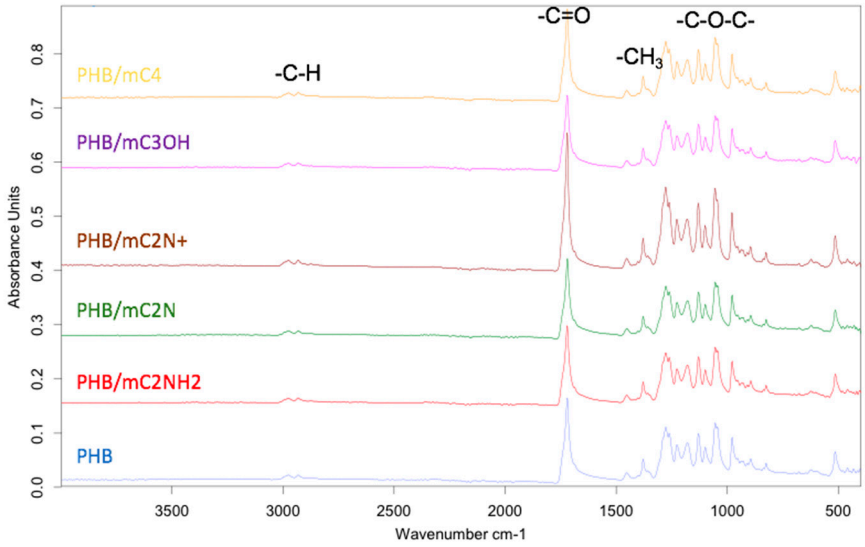

Figure 4. FTIR spectra of electrospun materials based on PHB with different chlorophyll derivatives: (**a**) PHB; (**b**) PHB/mC$_4$; (**c**) PHB/mC$_3$OH; (**d**) PHB/mC$_2$NH$_2$; (**e**) PHB/mC$_2$N+; (**f**) PHB/mC$_2$N.

The most notable for the identification and evaluation of PHB are the peaks: C=O carbonyl at 1720 cm^{-1}, -C-O-C- ester linkage at 1150–1300 cm^{-1}, -C-H bonds in methyl radical at 2900–3100 cm^{-1}, -CH3 at 1380 cm^{-1} [45]. The region 400-1300 cm^{-1} is responsible for a complicated series of absorptions due to all manners of bending vibrations of PHB [46].

In works by Luo et al. [47], Kovalcik et al. [48], Xu et al. [49] was proposed to evaluate the state of the crystalline phase of PHB with ratio of the peak intensities of 1225 cm^{-1} and 1453 cm^{-1}, which allows us to characterize changes in the size of the crystalline fraction of the PHB. The ratios were obtained: 3.63 for PHB; 3.64 for PHB/mC$_4$; 3.22 for PHB/mC$_3$OH; 3.59 for PHB/mC$_2$NH$_2$; 3.88 for PHB/mC$_2$N+; 3.13 for PHB/mC$_2$N. These ratios are very close values and indicate weak changes in the structure of the crystalline phase of PHB. Although this method should be subjected to some criticism, since the signal is taken from the surface and does not give a complete picture of the structural organization in the mass of fibers, and not only on their surface, which is more influenced by environmental factors during electroforming.

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3.3. Thermophysical characteristics of PHB/chlorophyll derivatives electrospun materials.

Chlorophylls and their derivatives are thermally stable in the range from 20 to 200 ˚C, and thermal decomposition begins no earlier than at 300 ˚C [50]. According to this, we can evaluate the effect of additives on the formation of the supramolecular structure of the PHB, focusing on the thermophysical properties of the polymer. Thus, the first heating allows us to assess the state of the polymer in the form of fibers, the supramolecular structure of which is largely determined by the peculiarities of the electroforming process [51]. The second heating allows us to see the thermophysical behavior of the native state of PHB formed under the influence of chlorophyll derivatives. DSC curves are shown on Figure 5.

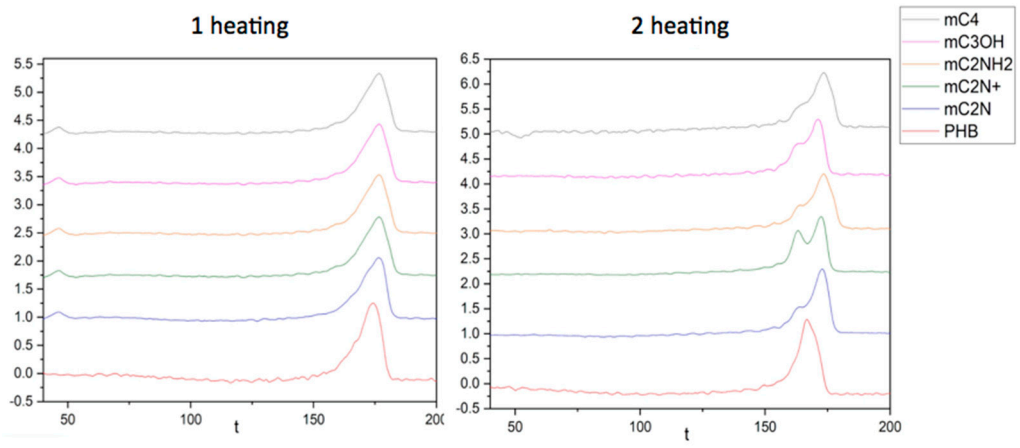

Figure 5. DSC curves of electrospun materials based on PHB with different chlorophyll derivatives.

The enthalpy and melting point of PHB/chlorophyll derivatives electrospun materials are shown in Table 3. Thus, the melting temperature changes slightly, and therefore the size of the crystallites remains quite close, because the larger crystallites melt at higher temperatures, and smaller ones at lower temperatures [52]. It is important to note that the introduction of all additives reduced the enthalpy of melting by 8-17% at the first melting and by 12-19% at the second melting. These data indicate the plasticizing effect of additives. Moreover, it is important to note that the type of the selected radical, as can be seen from the results, influenced the structure of the PHB in its own way. This is especially evident in the form of peaks during the second heating. it can be seen that when all additives are introduced, a low-temperature shoulder appears in the range of 155-165 degrees. This shoulder indicates the formation of defective crystallites or a fine crystalline fraction during the formation of fibers. And consequently, at the stage of curing of the polymer melt, additives can prevent the slow crystallization of a more regular fraction of PHB, acting as crystallization aggregates.

Table 3. Thermal Properties of PHB- chlorophyll derivatives, where χ - crystallinity degree Δ±2.5%, ΔH -melting enthalpy Δ±2.5%, Tm – melting temperature Δ±2%.

Sample	First heating run		Second heating run	
	T_m, ⁰C	ΔH, J/g	T_m, ⁰C	ΔH, J/g
PHB	175	93.1	170	90.8
PHB/mC_2NH_2	176	83.3	174	78.7
PHB/mC_2N	176	85.8	173	81.2
PHB/mC_2N+	177	80.6	173	79.8
PHB/mC_4	176	82.0	174	78.9
PHB/mC_3OH	175	77.4	171	74.9

Additives probably occupy a position in the amorphous phase of the polymer, influence the crystallization process, contribute to the formation of two fractions of PHB crystallites: large and small after the first heating. However, with slow curing during electrospinning, crystallization

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centers can act in all cases, except for mC_2N, which has a pronounced shoulder already at the first heating. This effect is probably manifested in the formation of defects in the form of glues and smudges on the material (Figure 2f). And the material that differed in the smallest number of defects and the smallest bulk density (Figure 2e) has the most pronounced peak consumption, which indicates the significant role of the additive in rapid crystallization under experimental conditions.

3.4. Wettability of PHB/chlorophyll derivatives electrospun materials.

The chemical structure of PHB contains hydrophobic methyl group and hydrophilic ester groups, but in general PHB is hydrophobic polymer [53]. PHB is a hydrophobic material. The control of its hydrophilicity is an important task, the solution of which can greatly expand the areas of biomedical introduction of this polymer [54]. Figure 6 shows the changes in wettability of PHB based electrospun materials with different chlorophyll derivatives.

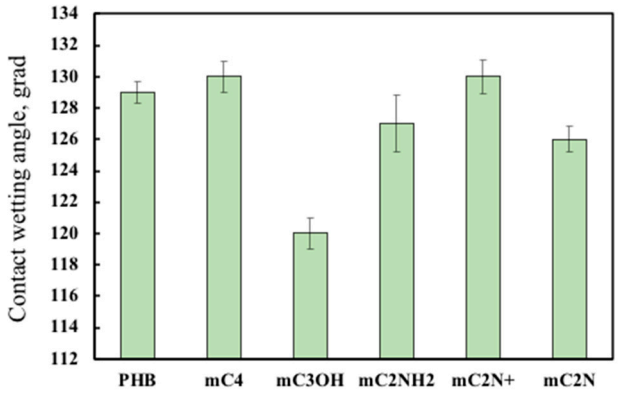

Figure 6. Wettability (contact angle) of electrospun materials based on PHB with different chlorophyll derivatives.

As can be seen from the Figure 6, chlorophyll additives affect the wettability of the material in different ways, leading to a change in its surface characteristics. In the works of Russie et al. it was possible to obtain hydrophobic materials based on chlorophyll derivatives [55]. The greatest increase in hydrophilicity can be seen for mC_3OH, to a less extent for mC_2N, which may be due to the appearance of open pores on the surface of fibers that contribute to wettability. The mC_2NH_2 additive has even less effect. An increase in the hydrophilicity of the material can occur due to the presence of a polar amino group, which is able to form hydrogen bonds with water molecules. In other cases, we see a slight increase in hydrophobicity, which is inherent in chlorophyll derivatives.

3.5. Antimicrobial properties of PHB/chlorophyll derivatives electrospun materials.

Many tetrapyrroles have notable antimicrobial characteristics [56–58]. Chlorophyll derivatives are considered good antimicrobial agents in the case of photodynamic therapy. Due to singlet oxygen the antimicrobial effect of chlorophyll derivatives has been shown in relation to Staphylococcus aureus, Escherichia coli, Candida albicans, and Artemia salina in works by Gerola et al., Indrawati et al., Suvorov et al. [59–61]. However, of interest is the possibility of tetrapyrroles to exhibit antimicrobial properties in the absence of excitation, but for the time being in the material. To answer this question, samples of PHB/chlorophyll derivatives electrospun materials were placed in medium with E. Coli. The results are shown in Figure 7.

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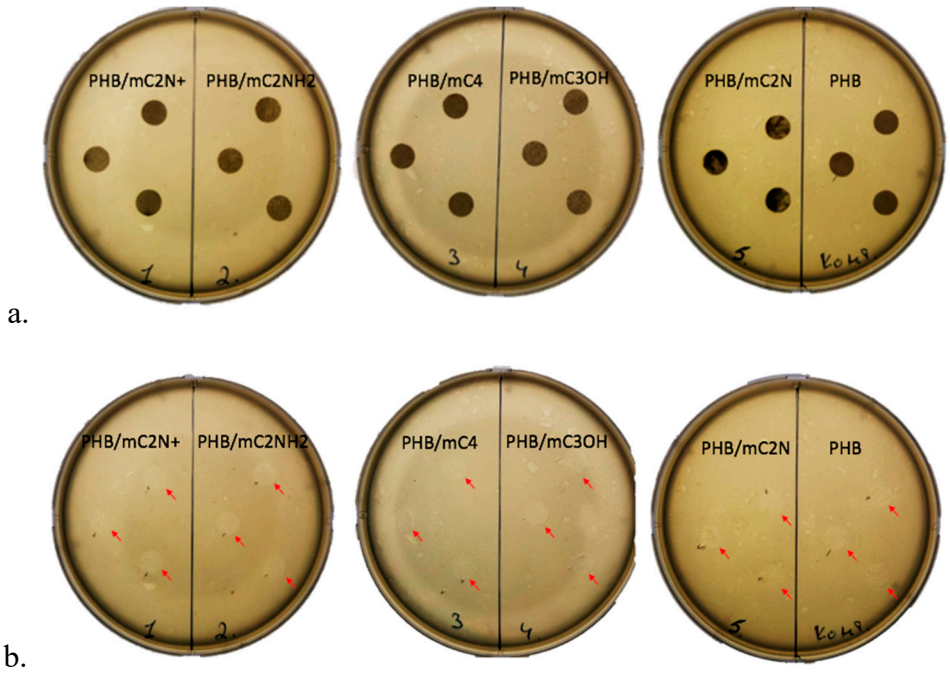

Figure 7. The microbiological test of electrospun materials based on PHB with different chlorophyll derivatives against E. coli (red arrows show lysis zone).

So, it is observed that the additives themselves do not have antimicrobial properties, but they suppress the activity of E. coli directly under the material layer. The lysis zone does not go beyond the boundaries of the material, moreover, it practically does not differ from the one obtained for pure PHB. This is probably due to the low concentration of the additive and the absence of photo excitation.

3.6 Mechanical properties of PHB/chlorophyll derivatives electrospun materials.

It is important to note that materials based on PHB are characterized by low strength, high brittleness. Often biomimetic medical materials are not required to withstand high loads, so this is not a problem. In many respects, high brittleness is a consequence of the semi-crystalline nature of the polymer, but also, due to the low glass transition temperature, the amorphous phase is in a glazed state and is quite fragile. Glass transition temperature of PHB is 4-7 °C [62].

Although a number of scientists note that various additives are able to affect the glass transition temperature of PHB, which indicates changes in the state of the amorphous phase and, as a consequence, the mechanical properties [63,64].

So with the introduction of additives, we see the appearance of a peak in the range 47-50 °C (Figure 5 1 heating). These peaks may indicate a shift in the glass transition temperature, which means that the additive is localized in the amorphous phase. Thus, at room temperature, the amorphous phase of PHB with chlorophyll derivatives is in an elastic rubber state. However, this did not allow to increase or improve the mechanical properties of nonwovens based on PHB. Mechanical properties (tensile strength and elongation at break) are shown in Table 4.

Unfortunately, only 2 samples – PHB/mC_4 and PHB/mC_2N+ were suitable for investigation on the test machine. Only these chlorophyll derivatives allowed to obtain a material with the least number of structural defects since these two additives resulted in a decrease of structural defects. Other additives (PHB/mC_3OH; PHB/mC_2NH_2; PHB/mC_2N) were ripped apart without any signals.

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Table 4. Mechanical properties of PHB/chlorophyll derivatives electrospun materials.

Sample	Tensile strength, MPa Δ ±0.02 MPa	Elongation at break, % Δ ±0.2 %
PHB	1.7	3.6
PHB/mC$_2$NH$_2$	-	-
PHB/mC$_2$N	-	-
PHB/mC$_2$N+	1.5	1.3
PHB/mC$_4$	1.5	0.6
PHB/mC$_3$OH	-	-

Examples of Stress-strain curves of PHB/mC$_4$ and PHB/mC$_2$N+ electrospun materials are shown in Figure 8. It can be seen that the mechanical properties are influenced by the strength of individual fibers, which are still brittle, although well cured (Figure 8 B).

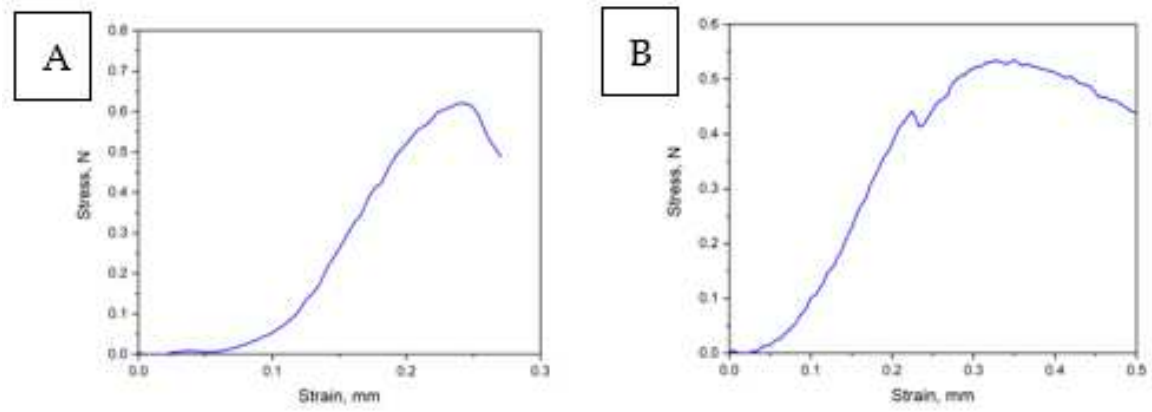

Figure 8. Mechanical analysis of PHB/chlorophyll with different chlorophyll derivatives: (**A**) PHB/mC$_4$; (**B**) PHB/mC$_2$N+.

From the data obtained, it can be seen that the strength of the material has changed slightly, remaining very low. Elongation decreased despite the fact that the number of defects decreased, and the state of the amorphous phase changed. Nevertheless, the materials are characterized by high porosity, have a controlled surface, which can offset their low mechanical properties sufficient for use in biomedicine.

4. Conclusions

In the study, new materials based on the biopolymer poly-3-hydroxybutyrate (PHB) modified with chlorophyll derivatives were proposed. These electrospun materials exhibit high environmental friendliness and possess biomimetic characteristics. All five examined chlorophyll derivatives enabled the production of nonwoven materials through the electrospinning method. However, mC$_2$N had a negative impact on the mechanical properties, making this additive unsuitable. All additives led to a reduction in the crystallinity of PHB while preserving the highly developed material structure. Additives mC$_4$ and mC$_2$N+ resulted in a decrease in structural defects, with mC$_4$ producing the thickest fibers and mC$_2$N+ achieving the lowest bulk density of the material. These additives can be employed to control the hydrophilicity and hydrophobicity of the produced material. Further research is planned to explore the biomedical properties of the new materials with the most successful combinations of properties relevant to solving healthcare and medical challenges.

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References

1. Pucci, C.; Martinelli, C.; Degl'Innocenti, A.; Desii, A.; De Pasquale, D.; Ciofani, G. Light-Activated Biomedical Applications of Chlorophyll Derivatives. *Macromol Biosci.* **2021**, *21*, 19.
2. Imran, M.; Ramzan, M.; Qureshi, A.K.; Khan, M.A.; Tariq, M. Emerging Applications of Porphyrins and Metalloporphyrins in Biomedicine and Diagnostic Magnetic Resonance Imaging. *Biosensors (Basel)* **2018**, *8(4)*, 95.
3. Tsolekile, N.; Nelana, S.; Oluwafemi, O.S. Porphyrin as Diagnostic and Therapeutic Agent. *Molecules* **2019**, *24(14)*, 2669.
4. Lukyanets, E.A. The key role of peripheral substituents in the chemistry of phthalocyanines and their analogs J. Porphyrins *Phthalocyanines*. **2010**, *14*, 40.
5. Staron, J.; Boron, B.; Karcz, D.; Szczygieł, M.; Fiedor, L. Recent Progress in Chemical Modifications of Chlorophylls and Bacteriochlorophylls for the Applications in Photodynamic Therapy. *Curr Med Chem* **2015,** *22(26),* 74.
6. Chen, M. Chlorophyll modifications and their spectral extension in oxygenic photosynthesis. *Annu Rev Biochem.* **2014,** *83,* 40.
7. Perez-Galvez, A.; Viera, I.; Roca, M. Chemistry in the Bioactivity of Chlorophylls: An Overview. *Curr Med Chem*. **2017**, *24(40),* 4515-4536.
8. Negishi, T.; Rai, H.; Hayatsu, H. Antigenotoxic activity of natural chlorophylls. *Mutat Res*. **1997**, *12,* 97-100.
9. Solymosi, K.; Mysliwa-Kurdziel, B. Chlorophylls and their Derivatives Used in Food Industry and Medicine. *Mini Rev Med Chem*. **2017**, *17,* 1194-1222.
10. Hoober, J.K.; Eggink, L.L.; Chen, M. Chlorophylls, ligands and assembly of light-harvesting complexes in chloroplasts. *Photosynth Res.* **2007,** *94,* 387-400.
11. Pogorilyy, V.; Ostroverkhov, P.; Efimova, V.; Plotnikova, E.; Bezborodova, O.; Diachkova, E.; Vasil'ev, Y.; Pankratov, A.; Grin, M. Thiocarbonyl Derivatives of Natural Chlorins: Synthesis Using Lawesson's Reagent and a Study of Their Properties. *Molecules*. **2023**, *20,* 4215.
12. Sigurdson, G.T.; Tang, P.; Giusti, M.M. Natural Colorants: Food Colorants from Natural Sources. *Annu Rev Food Sci Technol.* **2017**, *28,* 261-280.
13. Zhou, A.; Wei, Y.; Wu, B.; Chen, Q.; Xing, D. Pyropheophorbide A and c(RGDyK) comodified chitosan-wrapped upconversion nanoparticle for targeted near-infrared photodynamic therapy. *Mol Pharm*. **2012**, *4,* 9.
14. Dougherty, T.J. Photosensitizers: therapy and detection of malignant tumors. *Photochem Photobiol*. **1987,** *45,* 89.
15. Thanasekaran P, Chu CH, Wang SB, Chen KY, Gao HD, Lee MM, Sun SS, Li JP, Chen JY, Chen JK, Chang YH, Lee HM. Lipid-Wrapped Upconversion Nanoconstruct/Photosensitizer Complex for Near-Infrared Light-Mediated Photodynamic Therapy. ACS *Appl Mater Interfaces*. **2019,** *9,* 95.
16. Ferreira, D.P.; Conceição, D.S.; Calhelha, R.C.; Sousa, T.; Socoteanu, R.; Ferreira, I.C.F.R.; Vieira Ferreira, L.F. Porphyrin dye into biopolymeric chitosan films for localized photodynamic therapy of cancer. *Carbohydr Polym*. **2016**, *20,* 160-171.
17. Ferreira, L.F.V.; Machado, I.F.; Gama, A.; Socoteanu, R.P.; Boscencu, R.; Manda, G.; Calhelha, R.C.; Ferreira, I.C.F.R. Photochemical/Photocytotoxicity Studies of New Tetrapyrrolic Structures as Potential Candidates for Cancer Theranostics. *Curr Drug Discov Technol.* **2020,** *7,* 661-669.
18. Lin B, Xu X, Zhang X, Yu Y, Wang X. Photodynamic Treatment of Colorectal Cancer Using Chlorin e6-Loaded Poly(lactide-co-glycolide)- Based Nanoparticles. *J Biomed Nanotechnol.* **2021**, *1,* 1939-1950.
19. Mollaeva, M.R.; Nikolskaya, E.; Beganovskaya, V.; Sokol, M.; Chirkina, M.; Obydennyi, S.; Belykh, D.; Startseva, O.; Mollaev, M.D.; Yabbarov, N. Oxidative Damage Induced by Phototoxic Pheophorbide a 17-Diethylene Glycol Ester Encapsulated in PLGA Nanoparticles. *Antioxidants* **2021**, *10,* 1985.

20. McAdam, B.; Brennan Fournet, M.; McDonald, P.; Mojicevic, M. Production of Polyhydroxybutyrate (PHB) and Factors Impacting Its Chemical and Mechanical Characteristics. *Polymers (Basel).* **2020**, *4*, 2908.
21. Tyubaeva PM, Varyan IA, Zykova AK, Yarysheva AY, Ivchenko PV, Olkhov AA, Arzhakova OV. Bioinspired Electropun Fibrous Materials Based on Poly-3-Hydroxybutyrate and Hemin: Preparation, Physicochemical Properties, and Weathering. *Polymers (Basel).* **2022**,*12*, 4878.
22. Kliem, S.; Kreutzbruck, M.; Bonten, C. Review on the Biological Degradation of Polymers in Various Environments. *Materials* **2020**, *13*, 4586.
23. Tyubaeva, P.M.; Varyan, I.A.; Nikolskaya, E.D.; Mollaeva, M.R.; Yabbarov, N.G.; Sokol, M.B.; Chirkina, M.V.; Popov, A.A. Biocompatibility and Antimicrobial Activity of Electrospun Fibrous Materials Based on PHB and Modified with Hemin. *Nanomaterials* **2023**, *13*, 236.
24. Woods, I.; Flanagan, T.C. Electrospinning of biomimetic scaffolds for tissue-engineered vascular grafts: threading the path. *Expert Rev Cardiovasc Ther*. **2014**, *12*, 32.
25. Pramual, S.; Assavanig, A.; Bergkvist, M.; Batt, C.A.; Sunintaboon, P.; Lirdprapamongkol, K.; Svasti, J.; Niamsiri, N. Development and characterization of bio-derived polyhydroxyalkanoate nanoparticles as a delivery system for hydrophobic photodynamic therapy agents. *J Mater Sci Mater Med*. **2016,** *27*, 40.
26. Avossa, J.; Paolesse, R.; Di Natale, C.; Zampetti, E.; Bertoni, G.; De Cesare, F.; Scarascia-Mugnozza, G.; Macagnano, A. Electrospinning of Polystyrene/Polyhydroxybutyrate Nanofibers Doped with Porphyrin and Graphene for Chemiresistor Gas Sensors. *Nanomaterials (Basel).* **2019**, *17*, 280.
27. Tyubaeva, P.; Varyan, I.; Lobanov, A.; Olkhov, A.; Popov, A. Effect of the Hemin Molecular Complexes on the Structure and Properties of the Composite Electrospun Materials Based on Poly(3-hydroxybutyrate). *Polymers (Basel).* **2021**, *21*, 4024.
28. Ol'khov, A.A.; Tyubaeva, P.M.; Zernova, Y.N.; Kurnosov, A.S.; Karpova, S.G.; Iordanskii, A.L. Structure and Properties of Biopolymeric Fibrous Materials Based on Polyhydroxybutyrate-Metalloporphyrin Complexes. *Russ J Gen Chem.* **2021,** *91*, 546-553.
29. Gushchina O.I., Grishina M.Y., Larkina E.A., Lebedeva V.S., Mironov A.F. (2017) Synthesis of Hydroxyl Derivatives of Chlorin e_6. Macroheterocycles. 10(1). 81 – 83. https://doi.org/10.6060/mhc161072g.
30. Belykh, D.V., Kopylov, E.A., Gruzdev, I.V. et al. Opening of the extra ring in pheophorbide a methyl ester by the action of amines as a one-step method for introduction of additional fragments at the periphery of chlorin macroring. Russ J Org Chem 46, 577–585 (2010). https://doi.org/10.1134/S1070428010040238.
31. A.Yu. Rybkin, A.Yu. Belik, N.S. Goryachev, P.A. Mikhaylov, O.A. Kraevaya, N.V. Filatova, I.I. Parkhomenko, A.S. Peregudov, A.A. Terent'ev, E.A. Larkina, A.F. Mironov, P.A. Troshin, A.I. Kotelnikov Self-assembling nanostructures of water-soluble fullerene [60]–chlorin e_6 dyads: Synthesis, photophysical properties, and photodynamic activity // Dyes and Pigments. – 2020. – V. 180. P. 108411 ISSN 0143-7208, https://doi.org/10.1016/j.dyepig.2020.108411 (Scopus, http://www.sciencedirect.com/science/article/pii/S0143720820304265).
32. O. I. Gushchina, E. A. Larkina and A. F. Mironov, Makrogeterotsikly/ Macroheterocycles, 2014, 7, 414 (in Russian).
33. Hantel, M.M.; Armstrong, M.J.; DaRosa, F.; l'Abee, R. Characterization of Tortuosity in Polyetherimide Membranes Based on Gurley and Electrochemical Impedance Spectroscopy. *J. Electrochem. Soc*. **2016**, *164*, 334–339.
34. Lim, L.T.; Mendes, A.C.; Chronakis, I.S. Electrospinning and electrospraying technologies for food applications. *Adv Food Nutr Res.* **2019.** *88*,167-234.
35. Urbanek O.; Sajkiewicz P.; Pierini F. The effect of polarity in the electrospinning process on PCL/chitosan nanofibres' structure, properties and efficiency of surface modification. Polymer **2017**, 124, 168–175. doi:10.1016/j.polymer.20
36. Olkhov, A.A.; Tyubaeva, P.M.; Zernova, Y.N.; Markin, V.S.; Kosenko, R.; Filatova, A.G.; Gasparyan, K.G.; Iordanskii, A.L. The Influence of Technological Factors and Polar Molecules on the Structure of Fibrillar Matrices Based on Ultrafine Poly-3-hydroxybutyrate Fibers Obtained via Electrospinning. *Technologies* **2023**, *11*, 118.
37. Behere, I.; Ingavle, G. In vitro and in vivo advancement of multifunctional electrospun nanofiber scaffolds in wound healing applications: Innovative nanofiber designs, stem cell approaches, and future perspectives. *J Biomed Mater Res A*. **2022,** *110(2),* 443-461.
38. Thanh, N.H.; Olekhnovich, R.; Sitnikova, V.; Kremleva, A.; Snetkov, P.; Uspenskaya, M. PHB/PEG Nanofiber Mat Obtained by Electrospinning and Their Performances. *Technologies* **2023**, *11*, 48.

39. Borisova, I.; Stoilova, O.; Manolova, N.; Rashkov, I. Modulating the Mechanical Properties of Electrospun PHB/PCL Materials by Using Different Types of Collectors and Heat Sealing. *Polymers (Basel).* **2020**, *20*, 693.
40. El-hadi, A.M.; Al-Jabri, F.Y. Influence of Electrospinning Parameters on Fiber Diameter and Mechanical Properties of Poly(3-Hydroxybutyrate) (PHB) and Polyanilines (PANI) Blends. *Polymers* **2016**, *8*, 97.
41. Innes, N.; Johnson, IG; Al-Yaseen, W.; Harris, R.; Jones, R.; Kc, S.; McGregor, S.; Robertson, M.; Wade, WG; Gallagher, JE. A systematic review of droplet and aerosol generation in dentistry. *J Dent.* **2021**, *105*, 103556.
42. Rodríguez-Tobías, H.; Morales, G.; Ledezma, A.; Romero, J.; Saldívar, R.; Langlois, V.; Renard, E.; Grande, D. Electrospinning and electrospraying techniques for designing novel antibacterial poly(3-hydroxybutyrate)/zinc oxide nanofibrous composites. *J. Mater. Sci*.. **2016**, *51*, 8593–8609.
43. Katsogiannis, K.A.G.; Vladisavljević, G.T.; Georgiadou, S. Porous electrospun polycaprolactone (PCL) fibers by phase separation. *Eur. Polym. J.* **2015**, *69*, 284-295.
44. Wagner, A.; Poursorkhabi, V.; Mohanty, A.K.; Misra, M. Analysis of Porous Electrospun Fibers from Poly(l-lactic acid)/Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) Blends. *Chem. Eng.* **2014**, *2*, 1976–1982.
45. Shamala, T.R.; Divyashree, M.S.; Davis, R.; Kumari, K.S.L.; Vijayendra, S.V.N.; Raj, B. Production and characterization of bacterial polyhydroxyalkanoate copolymers and evaluation of their blends by Fourier transform infrared spectroscopy and scanning electron microscopy. *Indian J. Microbiol.* **2009**; *49*, 251-258.
46. Stuart, B.. Infrared Spectroscopy: Fundamentals and Applications, 2nd ed.; Publisher: University of Technology, Sydney, Australia 2004; pp. 208.
47. Luo, S.; Netravali, A.N. A study of physical and mechanical properties of poly(hydroxybutyrate-co-hydroxyvalerate) during composting. *Polym. Degrad. Stab*. **2003**, *80*, 59–66.
48. Kovalcik, A.; Obruca, S.; Kalina, M.; Machovsky, M.; Enev, V.; Jakesova, M.; Sobkova, M.; Marova, I. Enzymatic Hydrolysis of Poly(3-Hydroxybutyrate-co-3-Hydroxyvalerate) Scaffolds. *Materials (Basel).* **2020**, *13*, 2992.
49. Xu, J.; Guo, B.H.; Yang, R.; Wu, Q.; Chen, G.Q.; Zhang, Z.M. In situ FTIR study on melting and crystallization of polyhydroxyalkanoates. *Polymer*. **2002**, *43*, 6893–6899.
50. Berezin, D.B.; Makarov, V.V.; Guseinov, S.S.; Romanenko, Yu.V.; Khudyaeva, I.S.; Startseva, O.M.; Belykh, D.V.; Kustov, A.V. Thermal stability of chlorophyll a derivatives containing hydrophilic groups. *Russ. J. Gen. Chem*. **2017,** *87*, 1557–1561.
51. Yeo, J.C.C.; Muiruri, J.K.; Thitsartarn, W.; Li, Z.; He, C. Recent advances in the development of biodegradable PHB-based toughening materials: Approaches, advantages, and applications. *Mater. Sci. Eng. C* **2018**, *92*, 1092-1116.
52. Walle G.A.; Koning G.J.; Weusthuis R.A.; Eggink G. Properties, modifications and applications of biopolyesters. Adv. Biochem. Eng. Biotechnol. **2001,** 71, 263-91. doi: 10.1007/3-540-40021-4_9.
53. Moreira, J.B.; Kuntzler, S.G.; Vaz, B.S.; da Silva, C.K.; Costa, J.A.V.; de Morais, M.G. Polyhydroxybutyrate (PHB)-based blends and composites. *Biodegradable Polymers, Blends and Composites*. **2022**, 389-413.
54. Pantani, R.; Turng, L.S. Manufacturing of advanced biodegradable polymeric components. *J. Appl. Polym. Sci.* **2015**, *132*, 48.
55. Suvorov, N.; Pogorilyy, V.; Diachkova, E.; Vasil'ev, Y.; Mironov, A.; Grin, M. Derivatives of Natural Chlorophylls as Agents for Antimicrobial Photodynamic Therapy. *Int J Mol Sci.* **2021**,*22(12),* 6392.
56. Banfi, S.; Caruso, E.; Buccafurni, L.; Battini, V.; Zazzaron, S.; Barbieri, P.; Orlandi, V. Antibacterial activity of tetraaryl-porphyrin photosensitizers: An in vitro study on Gram-negative and Gram-positive bacteria. *J. Photochem. Photobiol. B, Biol*. **2006,** *85*, 28-38.
57. Nitzan, Y.; Ladan, H.; Gozansky, S.; Malik, Z. Characterization of hemin antibacterial action on Staphylococcus aureus. *FEMS Microbiol. Lett.* **1987**, *48*, 401-406.
58. Beyene, B.B., Wassie, G.A. Antibacterial activity of Cu(II) and Co(II) porphyrins: role of ligand modification. *BMC Chemistry* **2020**, *14*, 51.
59. Gerola, A.P.; Santana, A.; Franc¸a, P.B.; Tsubone, T.M.; de Oliveira, H.P.M.; Caetano, W.; Kimura, E.; Hioka, N. Effects of Metal and the Phytyl Chain on Chlorophyll Derivatives: Physicochemical Evaluation for Photodynamic Inactivation of Microorganisms. *Photochem. Photobiol*. **2011**, *87*, 884–894.
60. Indrawati, R.; Zubaidah, E.; Sutrisno, A.; Limantara, L.; Yusuf, M.M.; Brotosudarmo, T.H.P. Visible Light-Induced Antibacterial Activity of Pigments Extracted from Dregs of Green and Black Teas. *Scientifica (Cairo).* **2021**, *12*, 5524468.
61. Suvorov, N.; Pogorilyy, V.; Diachkova, E.; Vasil'ev, Y.; Mironov, A.; Grin, M. Derivatives of Natural Chlorophylls as Agents for Antimicrobial Photodynamic Therapy. *Int. J. Mol. Sci*. **2021**, *22*, 6392.

62. Turco R.; Santagata G.; Corrado I.; Pezzella C.; Di Serio M. In vivo and Post-synthesis Strategies to Enhance the Properties of PHB-Based Materials: A Review. Front Bioeng Biotechnol. **2021**; 8:619266. doi: 10.3389/fbioe.2020.619266.
63. Czerniecka-Kubicka, A.; Frącz, W.; Jasiorski, M. et al. Thermal properties of poly(3-hydroxybutyrate) modified by nanoclay. J. Therm. Anal. Calorim. **2017**, 128, 1513–1526. https://doi.org/10.1007/s10973-016-6039-9
64. Iordanskii, A.; Fomin, S.; Burkov, A.; Pankova, Yu.; Zaikov, G. Characterization of the structure and properties of the polymer blends based on poly-3-hydroxybutyrate and polyisobutylene Journal of Characterization and Development of Novel Materials. **2015,** 1937, 7975