

Surface Modification of Bioresorbable Polymer Scaffolds by Laser Treatment

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Abstract—The effect of laser irradiation on the properties of the surface of films prepared from a bioresorbable polymer poly(hydroxybutyrate) has been studied. To determine the spectral region of the polymer optimal for the effective action of radiation on electron molecular bonds, theoretical investigations have been performed, which have shown that, for modifying the surface of PHB scaffolds, it is expedient to use a vacuum laser at a wavelength of 160 nm. Using laser irradiation at a power from 3 to 30 W, a series of films with modified surface, from roughnesses to perforations, have been obtained. The microstructure and properties of the film surface depending on the mode of irradiation have been examined, and conditions have been found under which the contact marginal angles of film wetting with water can be decreased to 50° (compared with 76–80° in starting products). Thus, conditions of laser treatment of PHB scaffolds have been theoretically substantiated and experimentally realized that provide a beneficial effect on the properties of the surface without destroying the structure of the material.

Key words: laser irradiation, microbial biocompatible and biodegradable polymers, polyhydroxybutyrate, surface properties, hydrophilicity

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INTRODUCTION

The natural polyesters of hydroxy derivatives of alkanic acids—poly(hydroxyalkanoates) (PHA)—synthesized by microorganisms in a specialized biotechnological process have the property of being degradable in biological media under the action of enzymes and cell elements to final products (CO₂ and H₂O), are marked by high biocompatibility, mechanical strength, and thermo-plasticity, and for these reasons have broad prospects of application in various fields [1]. Especially promising is the use of PHA for constructing surgical elements and endoprostheses, and as a scaffold for functional cells in cell and tissue engineering [2]. The interdisciplinary approach accepted in the latter field is aimed first of all on creating novel materials for restituting the lost functions of separate tissues or whole organs. The main principles of the given approach consist in designing biodegradable carriers that can be implanted into a damaged organ or tissue, in combination with donor cells and/or bioactive substances [3].

Materials used in tissue engineering must possess special properties. First of all, the products of their breakdown should not be toxic; the construct must retain its shape and remain sturdy enough until the new host tissue at the site of implant fully recovers. The material must not be immunogenic, must sustain cell growth and organization into tissue, and the implant itself must provide for free

removal of metabolic products. In recent years, PHA have been actively studied as a material suitable for constructing biocompatible scaffolds, since PHA can be used to make 2D and 3D matrices, micro- and nanoparticles, ultrathin fibers, etc. For these purposes, use can be made of polymer solutions, latexes, melts and powders [4].

The scaffold surface properties have a weighty influence on cell adhesion and proliferation. To improve the adhesive properties and gas dynamics of the constructs as well as their permeability for substrates and products, various means are employed, including physical or chemical treatment. There are positive examples of enhancing the adhesive properties of PHA scaffolds via surface immobilization of collagen, interaction with chitosan and other polysaccharides, acrylic acid, etc. [5–7]. Another approach to surface modification is treatment with gas plasma [8, 9]. It has been shown that upon a 10-min exposure of a PHA film to ammonia plasma, up to 8% nitrogen is incorporated into the film structure [10]. This is attended by significant alteration of surface properties, as the contact marginal angles are reduced by 20–30°. The surface becomes hydrophilic, and this property is retained in time. Likewise it has been shown [11] that the surface of PHA films after plasma treatment with the use of ammonia gas becomes more hydrophilic, owing to which a greater number of endothelial cells attach to it

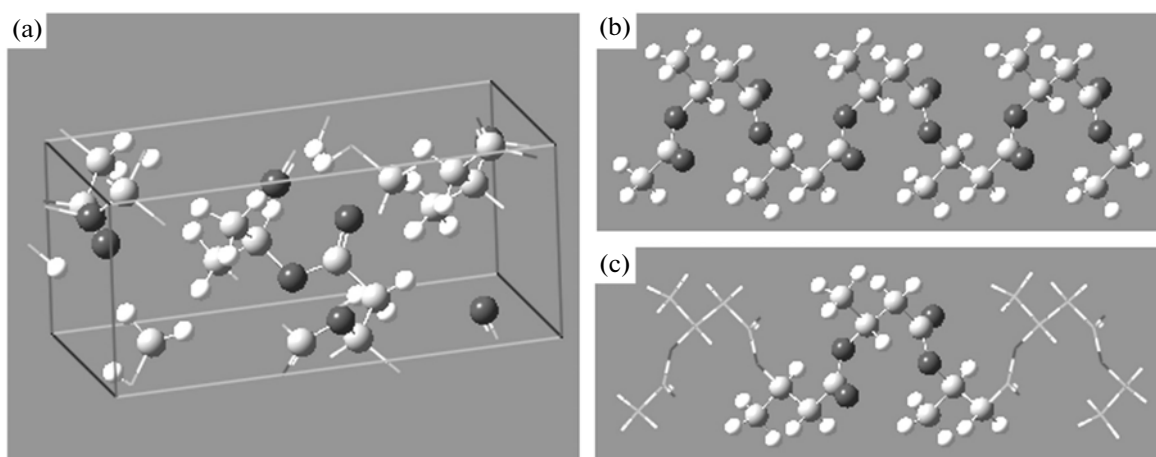


Fig. 1. Models of (a) PHB unit, (b) cluster containing six monomers, and (c) layers for ONIOM calculation.

and grow better, releasing a larger amount of fibronectin and forming a multilayer cell matrix.

Works can be found where laser treatment has been applied to improve the scaffold properties. Such treatment has advantages over other methods, permitting selective modification of the surface without destruction of the material or formation of toxic products. It is supposed that hydrophilicity rises in the laser-irradiated sites, thereby enhancing the adhesive properties. It has been shown [12] that laser irradiation of films made of poly(hydroxybutyrate) (PHB) gives rise to specific zones ranging in width from 10 to 20–40 μm with altered spherulite structure; the authors reported that murine L929 fibroblasts adhered and grew best at these zones. However, such studies for PHA are very scarce.

The aim of the present work was theoretical substantiation and experimental assessment of the influence of laser radiation on the surface properties of scaffolds prepared from biocompatible and bioresorbable PHA.

EXPERIMENTAL

PHB samples were produced in the Institute of Biophysics using the hydrogen-oxidizing bacteria *Ralstonia eutropha* B5786 [4]. PHB was extracted from the biomass with chloroform and precipitated with hexane. The composition of PHB was determined after methanolysis by the methyl esters of fatty acids using a Hewlett Packard GCD Plus chromatograph/mass spectrometer. Solutions of PHB in chloroform (or dichloromethane) were poured onto polished metal to produce flexible transparent films of thickness 0.016 ± 0.002 to 0.1 ± 0.01 mm, strength 4.0 ± 0.28 kg/mm², elasticity modulus 130 ± 28 kg/mm², elongation at rupture about 4%. The film surface was characterized by the contact angle of wetting with water: (θ , deg), the surface free energy (γ_s), the free energy of the interphase surface (γ_{SL}) and the cohesion (W_{SL} , erg/cm²), using well-known equations [16]. To determine the contact angle, film samples were placed on a glass slide, drops

of distilled water (100, 200, 300 μL ; 10 drops each) were applied on the surface with an automatic micropipette, photographed with a digital camera, and the mean angles were calculated.

To optimize the regime of laser treatment, mathematical modeling was used to examine the PHB spectral regions for the most effective action on electron molecular bonds of the polymer, using Gaussian 03 [13] and VASP [14, 15] packages.

The laser radiation power was varied from 3 to 30 W, the exposure from 1 to 3 ms.

RESULTS AND DISCUSSION

For a polymer link to enter a reaction, it must be provided with an initiatory active center: free radical, active ion or active coordination complex. The first to react are links containing a double bond (C=C, C=N, C=O), a triple bond, or a cyclic group that can open under the action of UV radiation. Since there is almost no experimental data on PHB absorption in this spectral range, we made a theoretical study to determine the wavelengths optimal for effective action on its electron molecular bonds. Available x-ray data for PHB [17] were used to model a unit cell (Fig. 1a), which was then multiplied lengthwise to obtain a chain of six hydroxybutyrate monomers (Fig. 1b). Bonds at both sides of the resulting cluster were completed with hydrogens to obviate unpaired electrons, with which the structure could have been treated as a radical.

The choice of a cluster model rather than a periodical structure is dictated by the lack of quantum-chemical methods for calculations of excited states of periodical systems. The cluster was examined in the layered approach ONIOM [18], whereby a molecule is partitioned into more important and less important parts that are calculated by accordingly more exact and less exact methods, and then the results are summed. In this way, larger molecular systems become amenable to quantum-chemical studies.

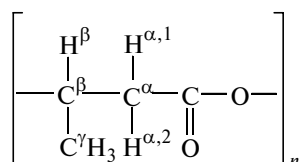
Table 1. Calculations for the excited states

No.	Excitation energy, eV	Wavelength, nm	Oscillator strength
1	5.51	224.90	0.0009
2	5.53	224.12	0.0017
3	6.65	186.40	0.0005
4	6.82	181.68	0.0043
5	7.41	167.41	0.0012
6	7.53	164.61	0.0163
7	7.73	160.43	0.0741
8	7.87	157.49	0.0388
9	7.92	156.50	0.0015
10	8.07	153.67	0.0007
11	8.31	149.18	0.0620
12	8.40	147.54	0.0009

Table 2. Atomic coordinates for the irreducible part of the PHB unit, fractions of translation vectors [17]

Atom	<i>a</i>	<i>b</i>	<i>c</i>
C ^α	0.193	0.140	-0.125
C (carbonyl)	0.357	0.078	0.023
O (ester)	0.284	-0.018	0.069
C ^β	0.442	-0.075	0.209
O (carbonyl)	0.540	0.115	0.089
C ^γ	0.593	-0.144	0.064
H ^β	0.553	-0.024	0.302
H ^{α,1}	0.071	0.179	-0.018
H ^{α,2}	0.295	0.194	-0.220

Our cluster model was accordingly partitioned as shown in Fig. 1c. The first layer was the central part, for which excited states were calculated by TDDFT [19] with an exchange-correlation potential B3LYP [20] in the 6-31G(p,d) basis set [21]. The second layer contained the flanking parts, which were calculated analogously but without the time-dependent procedure. Cluster geometry was not optimized as its electronic structure was examined. Demarcation of the first layer was due not so much to the impossibility of calculation over a larger structure as to its inexpedience. The cluster has repeating units, and

**Fig. 2.** Empirical formula of the PHB monomer.

the features of its electronic structure are determined by those of one monomer. On the other hand, an absorption spectrum calculated for a single monomer cannot be interpreted as a spectrum of the cluster or the polymer in whole. This follows from the auxochrome concept [22], according to which the presence of certain groups (e.g. carboxyls as in PHB) leads to a long-wavelength shift of the absorption bands. Such a shift would not be seen in a monomer calculation. Therefore, the first layer included two monomers and associated methyl groups, which are also auxochromes.

One more factor that influences the absorption spectrum of the calculated part of molecule is the polarizing electrostatic field created by its surroundings. This is why the second layer was considered. Calculation of its electronic structure yielded the effective charges on the atoms creating the electrostatic field in which the excited states of the first layer were calculated. As a result, we have obtained twelve excited states of the cluster (Table 1). Excitation energies correspond to the energies of absorption of light of indicated wavelengths. One can see that PHB absorbs in the UV and far UV regions. Consideration of the nature of molecular orbitals between which the electron transition occurs in light absorption indicates that they are mainly localized on the atoms of carboxyl groups; indeed the latter have readily polarizable electron pairs and C=O double bonds.

To confirm the validity of the cluster approach, calculation of the PHB unit cell under periodic conditions was performed with the VASP program. According to [17] this unit is orthorhombic with translation vectors $a = 5.76 \text{ \AA}$, $b = 13.20 \text{ \AA}$ and $c = 5.96 \text{ \AA}$, having $P2_12_12_1$ symmetry. The coordinates of atoms in its irreducible part (notation as in Fig. 2) are marked in Table 2. The state density plot thus obtained (Fig. 3) shows that the optical gap width is 5.33 eV. This result is very close to the energy of the first excited state arising on electron transition from the upper occupied molecular orbital to the lower vacant one or, which is equivalent, transition from the valence zone to the conduction zone.

The last step was to construct the electronic absorption spectrum as such. It was assumed that spectral line broadening obeys the Gauss distribution law:

$$f(\lambda) = \frac{1}{\sigma\sqrt{2\pi}} \exp\left(-\frac{(\lambda - \lambda_0)^2}{2\sigma^2}\right), \quad (1)$$

where f is oscillator strength; λ is wavelength; λ_0 is absorbed wave length upon cluster excitation; σ^2 is dispersion.

To build a spectrum with the given formula, one must know the dispersion. It has been determined assuming that the main contribution to line broadening comes from atom vibrations in the cluster (temperature was taken to be constant). Therefore, knowing just the minimal vibration energy E_{vib} and using the Boltzmann distribution in the form

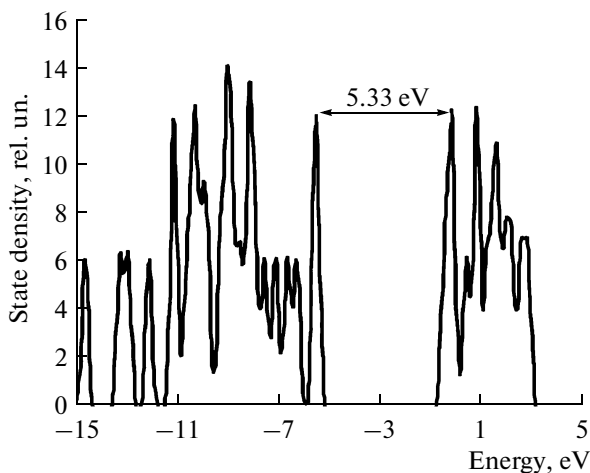


Fig. 3. Densities of PHB electronic states.

$$\frac{f(\lambda)}{f_0} = \exp\left(-\frac{E_{\text{vib}}}{kT}\right), \quad (2)$$

we can determine the ratio of $f(\lambda)$ and f_0 corresponding to λ and λ_0 , where

$$\lambda = \lambda_0 + \frac{hc}{E_{\text{vib}}}. \quad (3)$$

Above, Boltzmann constant $k = 1.38 \times 10^{-23}$ J/K; $T = 298$ K; Planck's constant $h = 6,63 \times 10^{-34}$ J s; speed of light $c = 3 \times 10^8$ m/s.

Substituting (2) and (3) into (1), we get:

$$f_0 \exp\left(-\frac{E_{\text{vib}}}{kT}\right) = \frac{1}{\sigma\sqrt{2\pi}} \exp\left(-\frac{1}{2}\left(\frac{hc}{E_{\text{vib}}\sigma}\right)^2\right). \quad (4)$$

In this expression we take the oscillator strength in cluster transition to the first excited state $f_0 = 0.0009$ (Table 1), while σ and E_{vib} remain unknown. The latter value was determined by additional calculation of polymer vibration energies with account of periodic conditions. The lowest vibration energy proved to be $E_{\text{vib}} = 0.292$ meV. In this way, we get an equation where only σ is

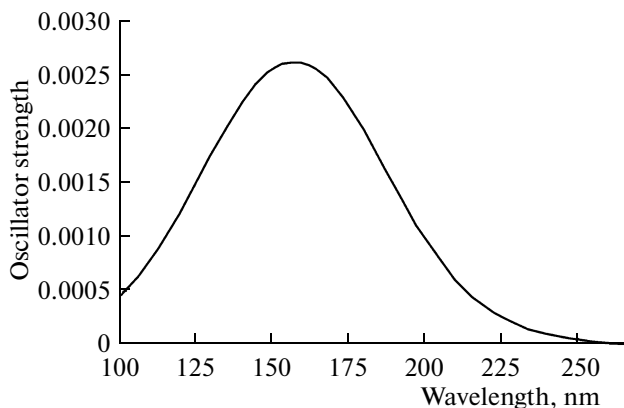


Fig. 4. Calculated electronic spectrum for PHB absorption.

unknown. Solving this equation yields $\sigma = 29.494$. Now distribution (1) is rewritten as:

$$f(\lambda) = \frac{1}{29.494\sqrt{2\pi}} \exp\left(-\frac{(\lambda - \lambda_0)^2}{2 \times 29.494^2}\right). \quad (5)$$

For each λ_0 from Table 1 in the range from 100 to 270 nm we obtain a set of $f(\lambda)$, summation of which gives a model PHB absorption spectrum (Fig. 4). The absorption maximum is about 160 nm, i.e. in the far UV, and corresponds to cluster transition into the seventh excited state. The latter testifies to the possibility of rupturing chemical bonds in PHB by laser irradiation at the indicated wavelength. Note that only vacuum lasers operate in the far UV range.

Thus, for PHB modification it is recommended to use a vacuum laser at 160 nm. This is economically expedient, because such a laser takes much less power than those operating in the far IR range. We have treated PHB films at radiation power of 3–30 W. The surface characteristics of the initial films calculated from the marginal wetting angles (θ) were: surface tension (γ) 34.66, interphase surface free energy 6.35, cohesion (W_{SL}) 100.81 erg/cm². Laser treatment yielded a series of films with altered surfaces, from pronounced roughness to perforations

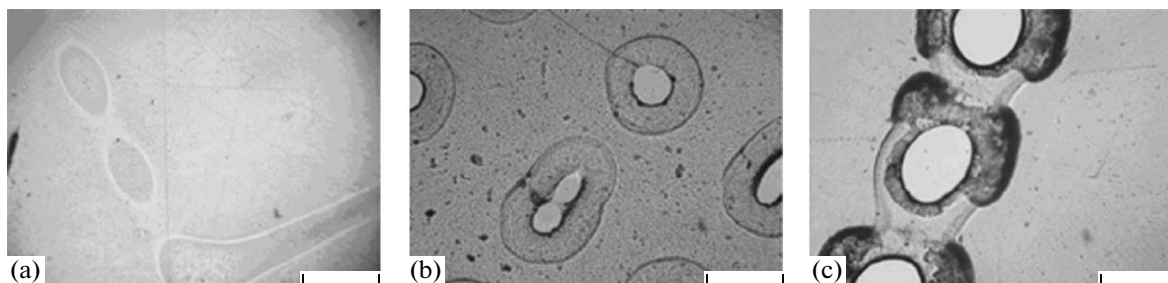


Fig. 5. Surfaces of PHB films treated with laser radiation of (a) 6.15, (b) 8.00, and (c) 9.00 W. Scale bar, 50 μm .

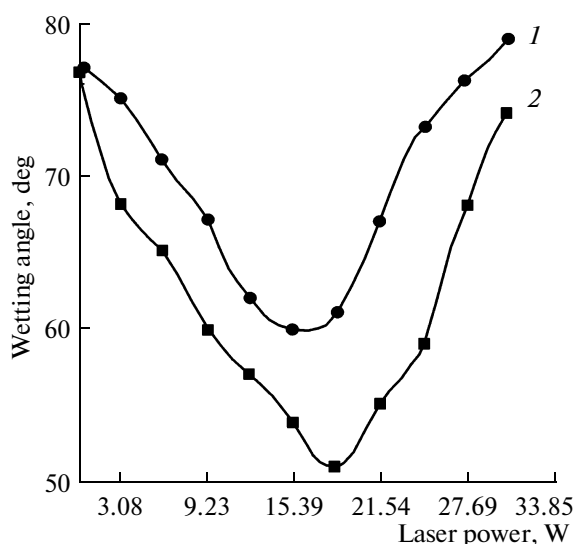


Fig. 6. Dependences of the marginal wetting angle for PHB scaffolds on pulse power at exposures of (1) 1 ms and (2) 3 ms.

(Fig. 5). The surface microstructure and properties were examined as dependent on the irradiation regime, and conditions were found whereby the marginal wetting angles could be reduced to 50° (versus 76 – 80° for initial specimens) (Fig. 6); i.e., scaffold hydrophilicity could be significantly increased without destroying the material structure. Analysis of cohesion between phases (plotted with Advanced Grafer ver. 2.06) revealed, for $\tau = 1$ ms, maxima at $\alpha = 63^\circ$ and 77° , corresponding to $W_{SL} = 0.11$ and 0.115 J/m 2 ; for $\tau = 3$ ms, the values were $\alpha = 51^\circ$ and 71° , $W_{SL} = 0.085$ and 0.095 J/m 2 .

Examining the mechanism of laser ablation, we calculated the possibility of shock waves in the polymer, which might lead to structural alterations by initiating polymerization of chains of other configuration on the PHB surface. On the strength of the known fact that generation of shock waves requires that the energy absorption time be shorter than the characteristic time of propagation of acoustical perturbations in the specimen, we could prove that shock waves and ensuing polymer destruction are impossible under the tested conditions.

Thus, we have theoretically substantiated and experimentally realized the conditions of laser treatment of PHB scaffolds that provide for improving the surface properties (increased hydrophilicity and adhesiveness) without destructive alteration of the material.

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