Structural Changes in Surface-Modified Polymers for Medical Applications

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Biological properties of synthetic polymers can be improved by surface modification with the use of liquid oxidizers. A resorbable biomedical polymer — poly(glycolide-\(\epsilon\)-caprolactone) (PGCL) was incubated in 0.1 M NaOH for 2, 6, 16, and 24 h, followed by excessive washing and drying in vacuum. Surface properties of the materials before and after modification were evaluated: wettability by contact angle measurements, topography by atomic force microscopy, and chemical functions by infrared spectroscopy. Applied modification improved wettability of PGCL due to creation of chemical oxygenated functionalities, and resulted in a slight alternation of the surface topography and roughness. In order to determine whether NaOH incubation caused structural changes in bulk of PGCL, positron annihilation lifetime spectroscopy, differential scanning calorimetry and viscosity measurements were performed. It was found that the \(\text{ortho-positronium lifetime}\) in PGCL declines as a function of modification time. It suggests that NaOH incubation causes structural changes in PGCL not only on the surface but also in bulk.

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1. Introduction

Resorbable polymers belonging to a group of aliphatic polyesters (polylactides, polyglycolide, poly-\(\epsilon\)-caprolactone) are very promising materials for various biomedical applications [1]. They have advantageous bulk properties such as strength, modulus, and degradability which can be also tailored to meet the needs of tissue engineering. Tissue engineering is a novel approach to create new tissues with the use of cells which are cultivated \textit{in vitro} on artificial scaffolding materials acting as a temporary extracellular matrix [2]. However, the surface

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of aliphatic polyesters is not favorable for cell culturing in vitro, mainly due to its relatively high hydrophobicity, which prevents optimal cell attachment and growth. Several methods have been developed to improve surface hydrophilicity of aliphatic polymers, such as plasma treatment [3], ion beam modification [4], graft polymerization [5] or soaking in liquid oxidizers (e.g. nitric acid, hydrogen peroxide, sodium hydroxide) [6]. Sodium hydroxide aqueous solutions have been used to create surface polar groups on the surface of aliphatic polyesters, which in turn decreased hydrophobicity and resulted in better adhesion and function of osteoblasts [7], fibroblasts [6] and chondrocytes in vitro [8]. On the other hand, applied modification can affect not only the surface and subsurface regions of polymeric materials but also the whole structure, which may deteriorate their bulk properties and biological performance.

Positron annihilation lifetime spectroscopy (PALS) is a powerful tool to probe atomic-scale free volumes in polymeric materials. Free-volume defects detected by PALS have been correlated with polymers’ gas permeability [9], degradation phenomena [10], crystallinity [11], etc. In our previous study we found that the average size of the molecular-level free-volume holes in the copolymer of glycolide and L-lactide decreased as a function of hydrolytic degradation, which was connected with adsorption of water molecules [12].

The objective of the present study is to modify the surface of poly(glycolide-\(\varepsilon\)-caprolactone) (PGCL) in sodium hydroxide aqueous solution in order to enhance its hydrophilicity. The surface properties were characterized by means of water contact angles, infrared spectroscopy (FTIR-ATR) and atomic force microscopy (AFM). In order to determine whether NaOH incubation caused structural changes in bulk of PGCL, PALS, differential scanning calorimetry (DSC) and viscosity measurements were carried out.

2. Experimental

2.1. Materials

A copolymer of glycolide and \(\varepsilon\)-caprolactone (PGCL), with molar ratio of glycolide to \(\varepsilon\)-caprolactone 10:90, was synthesized at the Centre of Polymer and Carbon Materials (Polish Academy of Sciences, Zabrze, Poland) according to the method described previously [13]. The number-average molecular weight \((M_n)\) of PGCL was 58 kDa, while the weight-average molecular weight \((M_w)\) was 110 kDa.

The PGCL films were cast from 10\% (w/v) polymer solution in methylene chloride on glass Petri dishes, followed by air drying for 24 h and vacuum drying for the next 72 h. Then the films were rinsed with ultrahigh purity water (UHQ-water produced by Purelab UHQ, Elga, UK) for 12 h. Afterwards, the films were air- and vacuum-dried for 24 h and 48 h, respectively.

Polymeric films (weight of 1.5 g, thickness of 150 \(\mu\)m) were soaked in 100 ml of 0.1 M NaOH (POCh, Gliwice, Poland) for 2, 6, 16, and 24 h at room temperature. After modification the films were extensively washed in UHQ-water,
followed by washing in the ultrasound bath (Sonic3, Polsonic, Poland). Effectiveness of washing was checked by pH and conductivity measurements of the water before and after contact with modified films.

2.2. Methods

The contact angle was measured by sessile drop method by an automatic drop shape analysis system DSA 10 Mk2 (Kruss, Germany). The UHQ-water droplet was 0.2 µL and each determination was obtained by averaging the results of 12 measurements.

Topography measurements were performed under ambient laboratory conditions with an Explorer atomic force microscope (ThermoMicroscopes, Vecco, USA). Contact mode topographic images were recorded using Si₃N₄ probes with a spring constant of 0.05 N/m and a nominal radius of curvature of 20 nm (Vecco NanoProbe™ Tips, model MLCT-EXMT-A). The images were recorded at scan areas of 50 µm × 50 µm for three randomly chosen places (300 × 300 data points) and with scan rate of 3 lines/s. All images were flattened using a third order polynomial algorithm provided with the instrument.

The surface chemical structure of the polymeric films was analyzed using a Digital FTS 60v (BioRad) spectrometer equipped with ZnSe crystal in the attenuated total reflection mode (FTIR-ATR). The samples were studied in the range of 600–4000 cm⁻¹ with a 4 cm⁻¹ resolution and averaging of 64 scans for each spectrum.

The intrinsic viscosity of polymer was measured by Ubbelohde viscometer in chloroform at 25°C.

Thermal properties, such as glass-transition temperature (T_g), melting temperature (T_m) and heat of melting (ΔH_m) were evaluated using differential scanning calorimetry with Netzsch DSC 200 apparatus calibrated with mercury and indium standards. The heating rate of 10°C/min was applied and argon was used as an inert gas with flow rate of 30 mL/min.

Positron lifetime spectra were measured using a conventional fast-fast spectrometer with BaF₂ scintillators. The time resolution of the system was 260 ps (FWHM). The 30 µCi activity positron source containing ²²Na isotope enveloped in 7 µm thick kapton foil was sandwiched between two polymer samples. Since the thickness of the polymer film investigated was equal to 150 µm, each sample consisted of the stack of 6 films. The density of the material was 1.28 g/cm³ and hence the mean positron implantation depth was equal to 177 µm. In this way 96% of positrons from the ²²Na source annihilated in the sample. Lifetime spectra containing ≈ 2 × 10⁶ counts were measured at room temperature. All obtained spectra were deconvoluted using the LT code, subtracting the background and the source component [14]. The obtained positron lifetime spectra were decomposed into three components. The first component value τ₁ was fixed at 0.125 ns. The value of τ₂ was ranged from 0.401 ns to 0.407 ns for all samples. In this study we are discussing the longest-lived component, τ₃. Average free volume was calculated
according to the semi-empirical model [15, 16] combining the pick-off lifetime \( \tau_3 \) with the average free-volume radius of a spherical hole \( V_f = \frac{4\pi R^3}{3} \):

\[
\tau_3 = \frac{1}{2} \left[ 1 - \frac{R}{(R + \Delta R)} + \left( \frac{1}{2\pi} \sin \left( \frac{2\pi R}{(R + \Delta R)} \right) \right) \right]^{-1},
\]

(1)

where \( R \) is a radius of the hole and \( \Delta R \) is an electron layer thickness. The value of \( \Delta R \) was estimated as 1.66 Å by fitting the longest lifetime value to known free volume sizes of molecular crystals [17]. Additionally, the Doppler broadening of the annihilation line was measured using the spectrometer with the HpGe detector with the energy resolution FWHM = 1.2 keV interpolated for 511 keV.

### TABLE

Properties of PGCL films as a function of modification time in 0.1 M NaOH.

<table>
<thead>
<tr>
<th>( t ) [h]</th>
<th>( \theta ) [°]</th>
<th>( R_{\text{RMS}} ) [nm]</th>
<th>( \eta ) [dL/g]</th>
<th>( T_g ) [°C]</th>
<th>( T_m ) [°C]</th>
<th>( \Delta H_m ) [J/g]</th>
<th>( S )-parameter</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>81.2 (2.1)</td>
<td>112 (25)</td>
<td>0.72</td>
<td>−57.6</td>
<td>53.8</td>
<td>77.8</td>
<td>0.5041 (0.0005)</td>
</tr>
<tr>
<td>2</td>
<td>71.6 (2.0)</td>
<td>111 (22)</td>
<td>0.71</td>
<td>−55.0</td>
<td>52.8</td>
<td>77.5</td>
<td>0.5050 (0.0004)</td>
</tr>
<tr>
<td>6</td>
<td>66.1 (1.7)</td>
<td>135 (31)</td>
<td>0.78</td>
<td>−</td>
<td>53.1</td>
<td>76.7</td>
<td>0.5045 (0.0004)</td>
</tr>
<tr>
<td>16</td>
<td>59.7 (1.6)</td>
<td>124 (15)</td>
<td>0.79</td>
<td>−</td>
<td>53.0</td>
<td>78.4</td>
<td>0.5053 (0.0004)</td>
</tr>
<tr>
<td>24</td>
<td>45.1 (2.3)</td>
<td>157 (24)</td>
<td>0.74</td>
<td>−</td>
<td>52.9</td>
<td>83.9</td>
<td>0.5049 (0.0002)</td>
</tr>
</tbody>
</table>

\( t \) — modification time in 0.1 M NaOH; \( \theta \) — water contact angle, \( n = 12 \), standard deviation in parentheses; \( R_{\text{RMS}} \) — root-mean square roughness, \( n = 3 \), standard deviation in parentheses; \( \eta \) — intrinsic viscosity; \( T_g \) — glass transition temperature; \( T_m \) — melting temperature; \( \Delta H_m \) — heat of melting; \( S \)-parameter — standard deviation in parentheses.

Fig. 1. FTIR-ATR spectra of PGCL before (0 h) and after modification in 0.1 M NaOH for 2 h, 6 h, 16 h and 24 h.
3. Results

Table presents the water contact angle and the root-mean-square surface roughness of raw PGCL films and after modification with 0.1 M NaOH for different periods of time. Applied modification resulted in a significant decrease in contact angle, indicating an increase in surface hydrophilicity.

Figure 1 presents FTIR-ATR spectra of PGCL films before and after 2, 6, 16, and 24 h modification in 0.1 M NaOH. In the spectrum of PGCL before modification the following bands are visible: in the range of 2800–3000 cm$^{-1}$ from CH$_2$ stretching vibrations, a band at 1720 cm$^{-1}$ from C=O stretching vibrations.

![AFM images](image)

**Fig. 2.** AFM topographical images of PGCL surface (scan areas of 50 µm × 50 µm): (a) raw surface (z = 700 nm), and (b) after NaOH treatment for 2 h (z = 840 nm), (c) 6 h (z = 660 nm), (d) 16 h (z = 920 nm), and (e) 24 h (z = 970 nm).
bands in the range of 1050–1250 cm$^{-1}$ from C–O and C–O–C stretching vibrations, and bands in the range 1300–1500 cm$^{-1}$ attributed to deformational CH$_2$ vibrations. Those bands are characteristic of aliphatic polyesters [18]. Applied modification resulted in shape changes of the bands attributed to C=O, C–O and C–O–C. The results indicate that NaOH treatment causes oxidation of polyester bonds in PGCL, and creation of oxygenated groups, which in turn increase surface polarity and improve hydrophilicity.

Figure 2 presents AFM topographical images of PGCL surface before and after modification in NaOH. On the surface of raw PGCL regular structures typical of semi-crystalline polymers, called spherolites, are present (Fig. 2a). Spherolites consist of crystalline lamella, which are better visible for smaller scan areas (data not shown). AFM images show that liquid oxidizer preferentially etches amorphous areas of PGCL situated between spherolites, which results in better exposition of crystalline lamella and spherolites (Fig. 2b–e). Treatment in NaOH provokes slight increase in the root-mean-square roughness of PGCL (Table).

The results of thermal properties and viscosity measurements are also presented in Table. They indicate that applied modification did not have an impact on viscosity of the polymeric films, suggesting that NaOH treatment did not influence molecular mass of PGCL. However, DSC analysis shows that for the raw sample the glass transition was at $−57.6\, ^\circ\text{C}$ with a corresponding specific heat change, $\Delta C_p$, equal to 0.81 J/(g$^\circ$C). After NaOH modification for 2 h glass transition appeared at $−55.0\, ^\circ\text{C}$ and a specific heat change, $\Delta C_p$, of 0.28 J/(g$^\circ$C) was measured. It is worth noticing that for all samples treated for longer periods of

![Graph](image-url)

Fig. 3. The lifetime of o-Ps ($\tau_3$) and its intensity ($I_3$) of PGCL as a function of treatment time in NaOH. The inset presents the dependence of the average free-volume hole size, $V_f$, according to the Tao–Eldrup [15, 16] model on treatment time of PGCL in NaOH.
time the glass transition was not present. Melting temperatures registered for all samples were the same taking into account resolution of DSC apparatus and experimental error, but a significant increase in heat of melting was detected for the sample incubated in NaOH for 24 h. It suggests that NaOH influences crystallinity of PGCL.

Figure 3 presents the lifetime of $\alpha$-Ps, $\tau_3$, and its intensity, $I_3$, as a function of modification time. The inset presents the dependence of the average free-volume hole size, $V_f$. The value of the $\alpha$-Ps lifetime and resulting average free-volume hole size decrease with treatment time, while the intensity, $I_3$, does not change within the experimental error. The $S$-parameter (defined as the integral in a central region of the annihilation peak divided by the peaks’ total integral) extracted from the Doppler broadening spectra, does not depend on the modification time.

4. Discussion

Liquid oxidizers applied to modify surface of aliphatic polyesters may also have a negative impact on their structure and bulk properties, thus precluding their further application in the field of medicine and tissue engineering. Therefore, in this study beside surface characterization, the structure of one particular resorbable polymer – PGCL was also characterized with the use of three different methods, namely PALS, DSC, and viscosity measurements.

The results show that indeed 0.1 M sodium hydroxide oxidizes PGCL surface and creates oxygenated functional groups. The intensity of the bands in FTIR-ATR spectra attributed to these functions increased with modification time. Presence of polar oxygenated functions on the surface of PGCL resulted in decrease in water contact angle and increase in surface hydrophilicity. NaOH treatment has also an impact on surface topography, because oxidative agent preferably etches amorphous parts located between crystalline spherolites and lamellas. As a consequence, spherolites and lamellas are better exposed, which is also reflected in the value of root-mean-square roughness.

Thermal analysis shows, however, that NaOH treats not only the surface but also the whole material, because glass transition temperature – being a sign of amorphous structures — increases after modification in NaOH for 2 h and disappears for the samples treated in NaOH for 6 h, 16 h, and 24 h. Concomitantly, a significant increase in the heat of melting is observed for PGCL sample soaked in NaOH for 24 h, indicating an increase in their crystallinity. On the other hand, treatment in sodium hydroxide does not influence significantly the viscosity of PGCL, suggesting that it does not cause chain-scission and decrease in molecular mass.

As it follows from the PALS spectroscopy, a decrease in the subnanometer free-volume holes size from 141 Å$^3$ to 133 Å$^3$ observed in PGCL as a function of modification time indicates reorientation of the polymer chains. It could be connected with an increase in crystallinity [19, 20]. However, decrease in the volume
of the polymer amorphous regions should be also accompanied by decrease in the $\alpha$-Ps intensity [20, 21]. However, in our case the $I_3$ does not change significantly with the modification time.

Another possible explanation could be that during treatment in liquid oxidizer hydroxyl ions, sodium ions or water molecules diffuse into the free volumes and react with polyester bonds in PGCL, thus decreasing the size of free volumes. This explanation is in accordance with our previous study on hydrolytic degradation of poly(L-lactide-co-glycolide) — another resorbable polymer belonging to a group of aliphatic polyesters. We found that the size of free volumes decreases within the first week of contact with water due to diffusion of water molecules inside the polymer structure [12].

In the present study we do not observe $S$-parameter changes as a function of the PGCL modification time. Constant value of $S$-parameter suggests that the momentum of annihilating electrons does not change. It seems thus that the chemical surrounding of a positron or Ps atom before annihilation is similar in all samples and the $\alpha$-Ps lifetime drop is connected with geometrical reduction of the free-volume hole size in the bulk of the polymer. This is in accordance with the value of mean positron penetration depth in PGCL estimated to be 177 $\mu$m.

To sum up, sodium hydroxide modification results in creation of polar oxygenated functions on the surface of poly(glycolide-co-$\varepsilon$-caprolactone) which in turn improve its hydrophilicity. In spite of this, reduction of the free volume hole size and increase in crystallinity indicate that liquid oxidizer affects not only the surface but also the bulk of the polymer.

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References


