Polymer drug release system for biofilm inhibition in medical application

Abstract: Bacterial biofilm formation on surfaces is still a critical challenge regarding the application of implants. Generally, in order to avoid this, an additional systemic administration of antibiotics is given, which can lead to side effects, such as the reduction of the intestinal flora. Continuous treatment may lead to antibiotics resistance. Within this study we investigated the local drug delivery of N-acetyl-L-cysteine (NAC) from a Poly-L-lactide (PLLA) coating, an isched biodegradable polymer and a polyetherurethane (PEU) coating, a promising representative non-degradable polymer for cardiovascular applications as alternative to the administration of antibiotics. The incorporation of NAC influenced the surface properties of PEU in contrast to that of PLLA. The in vitro NAC release is almost completed after 24 h for PEU. For PLLA only small amounts of incorporated NAC, depending on the NAC loading, is released after a short time. Both systems are rather useful as local NAC delivery system directly after implantation.

Keywords: NAC, PEU, PLLA, in vitro release.

1 Introduction

Biofilm formation of bacteria on medical surfaces, including implants, has become a worldwide and severe problem [1, 2]. Surface-associated bacteria, which are embedded in a complex matrix of extracellular polymeric substances (EPS), are highly resistant to antibiotics, to the human immune system and therefore hard to eradicate [3]. The inhibition of bacterial biofilm formation is therefore of utmost importance for artificial implants [4]. The administration of antibiotics can lead to side effects, such as reduction of the intestinal flora. Continuous treatment may lead to antibiotics resistance [5]. A promising alternative is the application of the mucolytic agent N-acetyl-L-cysteine (NAC). NAC is known to decrease biofilm formation by a variety of bacteria and reduces the production of an extracellular polysaccharide matrix, while promoting the disruption of mature biofilms [6, 7]. The coating and modification of implants, such as stents for the cardiovascular application, with biocompatible polymers in order to establish a local drug delivery is rather common. The advantages in contrast to the systemic drug application are the direct and local application of the substances and a much lower required drug amount for successful treatments. Thus, a promising approach for an efficient decrease of biofilm formation is the local delivery of NAC from modified implants.

In this study, we present the NAC incorporation in Poly-L-lactide (PLLA), a well stabilized biodegradable polymer for medical applications. A polyetherurethane (PEU) is used as a promising representative of a non-degradable polymer for cardiovascular applications. The influence of the NAC incorporation on the polymer properties, as well as the differences in the in vitro NAC release was investigated. In this context, the ability of both polymers for the short and long-term in vitro NAC release was examined.

2 Materials and Methods

2.1 Materials

The polymer Resomer L 210 S (poly(L-lactide), PLLA, intrinsic viscosity in chloroform: 3.8 dL/g), was received from Evonik Industries AG (Darmstadt, Germany) and commercially available polyether urethane (PEU) was used.
NAC was purchased from Sigma Aldrich (Munich, Germany). The 0.9 % saline solution was received from Fresenius (Bad Homburg, Germany). All other reagents and solvents were at least of analytical grade purchased from commercial suppliers.

2.2 Preparation and characterization of NAC containing PLLA or PEU coating

All polymer coatings were prepared using the following procedures: 0.22 g of PLLA or 0.24 g PEU was diluted in 80 mL chloroform or THF, respectively to get a final polymer concentration of 0.2 % (w/w) with respect to solvent weight. The coatings were loaded with 5 %, 10 % and 20 % (w/w) NAC with respect to polymer weight. The polymer/drug films were coated on cleaned glass slides via spray coating technology. The glass slides (Ø = 15 mm) were fixed in a self-made device. Then the slides were spray coated for 1500 s at a pressure of 0.3 bar. The distance between nozzle and slide was 40 mm. Then the slides were dried at 80 °C for PLLA and 40 °C for PEU under vacuum. In order to reach the required amount of 2000 µg polymer/NAC coating, the glass slides were weighed before and after coating and if necessary the spray coating process was continued.

The influence of modifications on the surface hydrophilicity was analyzed by contact angle measurements of sessile drops (ultra pure water) using a Contact Angle System (OCA 20, Dataphysics Instruments GmbH, Filderstadt, Germany). The resulting mean values (contact angle of water 9W) and standard deviations (SD) were calculated from (n = 5). The morphological changes were examined in a PEI Quanta FEG 250 (Philips, Eindhoven, The Netherlands) environmental scanning electron microscope (ESEM). Thermal analysis was carried out with a DSC 1 (Mettler Toledo GmbH, Greifensee, Switzerland) under a nitrogen purge. High purity indium and zinc were used for temperature calibration and indium standard was used for calibration of the heat of fusion (ΔH). The scans of n = 3 films per subgroup were performed from -20 to 180 °C at a heating rate of 20 °C/min. The data were analyzed with respect to glass transition (T_g).

3 Results

All investigated polymer surfaces were characterized via ESEM, contact angle measurements and DSC.

2.3 In vitro NAC release

The drug loading and in vitro drug release were quantified using a High Performance Liquid Chromatography System (HPLC) from Knauer, Berlin, Germany under the following conditions: column Eurospher 100 C18, 250 x 4 mm, mobile phase: 0.05 M KH_2PO_4, pH 4.7 (350/650 v/v), isocratic flow rate: 1 mL/min, temperature: 23 °C, detection wavelength: UV 210 nm, injected sample volume: 30 µL. The calibration was performed in a concentration range from 0.1 to 100 µg/L. The in vitro release studies were conducted in 0.9 % saline solution under sink conditions. The glass plates (Ø = 15 mm) coated with about 2 mg NAC containing PLLA or PEU were immersed in 1 mL saline solution and stored in an incubator at 37 °C and shaking conditions. At predetermined time intervals (1, 3, 5, 24, 48, 72, and 96 hours), the release solution was removed and replaced with 1 ml fresh saline solution. The quantities of the released NAC were determined by HPLC. The NAC concentration was determined as the sum of average released NAC divided by the total quantities of NAC remaining in the coating. Mean values and standard deviations were calculated from three individual specimens.

Figure 1: ESEM micrographs of PEU incorporated with (A) 0 %; (B) 5 %; (C) 10 %; (D) 20 % NAC.

The PLLA surface morphology seems not to be changed with the NAC incorporation (data not shown). In contrast, with increasing the percentage of NAC the PEU surface becomes more structured (Figure 1).

This is also reflected by contact angle measurements. The contact angles of all investigated PLLA surfaces are rather similar. For PEU, an increase of about 20° to the NAC loaded PEU were detected. The contact angles between the NAC loaded PEU themselves are not significantly different only a
slight trend towards a reduced contact angle with increased amount of NAC was determined (Figure 2).

DSC measurements were performed with pure PLLA and PEU in comparison with polymers incorporated with different weight percent (wt%) of NAC. For all experiments $T_g$ was determined (Table 1). $T_g$ of PLLA decrease from 74 °C to 65 °C (20 wt%) with increasing amount of NAC. For PEU, a decrease from 42 °C to 34°C (20 wt%) was detected.

![Figure 2](image.png)

**Figure 2**: Average water contact angle $\Theta_W$ ± standard deviation (SD) of pure and NAC (5, 10, 20%) loaded PLLA and PEU surfaces measured via sessile drops method (samples $n = 5$ each; *** $p<0.001$; ** $p<0.01$, * $p<0.05$ significantly different to pure PEU).

**Table 1**: Glass transition temperature $T_g$ (°C) ± standard deviation (SD) of NAC incorporated PLLA and PEU using differential scanning calorimetry.

<table>
<thead>
<tr>
<th>Polymer</th>
<th>$T_g$ (°C ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLLA</td>
<td>73.9 ± 0.8</td>
</tr>
<tr>
<td>PLLA_05 % NAC</td>
<td>70.3 ± 0.5</td>
</tr>
<tr>
<td>PLLA_10 % NAC</td>
<td>71.4 ± 2.4</td>
</tr>
<tr>
<td>PLLA_20 % NAC</td>
<td>68.3 ± 2.8</td>
</tr>
<tr>
<td>PEU</td>
<td>41.1 ± 2.5</td>
</tr>
<tr>
<td>PEU_05 % NAC</td>
<td>37.4 ± 3.2</td>
</tr>
<tr>
<td>PEU_10 % NAC</td>
<td>34.2 ±3.8</td>
</tr>
<tr>
<td>PEU_20 % NAC</td>
<td>38.6 ± 0.6</td>
</tr>
</tbody>
</table>

The in vitro release of NAC for both polymers is shown in Figure 3. For all systems a burst release within the first 24 h was determined. In the further course, only a moderate increase was detected.

![Figure 3](image.png)

**Figure 3** Cumulative in vitro NAC release from PLLA (A) and PEU (B) films in isotonic saline solution under shaken conditions and at 37 °C; (Ø = 15 mm; NAC 5, 10, 20 w%, $n = 3$.

For PLLA the released amount is increased with the incorporated amount of NAC. For PLLA_5 %, PLLA_10 % and PLLA_20 % a NAC release of approximately 20 % NAC, 68 % NAC and 80 % NAC within 7 days was determined, respectively. In contrast to PLLA, almost all incorporated NAC amounts from PEU, regardless the content of NAC, were released within the first 2 days.

### Discussion

PLLA and PEU were investigated regarding the incorporation and the in vitro release of NAC. NAC is known to decrease biofilm formation for a variety of bacteria and NAC may reduce the production of extracellular polysaccharide matrix while promoting the disruption of mature biofilm [6,7].

The incorporation of NAC only marginally influenced the surface morphology and the contact angles for PLLA in contrast to PEU. Here, the surface became more structured and the contact angle decreased with higher content of NAC. Thus, the surface hydrophilicity of PEU is increased.

Also the in vitro NAC release from the biodegradable polymers PLLA, and the non-degradable PEU showed
different release profiles. The released NAC amounts from PLLA are dependent from the incorporated amount of NAC. During the \textit{in vitro} release of 10 days only 20 % NAC for 5 wt% NAC, 68 % for 10 wt% NAC and 80 % NAC for 20 wt% NAC of the total content was released. Probably a small amount of PLLA is rather strongly embedded. Thus, the residual NAC located in the bulk will be released later with occurring PLLA degradation \cite{9}.

For PEU, almost all NAC was released within the first day independent from the incorporated amount. This fast NAC release is most probably related to relatively low T_g (in a range of 43 to 34 °C) in contrast to PLLA (in a range of 74 to 68 °C). The drug diffusion at 37 °C should be faster through a polymer when the polymers T_g is below or at this temperature and therefore the amorphous phase of the polymer is in a rubbery state. In contrast, drug diffusion will be slower when the polymers T_g is >37 °C and the amorphous phase of the polymer is in a glassy state \cite{10}.

\section{5 Conclusion}

The inhibition of biofilm formation from bacteria is important for artificial implants in cardiovascular application. In order to avoid this formation, the local drug delivery of NAC from a polymeric coating is a very promising approach. Both investigated systems released NAC very fast. Thus, for a fast local NAC delivery within a short-term after implantation, the incorporation in PEU or PLLA is useful and seems to be applicable also as coatings. For a drug release and prevention of the biofilm formation over a longer period of time the addition of substances such as polymers, which can act as a diffusion barrier have to be considered. This will be a topic in further investigations.

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\section{Author Statement}

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\section{References}

\begin{itemize}
  \item \cite{1} Donlan, R. M. Biofilms and device-associated infections. Emerging infectious diseases2001; 7:277-281.
  \item \cite{8} Zhao T, Liu Y. N-acetylcysteine inhibit biofilms produced by Pseudomonas aeruginosa. BMC Microbiol. 2010;10:140
  \item \cite{9} Alexis F. Factors affecting the degradation and drug-release mechanism of poly(lactic acid) and poly((lactic acid)-co-(glycolic acid)). Polym. Int. 2005;54:36–46. doi:10.1002/pi.1697.
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