Antibacterial poly(D,L-lactic acid) coating of medical implants using a biodegradable drug delivery technology

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Objectives: Biomaterial-associated bacterial infections present common and challenging complications with medical implants. The purpose of this study was to determine the antibacterial properties of a low molecular weight biodegradable poly(D,L-lactic acid) coating with integrated antibiotics gentamicin and teicoplanin.

Methods: Coating of Kirschner-wires was carried out by a solvent casting technique under aseptic conditions with and without incorporated antibiotics. Release kinetics of gentamicin and teicoplanin were studied in phosphate-buffered saline. Initial bacterial adhesion of Staphylococcus epidermidis on coated and bare implants was determined by radiolabelling and counts of detached viable organisms.

Results: The incorporated antibiotics showed a continuous release over a period of at least 96 h with an initial peak of release in the first 6 h. Attachment of non-viable microorganisms, detected by radiolabelled bacteria, was increased significantly by the polymer coatings (P < 0.05). In contrast, the number of viable bacteria was reduced by the pure polymer (P < 0.01) and further by the polymer–antibiotic combinations (P < 0.05).

Conclusions: Poly(D,L-lactic acid) coating of implants could offer new perspectives in preventing biomaterial-associated infections. Combinations with other drugs to formulate custom-tailored implant surfaces are feasible.

Keywords: polylactide, PDLLA, drug release, Staphylococcus, biomaterial

Introduction

Since the first applications of biomaterials in medicine, infections and deficient tissue-integration represent the most important complications, which still limit the unrestricted use of biomaterials in humans. Implant-associated infections account for nearly 50% of the estimated 2 million nosocomial infections in the United States per year, and infection rates up to 100% are reported for certain implants, like external fixation pins. Treatment of these infections is associated with high complication rates and places an enormous burden on both the patient and healthcare providers; prolonged hospital stay, increased morbidity and mortality, and serious economic sequelae being common consequences. It is possible that the risk of infection can be reduced by an antiseptic surface coating for medical implants. The purpose of this study was therefore to determine the anti-infective properties of a new biodegradable coating for medical implants with regard to Staphylococcus epidermidis, one of the most important pathogens in biomaterial-associated infections. This coating is based on a polymer of low molecular weight poly(D,L-lactic acid) (PDLLA) and can be combined with drugs like antibiotics or growth factors to establish a locally acting drug-delivery system.

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Materials and methods

The polymer coating

The commercially available Resomer R203 is a polymer of PDLLA with a molecular weight of 29,000 Da and was purchased from Boehringer Ingelheim (Ingelheim, Germany). The polymer is a racemic mixture of the D- and L-enantiomers of lactic acid and serves as a biodegradable coating for medical implants.

Implants were coated with PDLLA by a solvent casting technique. In brief, the drug-carrier was dissolved in ethylacetate (Sigma–Aldrich AG, Deisenhofen, Germany) at a concentration of 133.3 mg/mL. The coating solution was maintained on dry ice to prevent evaporation of the organic solvent and a subsequent increase in the polymer concentration. To create a local drug delivery system, 5% (w/w) of the antibiotics gentamicin sulphate (COM Pharma, Hamburg, Germany) and/or teicoplanin (Aventis, Frankfurt, Germany) were added to the polymer solution. The implants were coated by two dip-coating procedures to achieve a dense and regular polymer coating. All coating steps were carried out under aseptic conditions in a laminar air-flow.

Medical implants

Commercially available Kirschner-wires (K-wires) of stainless steel (ISO 5832-1; Synthes GmbH & Co. KG, Umkirch, Germany) and titanium alloy (TiAl6V4, ISO 5832-11 and ISO 5832-3; Synthes GmbH & Co. KG) were studied. The wires were cut into lengths, cleaned and sterilized by autoclaving. The wires used for microbiological and antibiotic release studies and their abbreviations are shown in Table 1.

Bacterial strains

A clinical isolate of a biofilm-forming strain of \textit{S. epidermidis} (strain SE 183) was used for the \textit{in vitro} studies. The test strain was susceptible to both gentamicin and teicoplanin (gentamicin MIC 2 mg/L, teicoplanin MIC 0.5 mg/L). Biofilm formation was demonstrated by qualitative assessment with the tube assay previously described by Christensen \textit{et al.} Stock cultures of the isolate were lyophilized or stored at \textit{−70°C}.

Preparation of bacteria

\textit{S. epidermidis} (SE 183) was cultured to late logarithmic growth phase on blood agar plates at 37°C for 18 h before testing. Bacterial cells were then resuspended in normal saline and adjusted to 5 \times 10^6 cfu/mL by visual comparison with a 0.5 McFarland standard. This suspension was diluted with normal saline to an inoculum of 2.5 \times 10^5 cfu/mL.

Antibiotic release

Drug release from antibiotic- and polymer-coated K-wires (\(n = 4\); size 1.5, length 150 mm; stainless steel) was studied in 10 mL of phosphate-buffered saline (PBS) at 37°C (pH 7.4). Samples were coated with the PDLLA polymer (133.3 mg/mL ethylacetate) including gentamicin or teicoplanin (5% w/w). PDLLA-coated samples without antibiotics served as a control. To assess antibiotic stability during the test period, PBS of the control groups was supplemented with the gentamicin and teicoplanin. Aliquots of 500 \(\mu\)L were taken at the time points 10 min, 1, 6, 24 and 96 h from the PBS and assayed for gentamicin and teicoplanin. Antibiotic concentrations were determined by the fluorescence polarization immunoassays TDx/TDxFLx Gentamicin assay (Abbott Laboratories, Abbott Park, IL, USA) and Innofluor Teicoplanin assay system (Opus Diagnostics, Fort Lee, NJ, USA).

Total bacterial adhesion

The total amount of live and dead adhering bacteria was studied in a radiolabelling experiment, as described by Christensen \textit{et al.} \textit{S. epidermidis} was grown in Mueller–Hinton broth (Oxoid GmbH, Wesel, Germany) substituted with \[^3\text{H}\]thymidine. After 24 h of incubation on a rotary shaker (100 rpm), inoculum suspensions were prepared as described earlier. Coated and bare K-wires of stainless steel and titanium alloy (\(n = 9\); size 1.8, length 12 mm) were incubated with the radiolabelled bacterial cell suspension for 2 h at 37°C under static conditions. Individual wires were removed with sterile forceps, washed three times with sterile normal saline and dried for 60 min at 37°C.

Table 1. Sample groups and surface coating of stainless steel (S) and titanium alloy (T) for the \textit{in vitro} tests

<table>
<thead>
<tr>
<th>Surface coating</th>
<th>Stainless steel</th>
<th>Titanium alloy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bare K-wires without coating (control group)</td>
<td>S1</td>
<td>T1</td>
</tr>
<tr>
<td>K-wires coated with PDLLA</td>
<td>S2</td>
<td>T2</td>
</tr>
<tr>
<td>K-wires coated with PDLLA and gentamicin (5.0% w/w)</td>
<td>S3</td>
<td>T3</td>
</tr>
<tr>
<td>K-wires coated with PDLLA and teicoplanin (5.0% w/w)</td>
<td>S4</td>
<td>T4</td>
</tr>
<tr>
<td>K-wires coated with PDLLA and gentamicin (1.7% w/w) and teicoplanin (3.3% w/w)</td>
<td>S5</td>
<td>T5</td>
</tr>
</tbody>
</table>
Antibacterial poly(D,L-lactic acid) coating

for 15 s and air-dried. After transfer to scintillation vials containing scintillation fluid, counts per minute were measured in a β-counter (1219 Rackbeta; LKB Wallac, Bromma, Sweden).

Adhesion of viable bacteria

Adhesion of viable bacteria was evaluated in a bacterial adhesion assay. Coated and bare K-wires of stainless steel and titanium alloy (n = 10; size 1.8, length 12 mm) were immersed in 2 mL of the bacterial suspension (2.5 × 10^5 cfu/mL normal saline) and incubated for 2 h at 37°C under static conditions. After washing in normal saline, the K-wires were placed in vials containing 2 mL of trypsin solution (1% w/w) and sonicated (Sonorex RK255H, 50 kHz; Bandelin Electronic, Berlin, Germany) for 15 min to remove the adhering microorganisms. Serial dilutions of each trypsin solution were plated on blood agar plates for quantification of viable organisms. Blood agar plates were incubated for 48 h at 37°C, and the cfu were counted visually.

Complete detachment of the adhering microorganisms by sonication was verified through scanning electron microscopy (SEM).

Morphological analysis—SEM

Representative K-wire specimens from the in vitro studies were prepared for SEM as follows. Specimens were fixed in an aqueous solution of 1% (v/v) glutaraldehyde (Serva, Heidelberg, Germany) in a buffer of sodium phosphate (dibasic) monohydrate (Merck KGaA, Darmstadt, Germany) and sodium hydroxide (Merck KGaA) for a minimum of 16 h. The buffer contained 18.8 g/L sodium phosphate (dibasic) monohydrate and 4.3 g/L sodium hydroxide. Dehydration was carried out in an ascending ethanol series (50–70–80–90–100%) and samples were dried in a critical point dryer (CPD 030; BAL-TEC AG, Balzers, Liechtenstein) with carbon dioxide. Once coated with gold palladium (Polaron Autocoating Unit SEM 5200; Quorum Technologies, Newhaven, UK) each specimen was examined through a low vacuum SEM (JEOL 5900; JEOL Germany GmbH, Eching, Germany).

Calculations and statistical methods

Data from bacterial adherence studies were compared for statistical significance using non-parametric methods and the method of closed testing procedure, with P < 0.05 considered significant (Kruskal–Wallis and Mann–Whitney tests).

Results

Morphological analysis

After the dip-coating procedure, a stable and regular PDLLA coating could be observed through SEM. Complete detachment of adhering bacteria was observed after sonication.

Antibiotic release

The concentrations of the released antibiotics gentamicin and teicoplanin in the elution buffer are shown in Figure 1. A continuous drug release could be demonstrated for at least 96 h for both antibiotics. After an initial peak of release in the first few hours, a slow and continuous drug release could be observed for the rest of the testing interval. Gentamicin showed a pronounced initial peak of release in the first hour. Teicoplanin release, in contrast, was delayed compared with gentamicin, the initial peak of release lasted ~6 h and the remaining teicoplanin was released to the PBS with kinetics similar to that of gentamicin. Both gentamicin and teicoplanin showed a marked variation of initial release between individual wires (between 3.87 and 10.58 mg/L for gentamicin and between 2.71 and 15.11 mg/L for teicoplanin after 6 h). Antibiotic concentrations of the control groups remained constant throughout the entire study interval and gentamicin and teicoplanin were stable in PBS in control experiments for at least 96 h.

Total bacterial adhesion

The total biomass of bacteria adhering to the wires was measured using the radioactively labelled bacteria method. In
this method, the number of counts per minute is directly proportional to the number of implant-adhering microorganisms, and independent of their viability (data not shown). The median counts and inter-quartile ranges (along with outliers) obtained for the different wires are shown in Figure 2. The titanium alloy used showed a slightly lower biomass of \textit{S. epidermidis} adhering to the wire than stainless steel alloys, although the results are not significantly different ($P > 0.05$). However, for both types of wire a marked increase in bacterial adhesion can be observed for all PDLLA-coated samples compared with the bare specimens. For all of the titanium alloy wires this increase was significant at the $P < 0.05$ level, whereas for the stainless steel wires the increases were statistically significant ($P < 0.05$) for all except the PDLLA/gentamicin-coated (S3) wire.

With regard to the effect of antibiotic integration, gentamicin showed a tendency ($P = 0.05 - 0.1$) to reduce bacterial biomass after 2 h compared with pure PDLLA. Integration of teicoplanin on the other hand significantly increased the biomass of \textit{S. epidermidis} compared with implants coated with pure PDLLA and the PDLLA/gentamicin combination ($P < 0.05$).

\textbf{Adhesion of viable bacteria}

The number of viable bacteria adhering to the stainless steel and titanium alloy implants was evaluated in a quantitative adhesion assay. Results are expressed in colony forming units per agar dilution plate (Figure 3), this measurement being a direct indicator of the number of viable counts adhering to the implants.
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The PDLLA-coated implants significantly reduced adhesion of viable staphylococci compared with bare K-wires made from either titanium or stainless steel alloy (P < 0.01). Combination of PDLLA with either gentamicin or teicoplanin or both antibiotics on the implant together reduced viable counts to almost undetectable levels (P < 0.05). Although there were no significant differences between gentamicin and teicoplanin (P > 0.05), there was a slight tendency towards less bacterial growth with gentamicin-containing coatings, and also between the titanium alloy and stainless steel (Figure 3).

Discussion

Bacterial adhesion to biomaterials and the capability of many microorganisms to form biofilms on foreign bodies are well-established steps in the pathogenesis of implant-associated infections.11-13 In a biofilm, bacteria are protected from the host immune defense11,14 and exhibit a marked but reversible increase in antibiotic resistance.11,15,16 Even high local drug concentrations beyond the MBC for planktonic microorganisms do not completely eradicate bacteria located in biofilms.15,17

As a result, prevention of bacterial colonization and biofilm formation is an important consideration and may be supported by the use of antiseptic surface coatings. In the present work, a new antibacterial surface coating using a biodegradable drug-delivery system was studied.

The chosen PDLLA coating enables the possibility of covering medical implants with a biodegradable and biocompatible surface coating. This polymer is applied to implants by a solvent casting technique that allows coating of alloys and plastics with polished, irregular or porous surface materials. Breakdown of the polyolactic acids is based on hydrolytic splitting of the polymer backbone over several months to form oligomers, and release of lactic acid, which is metabolized in the citric acid cycle of the organism.18-20

Incorporation of antibacterials into this coating to give a local drug-delivery system ensures high concentrations around the implant for long periods of time and the risks and side-effects for the host organism are minimized compared with systemic drug application.21

In this study, elution fluids were not changed, and an elution model that is broadly applied in the literature, especially with antibiotic loaded bone cement, was used.22,23 Powdered teicoplanin and gentamicin do not dissolve very well in the volatile organic solvents used, and the final coating on the implants consisted of antibiotic particles in the polymer. This leads to an initial peak of release and high variability between experiments, caused by fine drug particles located at, and washed out of the polymer coating surface, a process that is not well-defined. On the other hand, after the initial peak, subsequent release is very predictable and comparable for all samples, being primarily the result of diffusion of incorporated antibiotics from deeper levels of the coating.

In this study, we observed significant differences between the results of adhesion studies depending on whether total or viable bacteria were assessed. The coating increased the total amount of attached microorganisms but at the same time significantly reduced the number of viable bacteria even without antibiotics, suggesting that the bare PDLLA coating has bactericidal action against adhering S. epidermidis SE 183. Since most of the bacterial adhesion studies only employ one type of test, either counting the total number of attached microorganisms24-26 or counting only viable bacteria,27-29 results from these studies may be misleading. Since gentamicin and teicoplanin are integrated into the polymer as fine particles, drugs located at the surface can be washed out to leave small irregularities in the polymer. As one of the most important features in primary bacterial adherence is the topography texture,30,31 this may promote the bacterial adhesion seen in this study. Apart from morphological features of the polymer, physiochemical characteristics like surface charge influence bacterial adhesion as well. In the present study, teicoplanin increased the total number of adhering microorganisms compared with gentamicin. Gallardo-Moreno et al32 and Wilcox et al33,34 have previously described promotion of bacterial adhesion to implant surfaces by subinhibitory concentrations of glycopeptide antibiotics.32-34 Our results would suggest that this effect may also occur at inhibitory concentrations and highlight the importance of undertaking total as well as viable bacterial counts.

PDLLA has excellent features with respect to implant coating, with high mechanical stability,35 good osteoinductive potential36 and excellent biocompatibility in vivo.36 The material also shows good anti-thrombogenic characteristics.37 In this study we have been able to demonstrate that the antibacterials gentamicin and teicoplanin can be incorporated into the polymer to give local drug-delivery systems that reduce bacterial adhesion in vitro. We conclude that a combination of the antibiotic–polymer coating with other drugs to create a multifunctional and custom-tailored surface is therefore possible and could offer new opportunities in the use of established biomaterials.

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References

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