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# Antimicrobial surface coatings for a permanent percutaneous passage in the concept of osseointegrated extremity prosthesis

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## Abstract

The clinical implementation of percutaneous implants is still limited owing to infections at the side of the stoma. In our concept, this issue is addressed by designing copolymer surface coatings possessing biocompatibility and antimicrobial activity to improve the maintenance of a physiological skin seal at the skin-implant interface. Different copolymers with surface-active phosphonate and antimicrobial cationic groups were designed. Thus, coated titanium samples were cultured with bacterial strains or fibroblasts, respectively. Antimicrobial impact was evaluated by imaging the reduction of bacterial adherence. Biocompatibility was displayed by fibroblast proliferation and morphology. A variety of copolymers of 4-vinylpyridine with vinylbenzylphosphonate or dimethyl(2-methacryloyloxy-ethyl) phosphonate were prepared by free radical polymerization. The optimized polymer coating (copolymer D) showed a reduction of adherent bacteria up to 95%, with only a slight reduction in the adherence of human fibroblasts compared with blank titanium controls. In this study, we demonstrate *in vitro* that polymer surface coatings can be simultaneously antimicrobial and biocompatible. We consider this to be a promising technology for the realization of a permanent aseptic percutaneous passage as needed for the advancement of osseointegrated limb prosthesis.

**Keywords:** antimicrobial surface coatings; biocompatibility; copolymer coating; osseointegrated prosthesis; percutaneous implant.

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## Introduction

The percutaneous passage of medical devices is still an unsatisfactorily solved problem, mainly due to the permanent risk of infection at the side of the implant-skin interface. However, this approach appears to be a promising technology for many applications in medicine and is widely used today.

One interesting example for percutaneous implants is the osseointegrated amputation limb prosthesis. This concept was introduced in the 1990s by the Swedish group of Branemark and co-workers and means the direct anchorage of the device to the residual bone of the amputation socket [4]. At present, two of these systems are in clinical use in selected patients with limb loss [3, 6]. Meanwhile, around 200 patients have been treated in this way. The published studies indicate that this alternative prosthetic anchorage has the ability to improve the quality of life and mobility of amputees in comparison with conventional socket-attached devices [3, 6–8]. However, in both systems, the routine clinical implementation is still limited owing to infections at the side of the percutaneous passage [3, 17]. Although complications could be reduced by standardized treatment protocols and modifications of the implant design, infection or revision rates of about 50% are reported. Both introduced designs work with polished blank titanium at the side of the stoma. Thus far, both groups failed with the attempt to attach the skin and soft tissue to the implants. The use of a porous surface for the transdermal coupler did not lead to a dermal integration or a reduction of infections. The same applies to the use of solely antimicrobial surface coatings such as silver. It is reported that this impairs the secretion out of the depth and even lead to higher infection rates [2].

Other authors working on the realization of transcutaneous implants define the implementation of a stable dermal attachment to the device to be critical for long-term success of a permanent aseptic percutaneous passage [11–14]. This would be the key to prevent the superficial infection at the skin-implant interface and a subsequent sinus tract formation. Ideally, this should be combined with an antimicrobial active implant surface to prevent bacterial colonization and subsequent invasion into the depth. In our concept of osseointegrated limb prosthesis, we address this issue by designing surface coatings combining the characteristics of an antimicrobial effect with good biocompatibility and good fibroblast adhesion.

Synthetic polymers with antimicrobial activity are known [5, 10]. They are based on membrane-disrupting cationic macromolecules that often incorporate quaternary ammonium groups and a hydrophobic part that strongly interacts with the bacterial cell membrane. Besides the challenge to connect polymers covalently to implant surfaces without

loss of function, these antimicrobial active substances rarely provide biocompatible properties for the peri-implant host tissue. Targeting this issue, the development of copolymers that include functional groups to improve biocompatibility describes a promising approach.

Herewith, we present the synthesis and characterization of a copolymer system possessing biocompatibility and antimicrobial activity, which should help optimize the maintenance of a physiological skin seal at the skin-implant interface.

## Materials and methods

Titanium samples ($TiAl_6V_4$) were obtained from Otto Bock HealthCare GmbH (Duderstadt, Germany). Dimethyl(2-hydroxyethyl) phosphonate, 4-vinylpyridine (VP), and triethylamine were purchased from Acros Organics (Geel, Belgium). 2,6-Di-*tert*-butyl-4-methylphenol was obtained from Aldrich (Munich, Germany) and methacryloyl chloride from Alfa-Aesar (Karlsruhe, Germany).

Synthesis of dimethyl(2-methacryloyloxyethyl) phosphonate (DMMEP) and vinylbenzylphosphonate (VBP) was carried out according to previously described methods [9]. Copolymers with different ratios of VP and phosphonate monomers were synthesized through free radical copolymerization with azobisisobutyronitril as initiator. The copolymers VP-co-VBP and VP-co-DMMEP were characterized by nuclear magnetic resonance spectroscopy (NMR) and elemental analysis. The composition of the copolymers was calculated using carbon-to-nitrogen-ratio from elemental analysis, as reported earlier [16]. The following *N*-alkylation was achieved with 1-bromohexane in nitromethane, and the degree of *N*-alkylation was determined from the hydrogen-1 NMR peak ratios [16].

Immobilization of different copolymers was achieved by spin coating of copolymer solutions in methanol (10 mg/ml) on 13 mm polished and sonicated titanium samples. The plates were heated at 120°C for 24 h and then sonicated three times in methanol to remove uncoated polymer from the surface. Characterization of the immobilized films is performed by water contact angle measurements with the tilting plate method [19] (tilt angle of 45°) and ellipsometry with Multiscop (Optrel, Sinzing, Germany) to measure the film thickness. X-ray photoelectron spectroscopy (XPS) measurements were performed to determine the composition of the surfaces, as described earlier [16].

The antimicrobial capacity of the copolymer coatings was evaluated using the most relevant bacterial strains for the transcutaneous setting, *Staphylococcus epidermidis* and *Staphylococcus aureus*. The bacteria were seeded onto the sample plates at a density of $8.4\times10^5$ cells/mm$^2$. The bacterial suspension was incubated under gentle rotation for 1 h at 37°C. Polished blank titanium samples served as controls. Unattached cells were washed off, and the adherent cells were stained with 1% acridine orange. Bacterial adherence was analyzed through confocal laser scanning microscopy.

The copolymer with the best antimicrobial capacity was chosen for the biocompatibility testing. The samples were seeded with the relevant cell type, human dermal fibroblasts (HDFibs), at a density of $1.5\times10^4$ cells/sample. After an incubation period of 24 and 72 h, respectively, the number and viability of the cells attached were determined using a modified lactate dehydrogenase activity assay together with a corresponding standard curve as described elsewhere [9]. The data were displayed relative to the uncoated blank titanium control. Cell morphology of the fibroblasts attached was analyzed by scanning electron microscopy (SEM).

Statistical analysis was performed using the Scheffé test with a confidence level of ≤0.05. All values reported are given as the mean values and standard deviation.

## Results

A variety of copolymers of VP with VBP or DMMEP were prepared by free radical polymerization. These copolymers were *N*-alkylated with 1 bromohexane to form *N*-hexylpyridinium bromide groups (HexVP). The degree of *N*-alkylation reached values between 83% and almost completion. The elemental analysis showed a good correlation between the monomer ratio in the feed and the final copolymer composition (Table 1).

We attached the copolymers to titanium oxide surfaces by using the surface activity of the phosphonate groups [1]. The coating resulted in ultrathin polymer films at the nanometer scale, with thickness between 3 and 11 nm (Table 1). The

**Table 1** Copolymer composition and coating characteristics.

| Polymer | Inserted comonomer (ratio) | Resulting copolymer composition | Layer thickness (nm) | Contact angle (°) |
|---|---|---|---|---|
| A) VP:VBP | 0.75:0.25 | 0.78:0.22 | 4.5±0.6 | $\theta_{adv}$=56±2<br>$\theta_{rec}$=48±3 |
| B) VP:DMMEP | 0.75:0.25 | 0.79:0.21 | 3.1±0.1 | $\theta_{adv}$=63±1<br>$\theta_{rec}$=39±1 |
| C) VP:DMMEP | 0.50:0.50 | 0.59:0.41 | 3.5±0.3 | $\theta_{adv}$=67±1<br>$\theta_{rec}$=40±2 |
| D) VP:DMMEP | 0.30:0.70 | 0.24:0.76 | 10.9±2.9 | $\theta_{adv}$=74±1<br>$\theta_{rec}$=49±1 |
| X) Control [16] titanium blank | | | | $\theta_{adv}$=33<br>$\theta_{rec}$=22 |

average layer thickness depended on the copolymer composition as described before [16]. The contact angles of the copolymer coatings were determined to investigate the surface wettability of the films. They varied in the range of 56°–74° for $\theta_{adv}$ and in the range of 39°–49° for $\theta_{rec}$, depending on copolymer composition (Table 1). The higher the content of polar HexVP groups, the lower the contact angle values and therefore the lower the surface hydrophobicity.

The different distribution patterns for both *S. epidermidis* and *S. aureus* in comparison with blank titanium controls (Figures 1 and 2) clearly demonstrate an antimicrobial effect for some of the coatings. Copolymer A as well as copolymer B still showed a uniform colonization of bacteria on the surface, which was comparable to the blank titanium control. Only a slight increase in aggregation of bacteria could be observed. Copolymer C already showed a clearer ousting effect on the bacteria. The bacteria were concentrated in some areas. However, there was still no reduction in the overall number of bacterial cells. In contrast to this, the number of adherent bacteria was reduced by copolymer D to up to 95% compared with blank titanium samples.

Hence, biocompatibility testing was performed on copolymer D. After 24 h of incubation with HDFibs, only a slight, non-significant reduction in the amount of adhered cells on polymer coating in comparison with blank titanium controls could be observed. In the following 48 h, these differences stabilized as proliferation of HDFibs on coated samples proceeded in a comparable speed. No cytotoxic effect could be observed in the period of cultivation (Figure 3). Microscopic analyses of the samples were performed using SEM. The dried cells showed the same morphology as those on the blank titanium control. Thus, there were no adverse effects on cell morphology due to the polymer coatings (Figure 4).

## Discussion

The mentioned example of osseointegrated extremity prosthesis is a promising application for transcutaneous implants and illustrates the prospects of this technology. However, the clinical implementation of this concept is still limited owing to infection at the side of the percutaneous passage and the unsolved problem of implementing a stable stoma.

To improve the skin-implant interface, copolymers with antibacterial activity were designed in this study. The copolymer design had to be a compromise between the antibacterial activity and biocompatibility to enable an attachment of the skin to the implant. On the basis of the concept of membrane-disrupting cationic macromolecules reported in the literature [5, 10], we designed antimicrobial surfaces combining the biocompatibility and immobilization ability of phosphonate groups [1] with the antimicrobial effect of quaternized poly(4-vinylpyridinium) [16, 18].

In our study, copolymers of VP with VBP or DMMEP with different compositions were prepared by free radical polymerization. In this way, the influence of composition on antimicrobial activity as well as on biocompatibility was investigated. An increase of the HexVP content resulted in a decrease of the contact angle values (Table 1), leading to a decrease in surface hydrophobicity. An increase of the content of DMMEP, which is a relatively hydrophilic monomer and gives homopolymers with non-fouling properties, improves the antibacterial activity and simultaneously decreases the biocompatibility. In the *in vitro* test used, the hindered adhesion of cells to the very hydrophilic DMMEP gives the impression of a lower biocompatibility, although there is no evidence for toxicity. These effects demonstrate that a balance must be found between a good antibacterial activity and an acceptable biocompatibility by specifying a defined composition of the copolymer. This challenge was achieved best by copolymer D.

As demonstrated in Table 1, the elemental analysis showed a good correlation between the monomer ratio in the feed and the final copolymer composition. The corresponding copolymerization parameters could be determined as has been described before [16]. Thus, any user-defined copolymer composition can be adjusted.

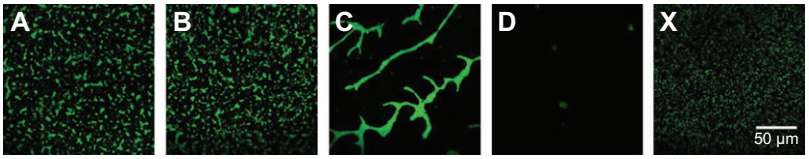


**Figure 1** The effect of various polymer coatings on titanium plates (A–D) regarding the adhesion of *S. epidermidis* after 1-h incubation *in vitro* in comparison with uncoated control (X).

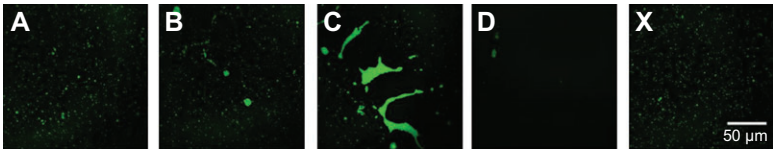


**Figure 2** The effect of various polymer coatings on titanium plates (A–D) regarding the adhesion of *S. aureus* after 1-h incubation *in vitro* in comparison with uncoated control (X).

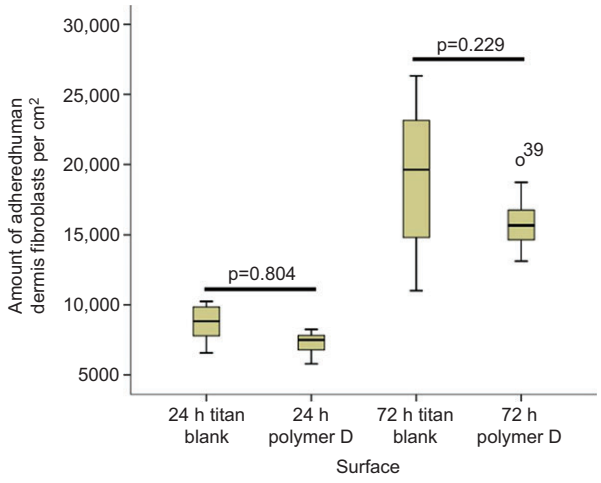


**Figure 3** Quantification of adhered HDFibs on polymer-coated titanium plates (copolymer D) in comparison with uncoated control (Titan blank) after 24 or 72 h of incubation (confidence level $p \leq 0.05$).

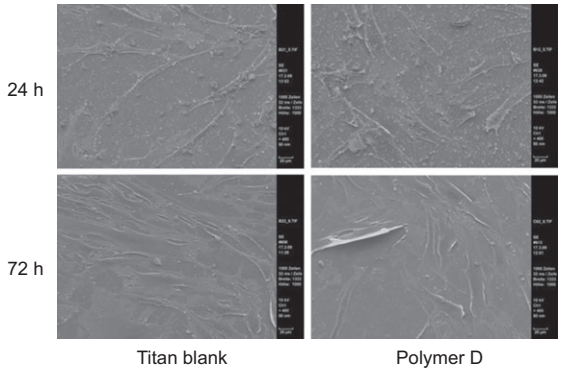


**Figure 4** SEM documentation of adhered HDFibs on polymer-coated titanium plates (copolymer D) in comparison with uncoated control (titanium blank) after 24 or 72 h of incubation.

In our bacterial model, copolymer D showed the desired high antimicrobial impact on different clinical relevant pathogens. Moreover, it showed a good biocompatibility to HDFibs, which is characterized by only slightly non-significant reduced initial adhesion, comparable morphology, and an appropriate proliferative activity on the coated material compared with blank titanium.

Admittedly, the limitation of this study is the *in vitro* design of the experiments under highly standardized conditions and with an isolated bacterial strain. Thus far, there is very limited experience with copolymer coatings in an *in vivo* setting. However, this criticism also applies for the other mentioned research efforts on bioactive surface coatings for the transcutaneous passage [11, 14, 15].

Thus far, our results confirm the possibility of designing copolymers as surface coatings that simultaneously show an antimicrobial effect and a good biocompatibility. Copolymer D seems to be suitable for further research to evaluate the aforementioned specifications in an *in vivo* setting. Further issues that must be addressed in the future would be the influence of the environment as well as of medical procedures and sterilization on the copolymer coating. We consider the proven basic characteristics of this copolymer composition and the overall concept of copolymer coatings to be a promising technology for the improvement of a permanent aseptic percutaneous passage as needed for the advancement of osseointegrated limb prosthesis.

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