Materials Science and Engineering C 61 (2016) 712–719

Contents lists available at ScienceDirect

Materials Science and Engineering C

journal homepage: www.elsevier.com/locate/msec

In vivo comparative study of tissue reaction to bare and antimicrobial polymer coated transcutaneous implants

Tilman Calliess [a,b], Ivonne Bartsch [a,b], Maike Haupt [a,b], Mattias Reebmann [a,b], Michael Schwarze [a,b], Meike Stiesch [c], Cornelia Pfaffenroth [d], Magda Sluszniak [d], Wibke Dempwolf [d], Henning Menzel [d], Frank Witte [e], Elmar Willbold [a,b,*]

[a] Laboratory for Biomechanics and Biomaterials, Hannover Medical School, Anna-von-Borries-Straße 1-7, 30625 Hannover, Germany
[b] CrossBIT, Center for Biocompatibility and Implant-Immunology, Feodor-Lynen-Straße 31, 30625 Hannover, Germany
[c] Clinic of Prosthetic Dentistry and Biomedical Materials Science, Hannover Medical School, Carl-Neuberg-Straße 1, 30625 Hannover, Germany
[d] Institute for Technical Chemistry, Braunschweig University of Technology, Hans-Sommer-Straße 10, 38106 Braunschweig, Germany
[e] Julius Wolff Institute and Center for Musculoskeletal Surgery, Charité — Universitätsmedizin Berlin, Augustenburger Platz 1, 13353, Berlin, Germany

ARTICLE INFO

Article history:
Received 3 February 2015
Received in revised form 3 December 2015
Accepted 31 December 2015
Available online 4 January 2016

Keywords:
Transcutaneous implants
Polymer coating
Antimicrobial
Epithelialization
In vivo

ABSTRACT

We coated transcutaneous implants made of titanium alloy Ti6Al4V with copolymer dimethyl (2-methacryloyloxy-ethyl) phosphonate and 4-vinylpyridine and investigated the tissue reaction with respect to its biocompatible and antimicrobial properties in vivo. We distinguished between clinically observable superficial inflammations and histologically detectable deep infections. The vinylpyridine moieties were transferred into cationic pyridinium groups by reaction with hexyl bromide. Thus polymers with both antimicrobial capacity and good biocompatibility were obtained. In a short-term study, we implanted specially designed bare or coated implants in hairless but immunocompetent mice and analyzed the tissue reaction histologically. No difference was found between bare and coated implants in the initial healing phase of up to 14 days; however, after 21 days the scar tissue formation was higher in the bare implant group. The degree of epithelial downgrowth was comparable in both groups at any time point. In a long-term study of up to 168 days, we analyzed resistance to infection. In the bare implant group, 7 of the 12 implantation sites became infected deep whereas in the coated implant group only two deep infections were observed. The other implantation sites showed only superficial signs of inflammation. These results generally accord with previous in-vitro studies.

1. Introduction

In modern medicine, transcutaneous implant devices are used in several applications, e.g. as catheters, dental implants, external hearing aid devices, ventricular assist devices or even orthopedic implants to replace lost limbs [1]. Some of these implants are used in sterile sites, but most of them penetrate the skin barrier. The latter ones come unavoidably in contact with environmental microorganisms thus making the implant and the implant tract a gateway for possible infections. In orthopedics, the concept of osseointegrated and transcutaneous implants began with Brånemark and co-workers, who introduced a direct anchorage of extremity prostheses to the residual bone of the amputation socket [2]. This approach was intended to serve as an alternative to the conventional socket attachment, mainly for patients who had insurmountable problems with the socket attachment and therefore had severely limited mobility [3]. First experiences with this new technique indicated that the direct anchorage of the prosthesis to the skeleton improved the quality of life and mobility of the amputees significantly. Today, different experienced groups [4–11], implant these prostheses worldwide with remarkable success and a high degree of patient satisfaction. However, for all used implant systems [12], routine clinical implementation is still limited owing to the occurrence of infections next to the transcutaneous passage [13–15]. In earlier studies, infections or revision rates of about 50% were still reported [16]; however, using modified implant designs and improved rehabilitation protocols, infections were significantly reduced [17].

Nowadays, much is known about tissue–material interactions at the bone–implant interface [18,19], but comparatively little is known about the skin–implant interface [20,21]. The transcutaneous passage is vulnerable to the function of the skin barrier and this interface is a possible gateway for infections, which can lead to severe complications up to implant failure or sepsis. The prosthetic designs, which are yet to be introduced, incorporate polished blank titanium next to the transcutaneous

* Corresponding author at: Laboratory for Biomechanics and Biomaterials, Department of Orthopaedic Surgery, Hannover Medical School, Anna-von-Borries-Straße 1-7, 30625 Hannover, Germany.
E-mail address: willbold.elmar@mh-hannover.de (E. Willbold).

http://dx.doi.org/10.1016/j.msec.2015.12.095
0928-4931/

passage. None of the research groups have yet succeeded in attaching the skin and soft tissue to the implants. The use of a porous surface for the transdermal coupler did not lead to soft-tissue integration or reduced infections, nor did the use of solely antimicrobial surface coatings such as silver [6,22,23]. Other research approaches for the realization of transcutaneous implants cite the implementation of a stable dermal attachment to the device as being critical to the long-term success of a permanent aseptic transcutaneous passage [24–28]. To overcome the problem of microbial infections of the transcutaneous passage, different implant surface modifications or coatings were proposed and evaluated [29–32]. Amongst these approaches, antimicrobial polymeric materials gained more and more attention during the last decades [33,34]. These polymers can be attached to surfaces without losing their biological activity [35,36] and therefore they are promising candidates for reducing local infections.

The study presented here is part of a research project designed to overcome existing limitations of osseointegrated limb prostheses [37–39]. Within the framework of this project, we focused on the production of multifunctional copolymer coatings that combined antibacterial activity and good biocompatibility with the aim of preventing deep implant infections and superficial inflammations as well as improving the integrity of the skin–implant interface. Here we used a copolymer in which the ability of phosphonate groups to immobilize the copolymer on titanium/titanium oxide [40] is combined with the antimicrobial effect of quaternized poly(4-vinylpyridinium) [41]. The copolymer is prepared by free-radical copolymerization of 4-vinylpyridine and dimethyl (2-methacryloyloxy-ethyl) phosphonate (DMMEP; Fig. 1) in a ratio of 30:70 and subsequent polymer-analogous reaction then creates N-hexylpyridinium groups (VP) along the polymer chain ([41]; further on called VP-co-DMMEP).

In an in-vitro study, the functionality and the bioactivity of VP-co-DMMEP had already been successfully proven [42]: Compared with bare implants, VP-co-DMMEP-coated implants reduced the number of adherent bacteria by up to 95% and showed a clear antimicrobial effect with respect to *Staphylococcus aureus* and *Staphylococcus epidermidis*, which represent the most common pathogenic bacterial species in clinical settings. Additionally, after 24 h of cultivation, human dermal fibroblasts showed normal morphology as well as normal adhesion and proliferation patterns, indicating good biocompatibility.

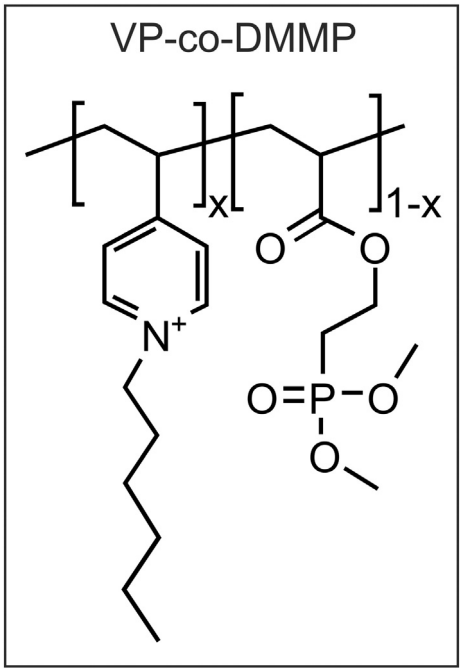

Fig. 1. Molecular structure of VP-co-DMMP.

The purpose of the present study was to further investigate these promising observations by an in-vivo study using VP-co-DMMEP-coated implants versus bare implants in hairless but immunocompetent mice. We focused especially on the structure of the transcutaneous passage and morphological features [43] such as epithelial downgrowth 7, 14 and 21 days after implantation. Possible infection patterns of the contact tissue in a long-term experiment were also studied. We hypothesized that the antimicrobial and biocompatible surface coating: (1) would lead to a reduction in the clinical apparent infections at the skin–implant interface, and (2) would improve the attachment of the soft tissue to the device.

2. Materials and methods

2.1. Preparation and production of transcutaneous implants

For the in-vivo simulation of a transcutaneous device, implants made of titanium alloy Ti6Al4V were designed and manufactured (Otto Bock HealthCare, Duderstadt, Germany; Fig. 2 (a)). A perforated round disc (10 mm diameter, and 1 mm thickness) with eight peripheral holes was designed as a subcutaneous soft-tissue anchor, where the central pin (3 mm diameter, 5 mm height) is serving as the transcutaneous part of the implant (Fig. 2(b)). The total weight of the implant was 0.5 g. These implants were then manually polished. Half of these polished implants were used as controls (further on called *bare implants*), the other half were coated with the polymers by dip coating in VP-co-DMMEP at a ratio of 30:70 (further on called *coated implants*). Synthesis of copolymer was carried out according to previously described methods [41,42,44]. The implants were then heated at 120 °C for 24 h and sonicated three times in methanol to remove any excess of non-bound polymer from the surface. This procedure results in polymer films with a thickness of approximately 5 to 7 nm. These thicknesses have been investigated via ellipsometry (Multiscope, Optrel, Sinzing, Germany) on polished Ti6Al4V references of the same size and geometry as the implants, but without the peripheral holes. These references have been prepared within the same passage as the implants. Using in contrast the spin coating procedure for the coating of titanium discs, a thickness of about 11 nm can reproducible be obtained [41,42]. Further characterization of the immobilized films was done by water contact angle measurements using the tilting plate method [45] (tilt angle of 45°) to determine the wetting behavior. The composition of surfaces coated with VP-co-DMMEP was determined exemplarily by X-ray photoelectron spectroscopy (XPS) measurements on titanium discs [41]. Prior to implantation, the implants were gamma-sterilized with 25–29 kGy of cobalt-60 radiation (BBF Sterilisationsservice, Kernen, Germany). No differences of the layer thicknesses could be observed.

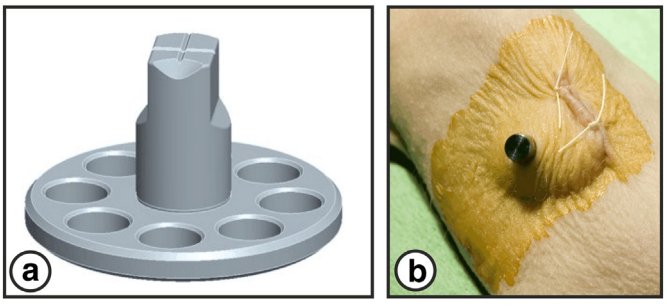

Fig. 2. The transcutaneous implant consists of a perforated disc for subcutaneous anchoring and a pin serving as the transcutaneous part (a). A longitudinal incision, a subcutaneous pocket and a round opening were created; the implant was then placed in the pocket (b).