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ScienceDirect
Acta Biomaterialia 5 (2009) 1905–1910
Acta BIOMATERIALIA
www.elsevier.com/locate/actabiomat

Antimicrobial effects of an NO-releasing poly(ethylene vinylacetate) coating on soft-tissue implants in vitro and in a murine model

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Received 3 November 2008; received in revised form 16 January 2009; accepted 26 January 2009
Available online 5 February 2009

Abstract

Infection of surgical meshes used in abdominal wall reconstructions often leads to removal of the implant and increases patient morbidity due to repetitive operations and hospital administrations. Treatment with antibiotics is ineffective due to the biofilm mode of growth of the infecting bacteria and bears the risk of inducing antibiotic resistance. Hence there is a need for alternative methods to prevent and treat mesh infection. Nitric oxide (NO)-releasing coatings have been demonstrated to possess bactericidal properties in vitro. It is the aim of this study to assess possible benefits of a low concentration NO-releasing carbon-based coating on monofilament polypropylene meshes with respect to infection control in vitro and in vivo. When applied on surgical meshes, NO-releasing coatings showed significant bactericidal effect on in vitro biofilms of *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and CNS. However, using bioluminescent in vivo imaging, no beneficial effects of this NO-releasing coating on subcutaneously implanted surgical meshes in mice could be observed.

Keywords: Abdominal wall reconstruction; Biofilm; Mesh infection; Monofilament polypropylene mesh; In vivo imaging

1. Introduction

Synthetic surgical meshes are widely used for the tension-free repair of abdominal wall defects and have clinically proven their value by lowering the recurrence rate of defects of the abdominal wall. Proper sterilization, introduction of laparoscopic techniques and strict guidelines have greatly reduced the likelihood of mesh infection [1]. Nevertheless, infection remains a serious concern in the application of surgical meshes for abdominal wall defects, which are costly, traumatic for the patient and associated with a high morbidity and even mortality. *Staphylococcus aureus* and *Staphylococcus epidermidis*, together with Gram-negative species including *Escherichia coli* and *Pseudomonas aeruginosa*, are held responsible for the majority of abdominal wall related mesh infections [2]. Bacteria can be found in approximately 90% of all implantation sites immediately after surgery and these organisms are held responsible for the occurrence of both early- and late-onset mesh infec-

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doi:10.1016/j.actbio.2009.01.041

tion [3]. Preferentially, bacteria on implants should be targeted before a mature biofilm can develop. In orthopedic implant surgery, for instance, local antibiotic-releasing bone cements and fleeces are applied to prevent and treat implant-associated infections [4]. The prevention of infection is of special interest in the case of revision surgery, since the risk for a secondary implant to become infected is much higher than for a primary implant [5,6]. Often, the infecting organisms have acquired antibiotic resistance [7] during the initial treatment of an infected primary implant, which makes the use of antibiotics as a preventive measure in revision surgery more difficult and alternative methods have to be considered.

Nitric oxide (NO) is an important mediator in the immune response. The arginine-dependent synthesis of NO by activated macrophages is responsible for both cytotoxic and cytostatic effects on pathogenic organisms [8]. These destructive effects are the result of oxidative and nitrosative stress generated by reactive intermediates of NO that result in damage to DNA, proteins and cell membranes [9]. The human body metabolizes NO very rapidly, preventing toxic levels from developing [10,11]. Nevertheless, localized high levels of NO in the vicinity of an implant may reduce the risk of both early and late infection as the NO will kill peroperatively introduced bacteria. It has been shown that coatings capable of releasing NO significantly inhibit adhesion and survival of *P. aeruginosa* on implant surfaces in vitro [12–15]. A number of studies have evaluated the effects of NO in vivo, as released from nitrogen-based (N-based) polymer coatings, such as N-based diazeniumdiolate coatings. Beneficial effects in vivo have been found on wound healing and tissue integration [16–18], as well as a remarkable decrease in bacterial presence on biomaterial surfaces [19]. However, a major disadvantage associated with all N-based diazeniumdiolates is their potential to form carcinogenic nitrosamines upon decomposition [20]. This limits the N-based diazeniumdiolate class of NO donors from consideration as therapeutic agents. In contrast, carbon-based (C-based) diazeniumdiolates are structurally unable to form nitrosamines while maintaining their ability to spontaneously release NO under physiological conditions, which makes them more suitable candidates for in vivo application.

A newly developed C-based NO-releasing polymer [21] coating applied on a surgical mesh could provide a safe first line of defense against invading pathogens. It is the aim of this study to evaluate potential benefits of a C-based NO-releasing polymer coating on surgical meshes with respect to their infection control in vitro as well as in vivo, using bioluminescent imaging. In vivo imaging is currently emerging as a technique to longitudinally monitor biomaterials-related infections in living animals [22–24]. Moreover, in vivo imaging enables longitudinal observation not only of actual mesh infection, but also of its spreading into surrounding tissue [22].

2. Materials and methods

2.1. NO-releasing coating of surgical meshes

Eight-millimeter-diameter pieces were stamped from a monofilament polypropylene mesh (SurgiPro™ Mesh, US Surgical, Norwalk, CT, US) using a dermal biopsy punch. A poly(ethylene-vinylacetate) copolymer (PEVA, 30% acetate) film was prepared by dip-coating the mesh samples with 10 mg ml^{-1} solution of PEVA in tetrahydrofuran (THF) as described by Kalivretenos et al. [21]. Dip-coating was done for 10 s, after which coated meshes were removed from THF and cured at 50 °C for 15 s, using a stream of warm air. Then the PEVA was converted to a C-based diazeniumdiolate by placing the samples in a 300 ml Ace pressure bottle and adding 50 ml dimethylformamide solution (DMF) and 1.07 g sodium trimethylsilanolate. The vessel was degassed with argon, pressurized with 76 psi NO gas and gently shaken for 18 h. At this time the vessel was purged with argon gas and the coated mesh samples were washed with THF and aspirated to dryness to yield a light yellow coating. The samples were then stored at room temperature in a dry environment. NO release was confirmed by a positive Griess reaction and utilizing an NO detector according to Smith et al. [25]. Briefly, coated meshes were placed in a closed reaction vessel with 4 ml of phosphate-buffered saline (PBS, 10 mM potassium phosphate and 150 mM NaCl, pH 7.4) at 37 °C. The vessel was flushed with nitrogen gas via a fritted glass tube at the bottom of the vessel at a flow rate of 0.7 l min^{-1} into a chemiluminescent NO detector (Thermo Environmental Instruments Model 42C, Franklin, MA, US) set to monitor NO content. The NO release was measured daily and expressed as pmol NO released min^{-1} mesh^{-1}. Measurements were continued until NO release was beyond detection. Prior to implantation, NO-releasing meshes were sterilized for 20 min in 70% ethanol, with the pH adjusted to pH 12 by addition of KOH in order to avoid premature NO release.

2.2. Biofilm formation and evaluation in vitro

Four different bacterial strains were isolated from implant related infections in patients: *S. aureus* AE8391, coagulase-negative staphylococcus (CNS) DN7334, *E. coli* AE8392 and *P. aeruginosa* DN7348. A bioluminescent mutant, *S. aureus* Xen29, was obtained from Xenogen Corporation (now: Caliper Life Sciences, Hopkinton, MA, USA). The *S. aureus* strain ATCC 12600 was made bioluminescent by stably integrating a modified *lux* operon onto its chromosome. This was achieved using the plasmid pXen-5 (pAUL-A Tn *4001 luxABCDE* Km^r) as described previously [23]. This modification enables the strain, designated Xen29, to produce luciferase and its substrate constitutively, resulting in a photon-emitting state when metabolically active. Its bioluminescent emission is stable for its progeny and can be detected transcutaneously. Bacteria were cultured from cryopreservative beads (Protect

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