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Nanostructured medical sutures with antibacterial properties

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ABSTRACT

Bacterial repellence in suture materials is a desirable property that can potentially improve the healing process by preventing infection. We describe a method for generating nanostructures at the surface of commercial sutures of different composition, and their potential for preventing biofilm formation. We show how bacteria attachment is altered in the presence of nanosized topographies and identify optimum designs for preventing it without compromising biocompatibility and applicability in terms of nanostructure robustness or tissue friction. These studies open new possibilities for flexible and cost-effective realization of topography-based antibacterial coatings for absorbable biomedical textiles.

1. Introduction

Sutures are natural or synthetic textile biomaterials used for tissue approximation during wound closure. For successful tissue fixation suturing materials need to have adequate mechanical strength, good tissue compatibility as well as biodegradation kinetics. Ideally the suture material should resist bacterial adherence to prevent wound infection. This property depends on the chemical composition of the suture itself and on supplementary antibacterial modifications [1]. Two major antibacterial strategies have been applied to sutures and biomaterials in general [2–9]: passive coatings based on cationic biopolymers that prevent bacterial attachment [10–13], or active strategies that release active compounds into the tissue and kill suspended bacteria (mainly silver [14–17], antimicrobial peptides [18,19] or antibiotics [20–22]). While passive strategies are preferred in terms of biocompatibility, active strategies are often more effective. However, toxicity issues and the development of resistant microbial strains compromise their application [23].

Particular surface micro- and nanotopographies have also shown the capacity of preventing bacterial attachment and biofilm formation [24–30], though the mechanism behind bacterial repellence and its correlation with the surface design remains to be unraveled. Reported studies on this topic mostly rely on surface nanostructures obtained by patterning techniques used in the microelectronic industry, which can hardly be implemented in biomaterials or in curved geometries as suture threads. Recently, high aspect ratio silicon nanopillars obtained by reactive ion etching (RIE) have been shown to kill bacteria upon contact [31]. Although a topography-based bactericide seems convenient because it avoids release of toxic components, silicon finds little use for low cost manufacture of biomedical devices and biomaterials. Alternative antibacterial strategies allowing cost-effective nanopatterning of established biomaterials in non-planar geometries would be desirable for real application.

Nanotopographies on textile fibers have recently been generated by plasma treatment [32]. Plasma treatment involves chemical changes, ablation and etching of the fiber surface, and eventually deposition of material. The extent of these processes depends on the plasma treatment conditions and the material itself [33,34].

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Recent studies have shown that oxidative plasma treatment of polymer surfaces of different compositions can lead to controlled etching of the polymer surface and formation of distinct nano-topographies with variable geometrical design and dimensions [35–45]. In this manuscript we apply this method to nanopattern commercially available suturing threads. We show nanopatterned sutures of different composition, including absorbable and non-absorbable ones. We demonstrate differential bacterial attachment depending on the suture nanotopographies and we identify optimum geometries for preventing attachment without compromising biocompatibility and applicability in terms of nanostructure robustness or tissue friction are identified. These studies open new possibilities for realization of topography-based antibacterial coatings.

2. Materials and methods

2.1. Materials

Different sutures USP 2/0 from the company B. Braun were used for these studies: non-absorbable monofilaments of polypropylene (PP, Premilene®) and poly(ethylene terephthalate) (PET, Miralene®) and absorbable monofilament sutures of polydioxanone (PDO, Monoplus®) and modified glycolic acid (Monosyn®).

2.2. Plasma treatment

Oxygen plasma treatment was carried out in a Plasma Activate Mini Flecto® (Plasma Technology GmbH, Rottenburg, Germany). It consists of a low pressure capacitive coupled plasma reactor operating at 24 kHz with a circular powered electrode of 65 mm diameter (Figure SI-1). The sutures were attached to the center of the electrode and a small weight (1.2 g) was placed at the free end of the fiber to prevent curling during treatment. Plasma treatment was performed at 0.1 mbar oxygen pressure and 80 W power. These conditions were taken from previously reported experiments [41–44,46]. Etching times between 0, 1, 2, 5, 10 and 20 min were tested. In order to avoid heating of the suture above 40 °C at longer plasma treatment times, the treatment was performed in pulses with 5 s oxygen plasma exposure followed by 60 s delay time. This was controlled by the software of the plasma equipment. The "etching time" in seconds is the number of cycles multiplied by 5. The temperature of the chamber did not surpass 40 °C during treatment.

2.3. Analysis of surface nanostructures

The surface structure of the sutures before and after plasma treatment was evaluated by scanning electron microscopy (SEM). Samples were placed on SEM pin stubs and sputtered coated with a 5 nm thick layer of platinum (Bal-Tec MCS 010 sputtering device) prior to SEM imaging. An accelerating voltage between 0.7 and 3 KV was used for taking images (LEO 1530 VP, Zeiss).

The top surface area fraction of the structures (the surface in contact with the bacteria) was analyzed from the SEM images using the open source image processing software Fiji (ImageJ 2.0.0-beta-7.5) [47] and a homemade macro.

Atomic force microscopy (AFM) was also used to characterize the topography of the treated surfaces. An AFM Bruker Dimension 3100 CL (Santa Barbara, USA) and Olympus non-contact mode cantilevers (OMCL-AC160TS-W2) with a nominal spring constant of 42 N/m were used. The RMS roughness values for the different samples were calculated through the following expression:

$$RMS = \sqrt{\frac{\sum_{i=1}^{N} (Z_i - Z_{ave})^2}{N}}$$

where Z_{ave} is the average Z value into the given area, Z_i is the current Z value and N is the total number of points in the area.

2.4. Bacterial assays

E. coli K12 wildtype (DSM A498, ATCC 23716) were grown aerobically in Luria Bertani (LB) medium at 37 °C overnight. The bacteria were diluted with LB medium to an optical density of 0.1 at 600 nm and shaken at 37 °C at 200 rpm until the mid-logarithmic phase was reached (OD_{600} ~ 0.5–0.6). The final cell density was adjusted to OD_{600} ~ 0.5, corresponding approximately to 1.25×10^8 CFU/mL. (CFU = colony-forming unit).

The suture samples (length 2 cm) were placed in a 6-well culture plate and 2.5 ml of the bacterial suspension (OD~0.5) were added. The samples were incubated at 37 °C with shaking at 30 rpm for 2 h. At the end of the incubation period, the samples were rinsed with PBS in order to eliminate non-adherent bacteria. The viability of adherent bacteria to the suture was tested by staining with a live/dead fluorescence stain (LIVE/DEAD BacLight Bacterial Viability Kit, Life Technologies) and imaging under the fluorescence microscope. For the quantification of the number of bacteria attached to the suture surface after incubation samples were vigorously shacked in tubes containing 1.5 ml of PBS vigorously for 10 min to resuspend attached bacteria. The viable organisms suspended in PBS were quantified by plating serial dilutions on LB-agar plates. LB-agar plates were incubated at 37 °C, and the CFU were counted using ImageJ software.

Each experiment was repeated threefold using three different plasma-treated suture samples. The one-way ANOVA test was used to determine significant differences in the results of the bacterial assays.

2.5. Characterization of adherent bacteria with SEM

Sutures incubated with bacteria were washed, immersed in a glutaraldehyde solution (4% in PBS) for fixation and dehydrated by immersion in ascending ethanol solutions (20%, 40%, 60%, 80% and 100%). Samples were placed on SEM pin stubs and sputter-coated with a 5 nm thick layer of platinum for SEM analysis.

2.6. Tissue drag test

The frictional forces encountered during passage through tissue were estimated by pulling 20 cm of suture through an artificial skin model (professional skin pad from Limbs and things). The tissue was mounted on a tensile testing machine (Zwick Roell Z005 with a maximum load of 50 N) with a homemade holder. Premilene and Monoplus sutures were passed transversally through the skin and attached to the upper jaw of the machine. Pulling tests were performed at an angle of 45°. The force required to pull the sutures through the tissue at constant rate of 400 mm/min versus length was recorded.

2.7. Evaluation of mechanical stability of the surface nanostructures during suturing

The mechanical stability of the surface nanostructures was evaluated by passing the suture through chicken skin. Monosyn sutures, plasma-treated for 0, 2, 5 and 10 min, were stitched and pulled through chicken skin at 400 mm/min at an angle of 45° using a tensile tester (Zwick Roell Z005 with a maximum load of 50 N). The topography of the surface was evaluated before and after the test using SEM.

2.8. In vitro degradation studies

The absorbable sutures were treated with plasma for 0, 2 and 10 min and immersed in LB medium and incubated at 37 °C for 7, 14 and 21 days. After incubation, the samples were washed with ethanol and dried at 37 °C. Changes in the surface morphology of the suture materials during degradation were analyzed by SEM.

2.9. Subcutaneous implantation

All animal experiments were previously authorized by the Committee on the Use of Live Animals in Teaching and Research of the State of Rhineland-Palatinate, Germany.

30 female, 6–8 week-old CD-1 mice (Charles River Laboratories, Germany) were randomly divided into four study groups. Three study groups were formed with four animals (n = 4) which underwent implantation of three different suture materials for 15 and 30 days. The fourth group with three animals (n = 3) served as control and underwent operation without biomaterial insertion in order to analyze the tissue reaction to the surgical procedure *per se*.

Subcutaneous implantation was conducted as described in previous publications [48–51]. In brief, the animals were anesthetized and the operation side was shaved and disinfected. An incision was made down to the subcutaneous tissue of the rostral interscapular region. A subcutaneous pocket was built in which the biomaterials were inserted and the wound was closed with stitches.

Animal housing was conducted at the *in vivo* Laboratory Animal Unit of the Institute of Pathology. The animals were kept under standard conditions, including artificial day/night-cycle as well as water *ad libitum* and regular rat pellet (Laboratory Rodent Chow, Altromin, Germany). Pre- and postoperative care was conducted according to established protocols. All experimental animals survived the implantation procedures without any postoperative complications.

2.10. Explantation and histological study of the explants

The explantation and histological study was conducted as described in previous publications [48–53]. The animals were euthanized with an overdose of anesthetics on day 15 after implantation. The implanted suture materials combined with their peri-implant tissue or the area of the control incision were explanted. The explanted tissue was fixed using a 4% formalin solution for 24 h and cut into three segments of identical dimensions including the left margin, the center and the right margin of the biomaterial. After dehydration via a series of increasing alcohol concentrations and xylol exposure, paraffin embedding was conducted. A rotation microtome (Leica, Wetzlar, Germany) was used to cut 3–5 μm thick sections.

The sections were stained with hematoxylin and eosin (H&E), Azan, Movat Pentachrome, Tartrate-resistant acid phosphatase (TRAP) and immunohistochemically with F4/80. A control slide was included for qualitative analysis of tissue reactions according to previously described methods [48–53].