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On-demand antimicrobial release from a temperature-sensitive polymer — Comparison with *ad libitum* release from central venous catheters

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ABSTRACT

Antimicrobial releasing biomaterial coatings have found application for instance in the fixation of orthopedic joint prostheses and central venous catheters. Most frequently, the release kinetics is such that antimicrobially-effective concentrations are only reached within the first days to weeks after implantation, leaving no local antimicrobial release available when a biomaterial-associated infection occurs later. Here we compare the *ad libitum* release of chlorhexidine and silver-sulfadiazine from a central venous catheter with their release from a new, on-demand release coating consisting of a temperature-sensitive copolymer of styrene and n-butyl (meth)acrylate. The copolymer can be loaded with an antimicrobial, which is released when the temperature is raised above its glass transition temperature. *Ad libitum* release of chlorhexidine and silver-sulfadiazine from a commercially-purchased catheter and associated antimicrobial efficacy against *Staphylococcus aureus* was limited to 16 days. Consecutive temperature-triggers of our on-demand coating yielded little or no antimicrobial efficacy of silver-acetate release, but antimicrobially-effective chlorhexidine concentrations were observed over a time period of 60–80 days. This attests to the clear advantage of on-demand coatings above *ad libitum* releasing coatings, that may have released their antimicrobial content before it is actually needed. Importantly, glass transition temperature of chlorhexidine loaded copolymers was lower (48 °C) than of silver loaded ones (61 °C), facilitating their clinical use.

1. Introduction

Antimicrobial releasing biomaterial coatings have found application for instance in the fixation of orthopedic joint prostheses [1] and central venous catheters (CVCs). CVCs are widely applied to offer vascular access for e.g. blood dialysis or administration of chemotherapeutics, parental nutrition or antibiotics. The use of CVCs however, is associated with a high incidence of infectious complications ranging from 0.5 to 26%, dependent on the type of catheterization. CVC-associated infections are expensive to treat [2] and moreover, extremely hazardous to patients, as they can easily evolve from a subcutaneous infection of the skin near the entry point into a CVC-associated bloodstream infection. It is estimated that CVC-associated bloodstream infections occur around 80,000 times per year in the United States alone [3]. CVC-associated infections can cause great clinical dilemmas as removal of the catheter is not an easy decision. The number of veins that can be used as entry point is limited (jugular, subclavian vein and femoral veins), while generally a once infected insertion site cannot be used for a second time. Thus removal of a CVC due CVC-associated infection for the administration of chemotherapeutics for instance, may disrupt the chemotherapy, while keeping the catheter in place may yield the risk of death due to sepsis of an already weakened patient.

Catheter-related infections are thought to arise mainly through two different routes of infection [4,5]. The first route is through the insertion site: bacteria, primarily from the skin, can migrate along the external catheter surface to cause subcutaneous infection and subsequent bloodstream infection. The second route is through the contamination of the catheter hub, leading to intra-luminal bacterial colonization and migration to the bloodstream. In order to reduce the incidence of CVC-associated infection, topical application of chlorhexidine gluconate as a skin disinfectant for catheter site care has proven to be effective [6]. As another strategy to prevent CVC-associated bloodstream infections, catheters have been equipped with antimicrobial coatings. Specifically catheters releasing chlorhexidine or a combination of chlorhexidine and silver have been demonstrated to reduce the number of CVC-associated bloodstream infections [7]. Most of the antimicrobials however, are released immediately upon contact with human tissue or fluids in a so-called “burst release” followed by a low-level tail-release that is only antimicrobially effective for a limited period of time [8]. As a consequence, whenever a CVC-associated infection develops in the later

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stages of catheterization, much of the antimicrobial content of these coatings may have been released at a time, when needed most.

These drawbacks in the use of antimicrobial-coatings can be avoided through the use of on-demand release coatings. On-demand release coatings can be externally triggered to release their content, either in a single episode or after multiple triggers, which is the preferred mode of on-demand release as it will allow triggered release at different instances over prolonged periods of time. Different on-demand mechanisms have been reported [9–12]. One such mechanism is to bring the carrier polymer in a more open state by increasing its temperature to or above its glass transition temperature to allow drug release, while when below its glass transition temperature the polymer structure is closed, impeding drug release [13]. Glass transition temperatures of materials suitable to be applied for triggered release of drugs are around 40–60 °C and clinically temperature-triggers in that temperature range can be given by infrared irradiation or the use of an electrical heating filament [13].

In this paper, a temperature-triggering mechanism based on bringing a material temporarily above its glass transition temperature, has been applied for the first time to offer an on-demand release of antimicrobials for potential use in CVCs to prevent catheter-associated infections. To this end, a copolymer of styrene (PS) and n-butyl (meth) acrylate (BA) was synthesized (abbreviated in the following as PS-BA) with temperature-dependent release characteristics of chlorhexidine and silver. In order to prevent release due to incidental rises in body temperature, polymer materials were designed with glass transition temperatures considerably higher than 40 °C. The polymer is intended to be used as a coating on catheters in the form of small beads loaded with chlorhexidine or silver-acetate, overlaid with an aromatic polyether-based thermoplastic polyurethane as a permeable, thermally insulating material (European Patent Application: EP 2732832 A2). It is the aim of this paper to compare the antimicrobial efficacies of the PS-BA polymer loaded with either chlorhexidine or silver over an extended period of time up to 60 days with the one of a commercially available, antimicrobial catheter, loaded with a combination of chlorhexidine and silver.

2. Materials and methods

2.1. Preparation of temperature-sensitive polymer and antimicrobial loading

In order to prepare a temperature-sensitive polymer with enhanced antimicrobial release characteristics above its glass transition temperature, T_g, a copolymer of styrene (PS) and n-butyl (meth)acrylate (BA) was synthesized (abbreviated PS-BA). For chlorhexidine loading, a solution of 1.92 mol styrene and 0.82 mol BA (styrene 99%, n-butylacrylate 99%, Sigma–Aldrich Chemie B.V., Zwijndrecht, The Netherlands) was prepared (70/30 mol% PS-BA) in 420 mL butyl acetate (99%, Sigma–Aldrich) and mechanically stirred at 69 °C in a 3-necked, 2 L round bottom flask under argon at 23 °C. A degassed suspension of 2.7 g azobisisobutyronitrile (98%, Sigma–Aldrich) in 20 mL butyl acetate was added three times at intervals of 2 h. For a silver loaded temperature-sensitive polymer, initial solutions of 1.60 mol PS and 0.40 mol BA (80/20 mol% PS-BA) were employed in the same amount of butyl acetate as for the chlorhexidine loaded polymer (420 mL). The reaction times were approximately 6.5 h and 40 h for polymers intended for chlorhexidine or silver loading, respectively. The solution was poured into 1 L methanol and stirred for 5 min, separated from the supernatant by decantation, covered with 500 mL ethanol, stirred overnight and subsequently for 1 h in 1.5 L ethanol. As a next step, drying was performed in vacuo, first at 23 °C for 5 h, then overnight at 80 °C resulting in 105 g of a colorless material, which was dissolved in 550 mL chloroform and re-precipitated in 2 L methanol by drop wise addition. Finally, the polymer was filtered, stirred in 1 L of methanol for 1 h (filtering and stirring were performed three times) and dried in vacuo for 96 h at 40 °C.

For antimicrobial loading of the polymer, the appropriate polymer was compounded in a custom-built twin-screw mini-compounder at 110 °C either with 20 wt.% or 30 wt.% chlorhexidine diacetate (70/30 mol% PS-BA) or with 2 wt.% Ag acetate (80/20 mol% PS-BA polymer) to produce strands of 100 mg with a diameter of 3.5 mm and a length of 2 cm. Glass transition temperatures of the antimicrobially loaded polymer strands were measured by differential scanning calorimetry (Perkin Elmer, Pyris Diamond, Waltham, Massachusetts, USA) on 10 mg samples in a temperature range from 0 to 100 °C, yielding a glass transition temperature of 48 °C and 61 °C for chlorhexidine and silver releasing coatings, respectively. The concentration of antimicrobial loading had no effect on the glass transition temperatures.

For determination of antimicrobial efficacy, strands were compression molded at 110 °C to prepare sample discs with a diameter of 2 cm and a thickness of 1.5 mm and average weight of 400 mg. Prior to evaluation of antimicrobial efficacies, discs were sterilized by 3 min sonication in 70% ethanol followed by air-drying.

2.2. Chlorhexidine and silver release

The release of antimicrobials was assessed from 2 cm PS-BA strands. For comparison, 2 cm sections of a commercially available, chlorhexidine- and silver-sulfadiazine loaded CVCs (Arrowg + ard Blue Plus) were employed.

Samples were immersed in 50 mL sterile demineralized water in glass containers under mild shaking at 37 °C, i.e. well below the glass transition temperature of PS-BA during a time period of 80 days. At regular time intervals, the PS-BA strands and CVCs were removed and placed for 1 h in a shaking bath at 60 °C. Aliquots (0.5 mL) were taken daily, but with a higher frequency immediately after a temperature-trigger, and the cumulative amounts of chlorhexidine or silver released were measured. Chlorhexidine concentrations were determined by UV–VIS at 229 nm, using a calibration based on measuring the optical density of a series of chlorhexidine solutions with known concentrations, while the concentration of Ag^+ was determined by Graphite Furnace Atomic Absorption Spectroscopy (GF-AAS) at 328 nm using a calibration based on measuring a series of Ag^+ solutions with known concentrations.

2.3. Antimicrobial efficacy

Staphylococcus aureus was selected because of its role in catheter-associated bloodstream infections [14]. *S. aureus* ATCC 49230 was cultured from cryopreservative beads (Protect Technical Surface Consultants Ltd., UK) onto blood agar plates at 37 °C in ambient air. For experiments, one colony was taken to inoculate 5 mL of tryptone soy broth (TSB, Oxoid, Basingstoke, Great Britain) at 37 °C for 24 h in ambient air. This pre-culture was diluted 1:20 in 200 mL TSB and grown statically for 16 h at 37 °C. Cultures were harvested by centrifugation for 5 min at 5000 *g* and bacteria were re-suspended in 10 mL phosphate buffered saline (PBS, 10 mL potassium phosphate and 150 mM NaCl, pH 7.0). Centrifugation was done twice in order to remove all traces of growth medium after which staphylococci were re-suspended in PBS to a concentration of 2×10^4 bacteria/mL, as determined in a Bürker-Türk counting chamber. Viability of the staphylococci in suspension amounted 90%, as determined using a Petrifilm Aerobic Count Plate® (3M Microbiology, St. Paul, MN, USA; see below). Next, 50 μL droplets of demineralized water were deposited on a sample disk and a temperature-trigger was applied by placing the sample discs in an incubator at 60 °C for 1 h. In order to limit evaporation and to retain constant conditions during antimicrobial release into the droplet, incubation was executed under saturated vapor conditions (indicated as the "ON"-state in Fig. 1). Subsequently, the sample discs were cooled and left at 37 °C for 24 h, also under saturated vapor conditions during which small