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Co-release of dicloxacillin and thioridazine from catheter material containing an interpenetrating polymer network for inhibiting device-associated *Staphylococcus aureus* infection

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ABSTRACT

Approximately half of all nosocomial bloodstream infections are caused by bacterial colonization of vascular catheters. Attempts have been made to improve devices using anti-adhesive or antimicrobial coatings; however, it is often difficult to bind coatings stably to catheter materials, and the low amounts of drug in thin-film coatings limit effective long-term release. Interpenetrating polymer networks (IPNs) are polymer hybrid materials with unique drug release properties. While IPNs have been extensively investigated for use in tablet- or capsule-based drug delivery systems, the potential for use of IPNs in drug release medical devices remains largely unexplored. Here, we investigated the use of silicone-hydrogel IPNs as a catheter material to provide slow antibacterial drug-release functionality. IPN catheters were produced by the sequential method, using supercritical CO_2 as a solvent to polymerize and crosslink poly(2-hydroxyethyl methacrylate) (PHEMA) in silicone elastomer. The design was tested against *Staphylococcus aureus* colonization after loading with dicloxacillin (DCX) alone or in combination with thioridazine (TDZ), the latter of which is known to synergistically potentiate the antibacterial effect of DCX against both methicillin-sensitive and methicillin-resistant *S. aureus*. The hydrophilic PHEMA component allowed for drug loading in the catheters by passive diffusion and provided controlled release properties. The drug-loaded IPN material inhibited bacterial growth on agar plates for up to two weeks and in blood cultures for up to five days, and it withstood 24 h of seeding with resilient biofilm aggregates. The combined loading of DCX + TDZ enhanced the antibacterial efficiency in static *in vitro* experiments, although release analyses revealed that this effect was due to an enhanced loading capacity of DCX when co-loaded with TDZ. Lastly, the IPN catheters were tested in a novel porcine model of central venous catheter-related infection, in which drug-loaded IPN catheters were found to significantly decrease the frequency of infection.

1. Introduction

Despite increased hygiene precautions and the use of antibiotic-coated vascular catheters, approximately 250,000 of vascular catheter-related bloodstream infections occur annually in the US, associated with a mean increase of 22 days in the hospital length of stay, increased hospital cost from US$ 3000 to 56,000 per patient, and mortality rates of 12–25% for critically ill patients [1,2]. Methicillin-sensitive and methicillin-resistant *Staphylococcus aureus* (MSSA and MRSA, respectively) are frequently isolated from colonized vascular catheters [3–6] and are associated with a high risk of spreading to the bloodstream, which may lead to sepsis and metastatic infections [3,7,8]. To address this problem, new anti-infective biomaterials are needed that can effectively prevent bacterial adhesion and biofilm growth.

In designing anti-infective biomaterials, studies have traditionally focused on utilizing coatings that passively repel or directly kill microorganisms at the point of contact *via* slow drug-release [9–11]. However, slow drug-release from coatings entails the dilemma of incorporating adequate amounts of drug while limiting the coating thickness to ensure that the physical properties of the device are not compromised.

Interpenetrating polymer networks (IPNs) have been studied extensively since the 1960s [12,13]. IPNs consist of two or more interlaced but non-covalently bonded polymer networks with at least one of the systems synthesized in the presence of the other [12–14]. The result is a composite material with advantageous properties acquired from both

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precursors [13,15]. If hydrogels are used in IPNs, *e.g.*, with medical device rubber materials, drug loading and release properties can be achieved, thus providing a conceptual alternative to drug-release coatings [16,17].

The basic concept of IPNs has garnered much interest in pharmaceutical sciences and has, due to parallel advances in polymer sciences, led to the development of several novel drug delivery systems [15,18–22]. However, thus far, the research and development of slow-release hydrogel IPNs have primarily focused on carrier functions in tablet- and capsule-based formulations or biodegradable implants with site-specific controlled drug-delivery properties [15,22–24]. These applications are mainly microscale particle-based and hence less suitable as a base material for medical devices.

In the current study, we explored the potential of a novel type of IPN material for use in anti-infective vascular catheters. The applied IPN technology is a post-treatment for pre-molded or -extruded rubber materials [16,17], and thus, enables the modification and functionalization of rubber-based medical devices. The drug-release and anti-infective properties of the IPN material were analyzed using the traditional beta-lactam antibiotic dicloxacillin (DCX). In addition, the release properties were analyzed after co-loading with an additional drug, the neuroleptic drug and non-antibiotic helper compound thioridazine (TDZ). This second drug was included to investigate the effect of co-loading on the overall kinetic release properties of the material. Combined treatments with drugs that exert different modes of action on the bacterial cell are increasing being used in the treatment of bacterial infections and could also be advantageous in slow release devices, to potentiate efficiency and target a wider spectrum of microorganisms. TDZ was chosen as a helper compound because it has previously been shown to synergistically potentiate the antibacterial effect of DCX against both MRSA and MSSA in broth cultures and in *C. elegans* [25–29]. However, relatively high TDZ concentrations are needed for obtaining synergetic effects [30–32], suggesting that this combination is suited for local rather than systemic treatment applications to avoid potential dose-related adverse effects.

Here, the anti-biofilm properties of the drug-loaded IPN material were tested in static and flow-based *in vitro* experiments using both MSSA and MRSA as the colonizing pathogen. Furthermore, the inhibitory efficacies of unloaded, DCX-loaded, and DCX + TDZ-loaded IPN catheters were tested against MSSA colonization in a porcine central venous catheter infection model.

2. Material and methods

2.1. Production of IPN samples

Silicone elastomer catheters (ID: 0.76 mm; OD: 1.65 mm. Helixmark silicone tubing, Freudenberg Medical) or discs/slabs (cut from a 2-mm-thick extruded band, PE4062, LEBO Production AB, Sweden) were exposed to supercritical carbon dioxide ($scCO_2$) in pressurized reactors to swell the materials. IPNs were produced as previously described by introducing 2-hydroxyethyl methacrylate (HEMA), diethyl peroxydicarbonate (DEPDC), and ethylene glycol dimethacrylate (EGDMA) into the chambers to facilitate polymerization and crosslinking of HEMA in the $scCO_2$-swollen silicone [16,17,33]. After extraction of residual monomers and uncrosslinked polymer in 96% EtOH for 1 week at ambient temperature, the mean hydrogel content was determined based on weight measurements as 41.68 wt% (SD = 1.55%) in the IPN catheters and 25.29 wt% (SD = 1.13%) in the IPN discs.

2.2. Sample loading

For initial drug loading and concomitant sterilization, the IPN specimens were placed in 96% ethanol containing 10 mg/mL dicloxacillin (DCX; Bristol-Myers Squibb) and/or thioridazine hydrochloride (TDZ; Sigma-Aldrich Corporation) for 7 days. After drying, the samples were additionally loaded for 7 days in sterile phosphate buffered saline solution (PBS; pH 7.4) containing the same drug concentrations. This subsequent loading in aqueous buffer was found to increase the total loading capacity, as described below. Unless otherwise stated, double-loaded specimens were used in the experiments. The loaded IPN materials were kept hydrated until use because complete drying of the loaded samples was found to delay the initiation of release for 1–2 days upon rehydration (data not shown).

2.3. Bacterial test strains

MSSA (ATCC 29213) with minimum inhibitory concentrations (MIC)/minimum bactericidal concentrations (MBC) of 0.5/2 μg/mL and 64/64 μg/mL for DCX and TDZ, respectively, was used for the *in vitro* and *in vivo* experiments. MRSA (ATCC 33591) with MIC/MBC values of >512/>512 μg/mL and 64/64 μg/mL for DCX and TDZ, respectively, was used only for the *in vitro* experiments. The MIC/MBC values were determined according to the guidelines described in ISO 20776-1:2006(E). Both strains exhibited confirmed biofilm formation capacities (data not shown). Furthermore, the *in vitro* synergy effects of the combination treatment (DCX + TDZ) for both strains in broth culture were confirmed by growth and viability assays as described previously [26,29].

2.4. Blood plasma collection

Blood was drawn in batches from a minimum of 5 healthy volunteers. The blood was collected in lithium-heparin collection tubes (9 mL, Vacuette, Greiner Bio-One, Frickenhausen, Germany) followed by centrifugation at 2500*g* for 15 min to isolate the plasma. Pooled batches of plasma were stored at −18 °C until use. After thawing, the plasma was centrifuged at 2000*g* for 5 min to remove any precipitate.

2.5. Evaluation of compound solution uptake ability

IPN and pristine silicone disc samples were immersed in 0.13 mg/mL fluorescein (Fluka, MO, USA) in 96% ethanol for up to 5 days at room temperature. Each day, the samples were removed from the solution, washed three times by vortexing in 15 mL distilled water for 15 s, and dried overnight in the dark. Cross-sections cut through the center of the discs were analyzed using a Zeiss Axiovert 100 M confocal laser scanning microscope with the following microscope settings: scan mode: plane; multitrack: 8 bit; stack size: 512 × 512, 9213.6 μm × 9213.6 μm; scaling: 18.00 μm × 18.00 μm; pixel time: 1.60 μs; objective: A-Plan 5×; beam splitters: MBS HFT 488, BBS2 NFT545; wavelength: 488 nm, 10%; filters: Ch1 LP505 and Pinhole Ch1 1000 μm.

2.6. Drug release profiles

One-hundred-millimeter-long IPN catheters loaded with DCX, TDZ, or the DCX + TDZ combination were placed in closed, dark containers containing 100 mL PBS. The release of DCX and TDZ were quantified by measuring the UV absorbance at 203 nm and 261 nm, respectively, using a Thermo Scientific Evolution 220 UV-vis spectrophotometer with INSIGHT software. The release media were regularly changed to ensure sink conditions throughout the experimental period of 28 days.

2.7. Disc diffusion assay

Agar plates were plated uniformly with a suspension of either MSSA or MRSA adjusted to McFarland 0.5. Material test slabs (measuring 1.2 cm × 1.2 cm) were placed onto the agar plates and incubated overnight at 37 °C. The following day, the inhibition zones were measured, and new plates were prepared for incubation with the same test slabs. Silicone, unloaded IPN, and IPN loaded with