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Chlorhexidine sustained-release varnishes for catheter coating – Dissolution kinetics and antibiofilm properties

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ABSTRACT

Catheter-associated urinary tract infections are difficult to eradicate or prevent, due to their biofilm-related nature. Chlorhexidine, a widely used antiseptic, was previously found to be effective against catheter-related biofilms. For the present study, we developed sustained-release chlorhexidine varnishes for catheter coating and evaluated their antibiofilm properties and chlorhexidine-dissolution kinetics under various conditions. The varnishes were based on ethylcellulose or ammonio methacrylate copolymer type A (Eudragit® RL). Chlorhexidine was released by diffusion from a heterogeneous matrix in the case of the ethylcellulose-based formulation, and from a homogeneous matrix in the case of Eudragit® RL. This dictated the release pattern of chlorhexidine under testing conditions: from film specimens, and from coated catheters in a static or flow-through system. Momentary saturation was observed with the flow-through system in Eudragit® RL-based coatings, an effect that might be present *in vivo* with other formulations as well. The coatings were retained on the catheters for at least 2 weeks, and showed prolonged activity in a biological medium, including an anti-biofilm effect against *Pseudomonas aeruginosa*. The current study demonstrates the potential of catheter coatings with sustained release of chlorhexidine in the prevention of catheter-associated urinary tract infections.

1. Introduction

Catheter-associated urinary tract infections (CAUTIs) are the most common infections acquired in hospitals and other healthcare facilities. These infections can cause discomfort, prolong hospitalization and, most importantly, lead to serious complications, such as pyelonephritis and bacteremia. Common pathogens include *Escherichia coli*, *Pseudomonas aeruginosa* and *Candida* species (Nicolle, 2014; Warren, 2001). In general, CAUTIs increase morbidity and mortality and place an economic burden on the healthcare system (Meddings et al., 2013; Nicolle, 2014; Shapur et al., 2012; Stamm, 1991; Trautner and Darouiche, 2004a).

The microorganisms involved in CAUTIs are found on catheters predominantly in the form of biofilm, a structure providing them with a protective environment and making them more virulent and less sensitive to antimicrobial therapy. Being a foreign body, the catheter usually supports biofilm formation on its surface, and it has been suggested that catheters impair the natural defense mechanisms of the host (Trautner and Darouiche, 2004b). Common antibiotic therapies have low efficacy against the biofilm and are associated with a high incidence of adverse effects.

As of today, there is no proven strategy for the prevention of CAUTIs (Tenke et al., 2014). Sanitary handling of catheters has hitherto been the best available option, and recommendations include eliminating unnecessary catheter use and removing the catheter as soon as possible (Cho et al., 2017; Meddings et al., 2013). There have been various attempts to prevent biofilm formation by impregnating catheters with different antimicrobial or antifungal agents, but these have been met with limited acceptance (Shapur et al., 2012; Tenke et al., 2014).

Chlorhexidine (CHX), a quaternary ammonium antiseptic, is in wide use due to its broad spectrum of antimicrobial and antifungal activities and low toxicity to mammals. Because of its nonspecific mechanism of action, CHX is less likely to cause bacterial resistance than conventional antibiotics. CHX is effective against both existing biofilms and biofilm formation (Darouiche et al., 2008; Lamfon et al., 2004; Redding et al., 2009). Therefore, it can potentially be used to prevent CAUTIs.

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For effective prevention of CAUTIs, the active agent has to be present at the target site and maintain its pharmacological activity as long as the catheter is in use. Conventional delivery systems usually fail the task due to rapid release of the active agent and its elimination from the site of action. Sustained-release delivery systems for local application can overcome this problem as they prolong the residence time of the drug at the target site and thus lower the required dose; consequently, side effects are reduced, thereby enhancing the drug's therapeutic potential and increasing its safety (Kanjickal and Lopina, 2004). Sustained-release varnishes based on polymers are an example of such delivery systems.

Characterization of *in-vitro* dissolution is an important aspect for study, especially when dealing with such unique systems as urinary catheters. Kinetic assessment, including evaluation of the release mechanism of the active agent, can lead to a better understanding of the system and of the ways to control its behavior (Grin et al., 2009; Siepmann and Peppas, 2001).

We previously reported some encouraging results with one type of sustained-release CHX-coated catheter in dogs (Segev et al., 2013). In general, coating the catheter with a sustained-release varnish of CHX has the potential to effectively prevent CAUTIs (Shapur et al., 2012).

The objective of this study was to develop sustained-release delivery systems of CHX for catheter coating and characterize their release profiles *in vitro* in various dissolution systems and their microbiological activity, as a potential means of preventing CAUTIs.

2. Materials and methods

2.1. Materials

The following materials were used: chlorhexidine diacetate (Sigma, Israel; PubChem CID: 9562059), polyethylene glycol (PEG-400, Sigma-Aldrich, Belgium), ethylcellulose (Ethocel® N-100, Hercules Inc., USA; Ashland, USA), ammonio methacrylate copolymer type A NF (Eudragit® RL, Evonik Röhm GmbH, Germany), ethanol (J.T. Baker, Netherlands), LB broth, Lennox (Acumedia, Lansing, MI, USA), siliconized latex Foley catheters, 8 Fr/Ch, 2.7 mm (Well Lead Medical Co., China).

2.2. Preparation of varnishes

CHX varnishes were prepared by dissolving the ingredients in absolute ethanol until a homogeneous solution (7.5% on a dry basis) was obtained (Czerninski et al., 2010). Varnishes used in retention studies were stained with 0.1% (w/v) erythrosin B (Sigma, Israel) for visualization. Selected formulations are presented in Table 1.

2.3. Dissolution studies

In-vitro dissolution studies were performed on film specimens, coated catheter pieces in a static system and coated catheter pieces in a flow-through system.

Film specimens were obtained by casting 5-ml aliquots of the varnish into 6-cm Ø PTFE molds and letting them dry overnight at 37 °C. The dry film was removed from the mold, and its thickness was measured at 10 different points using a micrometer (Mitutoyo, Japan). The film was then cut into 1.7 × 1.7 cm pieces.

Coated catheters were prepared by cutting the catheters into *ca.* 3-cm long pieces and sealing their edges with molten paraffin. The catheters were then immersed in the varnish and dried overnight. CHX content in each catheter piece was calculated as its percentage of the coating, determined by gravimetry.

Table 1
Composition of selected varnish formulations (in percentage of dry weight).

Composition	Chlorhexidine	PEG-400	Ethylcellulose	Eudragit® RL
F.I	5	16	79	–
F.II	5	16	30	49

The static dissolution studies were carried out at 37 °C on a shaker plate at 50 rpm. The film specimens and catheter pieces were immersed in 50 ml and 25 ml water, respectively. Sink conditions were maintained. Aliquots of the solution were withdrawn at designated time points and replaced with fresh dissolution medium. The percentage of CHX released at each time point was determined.

For flow-through dissolution studies, each catheter piece was inserted into a 4-mm Ø, *ca.* 7-cm long silicon tube attached to a 30-ml syringe filled with water. The syringes were then inserted into a syringe pump (Harvard Apparatus 22, USA) that forced the liquid through the catheter-containing silicon tube and into a collecting tube at a rate of 1.25 ml/h. The collecting tubes were replaced at designated time points, and CHX content in the samples was determined. The experiment was performed at 37 °C.

2.4. Analysis of CHX

The amount of CHX released was determined spectrophotometrically (UVIKON XS, Secomam, France) at 255 nm (Gong et al., 2007). The calibration curve in water was linear at 0.4–50 μg/ml.

2.5. Morphological studies

The morphology of the film specimens was determined using a Magellan 400 L extra-high resolution scanning electron microscope (SEM) (FEI, Netherlands). The accelerating voltage was 2.0 kV and the emission current was 50 pA.

2.6. Retention of the coating on catheters

The coated catheters were immersed in 0.05 M phosphate buffer pH 6.8. The study was carried out at 37 °C on a shaker set at 50 rpm. Visual examination of the catheters was performed every 24–72 h for 14 days. Uncoated catheter stained with an ethanolic solution of the dye served as a control.

2.7. Microbiological studies in a solution (planktonic)

Sterile catheters were cut into 0.9 × 0.9 cm pieces under aseptic conditions, and then immersed in a CHX-containing or placebo varnish and dried overnight. Each catheter piece was weighed before and after coating, to calculate the amount of varnish applied. Each sample was placed in a test tube with 50 μl of a suspension of overnight-grown *P. aeruginosa* (strain PAO1) diluted to 2 ml with the LB growth medium to an optical density at 600 nm (OD_{600}) of about 0.05. Uncoated catheter pieces immersed in the growth medium with and without *P. aeruginosa* served as positive and negative controls, respectively. The samples were incubated for 72 h at 37 °C. At 24 h and 48 h, the catheter pieces were removed, washed gently with phosphate buffered saline (PBS, Sigma-Aldrich, USA), and placed into a fresh suspension of diluted *P. aeruginosa*, as described above. The OD_{600} of each post-incubation suspension (at 24, 48 and 72 h) was measured using an Ultrospec 10 Cell Density Meter (Amersham Biosciences).

2.8. Biofilm model

Catheter pieces were prepared and coated as described in Section 2.7. They were then placed in a 48-well microplate. Each 1-ml well contained overnight-grown culture of *P. aeruginosa* diluted 1 to 10 with the growth medium. Uncoated catheters served as positive controls. The plate was incubated for 48 h at 37 °C, with a medium change at 24 h.