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Research Paper

Antibacterial and anti-encrustation biodegradable polymer coating for urinary catheter

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ABSTRACT

Bacterial biofilm and crystalline deposits are the common causes of failure of long-term indwelling urinary catheter. Bacteria colonise the catheter surface causing serious infections in the urinary tract and encrustations that can block the catheter and induce trauma in patients. In this study, the strategy used to resist bacterial adhesion and encrustation represents a combination of the antibacterial effects of norfloxacin and silver nanoparticles and the PLGA-based neutralisation of alkali products of urea hydrolysis gained through the degradation of the polymer in an aqueous milieu. Silver nanoparticles were coated with tetraether lipids (TEL) to avoid aggregation when dispersed in acetone and during the film formation. The polymer films loaded with the two antibacterial agents were applied on Polyurethane (PUR) and Silicon sheets. We demonstrated the antibacterial and anti-adhesion effectiveness of the coatings whereby commercially available biocompatible polymers PUR and Silicon were used as controls. Using artificial urine and an *in vitro* encrustation model, it was shown that the coatings resist the encrustation for at least 2 weeks. This combination of a biodegradable polymer and wide-range antibacterial agents represents a potentially attractive biocompatible coating for urinary catheters.

1. Introduction

One of the serious complications related to urinary catheterisation is the catheter-associated urinary tract infections (CAUTI). Millions of CAUTI occur per annum, out of which two million nosocomial infections occur in the United States with 40% involving urinary tract infection (Jarvis, 1996). In order to cause an infection, the bacteria must first adhere to the urinary tract and/or catheter surface. Adhesion of bacteria onto the catheter surface can also take place on the host-derived protein and other molecules adsorbed onto the catheter surface after catheterisation and the adhered bacteria form biofilm (Thomas et al., 2004). This biofilm provides protection for the bacteria against antibiotics, antibodies and defences of the human body which make it a serious problem (Stickler, 2008). Biofilm bacteria are up to 1000-fold more resistant to antimicrobial agents compared to planktonic bacteria (Vergidis and Patel, 2012). Encrustation of urinary catheter is another common problem combined with CAUTI (Denstedt et al., 1998; Talja et al., 1990). Among the bacteria related to CAUTI, *Proteus mirabilis* has a dominant role in the encrustation process, other urease producing bacteria are also responsible for crystalline biofilm (Kunin, 1989; Liedl, 2001; Mobley and Warren, 1987). Urease producing bacteria can hydrolyse urea in the residual bladder urine resulting in two molecules of ammonia to every molecule of carbon dioxide which leads to rise in pH of the urine and this, in turn, causes the crystallisation of magnesium and calcium phosphate (Cox and Hukins, 1989; Morris et al., 1999). These crystalline deposits can scratch the urethral mucosa when the catheter is withdrawn causing pain and haematuria. It can also block the catheter which is a major problem in patients undergoing long-term indwelling bladder catheterisation (Stickler and Feneley, 2010). Due to these complications related to urethral catheters, scientists, clinical investigators and manufactures are attempting for more than 50 years to optimise the development process of the catheters and to modify their surfaces to reduce the crystalline film formation and bacterial adhesion onto catheter surface (Kunin, 2001; Wu and Grainger, 2006). These attempts have focused on combining the catheter with antimicrobial agents. The most effective choice is coating of catheter surface with antimicrobial agents or polymer film loaded with antimicrobial agents (Mendez-Probst et al., 2010).

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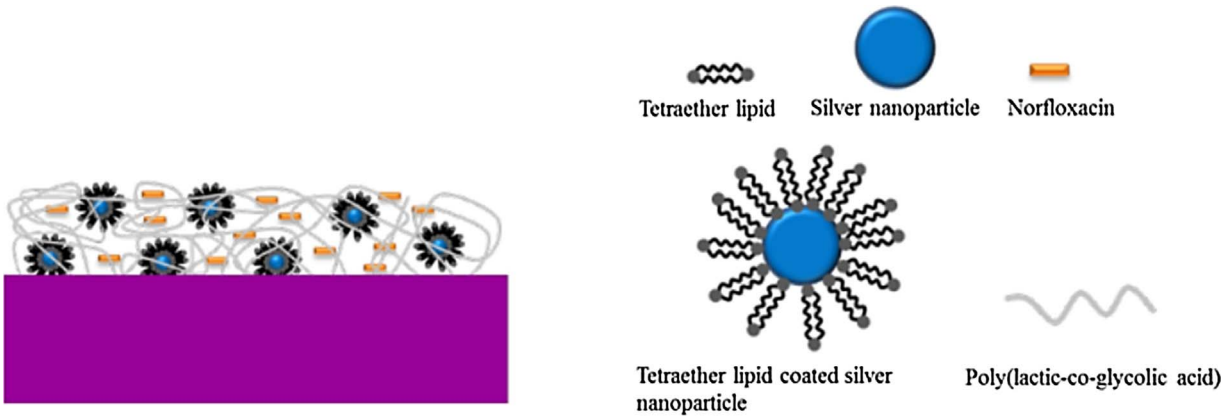

Fig. 1. Schematic representation of PLGA//TEL-Ag/NF construction.

The aim of this work was the development of TEL-coated silver nanoparticles distributed in a hydrophobic film of poly (lactic-co-glycolic acid) (PLGA) loaded with the broad-spectrum antibiotic drug norfloxacin (1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid - NF). Fig. 1 (Graphical Abstract) shows schematic description of the film. Silver and it salts have been the most commonly applied antimicrobial agents for coating of catheter surface (Ahearn et al., 2000; Cormio et al., 2001, 1997; Liedberg and Lundeberg, 1989; Mendez-Probst et al., 2010; Roe et al., 2008; Wu and Grainger, 2006). In the USA, three antimicrobial catheters coated with a silver alloy were launched to the market (Stobie et al., 2008). The ionised form of silver is well-known as broad-spectrum antibacterial agent against both gram-positive and gram-negative strains. It can attack broad sites within the bacterial cell and therefore it is improbable that bacteria can develop resistance against it (Melaiye and Youngs, 2005; Rai et al., 2009). Its antibacterial effectiveness, when it is embedded into coatings, was found to be higher than the coating alone since surface silver can be rapidly de-activated by protein anions and the impregnation of silver facilitates continuous release of silver ions (Furno et al., 2004; Rai et al., 2009). Researchers investigated numerous number of methodologies to construct silver impregnated coatings, these trials involve the use of silver nanoparticles distributed in a hydrogel coating and silver nanoparticles embedded in a polyelectrolyte multilayer (Fu et al., 2006; Murali Mohan et al., 2007; Thomas et al., 2007; Vimala et al., 2009; Yu et al., 2007). Embedding of silver nanoparticles in hydrophobic matrix mostly requires further modification with hydrophobic stabilizer. TEL are the main part of cell membrane of archaeon *Thermoplasma acidiophilum*, this kind of archaea grow at pH 2 and 56 °C and since they have no cell wall, it is the lipid composition of their membrane that provides high chemical and thermal stability (Bakowsky et al., 2000; Dayyoub et al., 2008; Frant et al., 2006; Ozcetin et al., 2011, 2010; Vidawati et al., 2010). The hydrocarbon chains of these lipids have no double bonds and are bond to the glycerol residues via ether bonds instead of ester bonds. These properties provide long term resistance against both hydrolytic and oxidative agents and (bio)chemical degradation which make these lipids suitable candidate to be used in urinary tract conditions (Bakowsky et al., 2000; Dayyoub et al., 2008). Norfloxacin is broad-spectrum fluoroquinolone antibacterial agent which is frequently used for the treatment of urinary tract infections (UTI) caused by both gram-positive and gram-negative bacteria (Appelbaum and Hunter, 2000; Hooton, 2003). This bactericidal agent builds a complex with enzyme DNA-gyrase enzyme which is required for synthesis of the bacterial DNA (Oliphant and Green, 2002).

It is of high importance to select the optimum coating formulation by choosing the compatible and suitable polymers which have the potency to control the release rate over the whole catheterisation period and reduce crystalline deposition. Various studies have focused on producing hydrogel coatings for urinary catheter (Thomas et al., 2007; Vimala et al., 2009; Yu et al., 2007). Hydrogel coatings can significantly decrease the damage of the urethral mucosa and the trauma when the catheter is withdrawn. It also unlikely to cause discomfort to the patient due to its soft and lubricant nature (Nacey and Delahunt, 1991; Nickel et al., 1987; Stensballe et al., 2005). However, it is still not evident that they promote the anti-encrustation properties(Cox et al., 1989, 1988; Tunney et al., 1996). We employed PLGA as anti-encrustation coating and for incorporation of the above mentioned anti-bacterial agents. PLGA is an FDA-approved, biocompatible and biodegradable polymer (Athanasiou et al., 1996; Emerich et al., 2017; Emerich et al., 1999.; Kitchell and Wise, 1985; Shive and Anderson, 1997). It degrades in water at pH $>$ 8 via chemical hydrolysis of the ester bonds resulting in oligomers with carboxyl end groups or lactic and glycolic acids. The yielded acids have the ability to decrease the pH in the surrounding microenvironment (Xie et al., 2010). This effect can be exploited to neutralise the alkaline products produced from urea hydrolysis and upgrade the coating effectiveness against encrustation. This study presents the development of new methodology to design new and effective anti-bacterial and anti-encrustation coating for urinary catheter. These coatings can be applied on varying types of catheter materials. In this study, we developed a new methodology to design anti-bacterial and anti-encrustation coating for urinary catheter. Commercially used PUR and silicon sheets were used in this study and coated with PLGA. The films were loaded with both norfloxacin and TEL-coated silver nanoparticles and characterised regarding uniformity and wettability. The anti-encrustation potent of the films was tested in synthetic human urine. Finally, quantitative assays of both dead and live adhered bacteria (five infection strains, one specific strain) were performed in an *in vitro* urinary tract infection model.

2. Materials and methods

2.1. Materials

Freeze-dried biomass from *Sulfolobus acidocaldarius* was purchased from BHP Billiton, Global Technology (Perth Technology Center, Australia). Poly ($_{D,L}$-lactide-co-glycolide) (PLGA), Types Resomer® RG 503H was purchased from Boehringer Ingelheim (Ingelheim, Germany). Norfloxacin, sodium dodecyl sulphate (95%) and urease (type II from jack beans) were obtained from Sigma-Aldrich (Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany). Silver nitrate was purchased from Carl Roth (Karlsruhe, Germany). Primed Medizintechnik Halberstadt GmbH supplied samples of commercially used silicone tubes (inner diameter 2 mm, outer diameter 4.2 mm) and sheets (20 mm × 10 mm × 1.6 mm). Medical grade polyurethane samples were delivered by Novoplast Schlauchtechnik GmbH: tubes (inner diameter 1 mm, outer diameter 1.7 mm) and sheets (20 mm × 10 mm × 1 mm). All microorganisms were purchased from DSMZ (Braunschweig, Germany):