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Biodegradable Tocopherol Acetate as a Drug Carrier to Prevent Ureteral Stent-associated Infection

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Abstract: Biomaterial-centred bacterial infections present common and challenging complications with medical implants like ureteral stent which provide substratum for the biofilm formation. Hence the purpose of this study is to make antibacterial stent surface with biodegradable polymer (tocopherol acetate) and anti-infective agents (norfloxacin and metronidazole) using a modified dip-coating procedure. This is done by impregnating the stent pieces in the anti-infective solution (a mixture of norfloxacin-metronidazole and polymer) for uniform surface coating (drug-carrier-coated stents). After coating, agar diffusion test was performed as qualitative test to find out the sensitivity of coated stents against the clinical isolates, *Staphylococcus epidermidis* and *Escherichia coli*. Quantitative test was measured by calculating the numbers of adhered bacteria on coated and uncoated stents by incubating the stent pieces in artificial urine. Difference in the number of viable bacteria adhered on the surface of coated and uncoated stents were statistically calculated using chi square test with $p < 0.05$ considered significant. The stent colonising ability of *Staphylococcus epidermidis* and *Escherichia coli* in a controlled environment chamber was determined using two-challenge dose of the isolates by *in vitro* challenge test. In qualitative test, the zone of inhibition around the coated stents showed sensitivity against the clinical isolates. In quantitative test, the number of adhered bacteria on the surface of coated stents was reduced to a significant level ($p < 0.05$). The polymer, tocopherol acetate is highly biodegradable in nature. Due to its degrading ability in body tissues, it releases the anti-infective drugs at a constant and sustained rate.

Key words: Implant, *Staphylococcus epidermidis*, *Escherichia coli*, norfloxacin, metronidazole, dip-coating

INTRODUCTION

The implant-associated infections are caused by different types of microorganisms that are capable of growing on the surface of medical devices (Darouiche, 2004) such as ureteral stents made of silicone. Ureteral stenting is a cheap and easy way for post-operative management for uncomplicated transurethral uretero-lithotomy (TUL) using ureteroscopy (removal of stones). The need for post-operative stenting showed that the non-stented patients experienced flank pain or colic due to transient ureteral edema (Lingeman *et al.*, 2003). The post-operative patients with ureteral stents were reported to have other complication of bacterial colonization on the stent surfaces. The colonization may due to potent biofilm forming bacteria, which are highly associated with biomaterial-centred infections. More than half of the nosocomial infections are linked with the use of medical implants (Schierholz and

Beuth, 2001). This puts the patient a set of complicated procedures and their associated problems like biofilm formation on the surface of urinary-ureteral stents due to several pathogenic and non-pathogenic microorganisms like *Staphylococcus aureus*, coagulase negative *Staphylococci* and *Escherichia coli*. In a biofilm, bacteria are well protected from the host immune defence. In order to be successful, stents should be biocompatible and coated with anti-infective agents using biodegradable carrier. Common carriers used to coat anti-infective agents onto the surfaces of different medical grade implants are poly D, L-lactic acid, diglyceride and triglyceride (Matl *et al.*, 2008). Wang *et al.* (2010) used a new biodegradable paclitaxel-eluting urethral stents for the treatment of urethral stricture in rabbit. El-Rehewy *et al.* (2009) used ureteral catheters impregnated with ciprofloxacin/N-acetylcysteine by instant dip-method which showed the highest inhibitory effect on microbial adherence to stent

surfaces. Bayston *et al.* (2009) reported that silicone catheter material impregnated with rifampicin, trimethoprim and triclosan showed long lasting activity to retain approximately 99% of principal pathogens associated with infection in patients on complications of peritoneal dialysis (CAPD). Shaheen *et al.* (2006) used liposome as a carrier for drug delivery system. They reviewed liposome for its nature, type, composition, preparation, mechanism of drug-transport, strategy of targeting and potential applications in respect with the advanced drug delivery system. These polymers even though provide a better drug-delivery system still it is not considered to be economic. Hence in the present research, the capability to improve implant biocompatibility by coating with anti-infective agents was studied using a cheaper biodegradable polymer called tocopherol acetate (Vitamin E). TA provides a sustainable release of anti-infective agents around the implant-associated tissues for the prevention of biofilm formation. The major biological function of tocopherol acetate is that of a lipid soluble antioxidant preventing the propagation of free-radical reactions. Due to its properties like inhibition of platelet adhesion and aggregation, resistance to oxidation and biocompatibility (Brody, 1999), in the present study it was used as a coating material in silicone implants for sustained release of antimicrobial drugs. The anti-infective agents (norfloxacin and metronidazole) for the study showing good synergistic activity when tested with a normal checker board method were selected (Bharadwaj *et al.*, 2003). In order to prevent the growth of aerobic and anaerobic bacteria on the surface of stent, a fluoroquinolone compound (norfloxacin) and a nitroimidazole compound (metronidazole) were mixed and coated on the stent surface. Two hundred milligram of each drug mixed and tested under *in vivo* conditions shows good bactericidal activity. Norfloxacin inhibits the activity of the bacterial enzyme DNA gyrase, which is responsible for the negative supercoiling of DNA, an essential conformation for DNA replication in the intact cell (Dollery, 1999). The 5-nitro group of metronidazole undergoes reductive transformation to an active intermediate which then exerts an inhibitory or lethal effect against DNA. Not only the DNA synthesis inhibited but also causes a loss of the helical structure of DNA with subsequent DNA strand breakage (Kucers *et al.*, 1997). Since both drug acts on the similar target site (DNA) of bacteria, it proves the character of synergism. Based on dip coating method described by Matl *et al.* (2008), the silicone stent surfaces were modified to release the anti-infective agents at a sustained rate. Antibacterial activity for both coated and uncoated stents were calculated based on the number of bacteria adhered on the c surface of stents. Adhered bacteria

were released using waterbath-sonicator and colony forming units were calculated by 1:10 dilution series (Gollwitzer *et al.*, 2003). The stent colonising ability of *Staphylococcus epidermidis* and *Escherichia coli* in a controlled environment chamber was determined using *in vitro* challenge method using two or more challenge doses of clinical isolates (Bayston and Barsham, 1988). With the above background information, as a preliminary study, the main objective of this research was,

- To make an anti-infective coating of stent using a cheaper drug carrier called tocopherol acetate
- To use target antibiotics like norfloxacin and metronidazole as anti-infective agents
- To study the antibacterial activity of coated stents,
- To determine the stent colonization, the test stent is processed in a controlled environment chamber with the challenge organisms (stent isolate) using *in vitro* challenge method

MATERIALS AND METHODS

In the present research, dip-coating methodology, qualitative and quantitative analysis of antimicrobial activity was carried out in Microbiology laboratory, CMS College of Science and Commerce, Coimbatore, India, from October, 2010 to December, 2010. *In vitro* challenge test was carried out in Microbiology laboratory, PSG College of Arts and Science, Coimbatore, India, in December, 2010.

Medical implants: Commercially available stent was used for the present study. Sterile stent was cut into pieces (n = 6, length-1 cm, diameter-5 mm) under aseptic conditions in a laminar airflow. Weighing each sample three times assessed the average weight of the stent samples, because weight standards are more accurate than length measurements.

Anti-infective coating

Drug carrier tocopherol acetate: A defined mass of the drug carrier, Tocopherol Acetate (TA) was weighed out in a glass vial. Tocopherol acetate (Merck, India) was considered to be an effective and cheaper drug-carrier due to their surface binding properties in living tissues, biodegradable in nature and sustained release of drugs at high concentrations.

Preparation of anti-infective agents (drugs): The drugs (norfloxacin and metronidazole) used in the study were checked for their synergistic activity based on the checker board method described by Bharadwaj *et al.* (2003). Preparations of Norfloxacin (Merck, India) and metronidazole (Merck, India) suspension with drug

carrier were made according to the method described by Matl *et al.* (2008). In brief, the anti-infective agents were suspended in 99% ethanol (Sigma chemicals). The resulting suspension was added to the drug carrier (TA), for building up a drug concentration of 10%.

Coating process: The commercial stent was cut into pieces (n = 6, length-1 cm, diameter-5 mm) before coating with anti-infective agents. Sterile stent pieces were coated with the carrier containing drug by dip-coating method as described by Matl *et al.* (2008). The coated stents were referred below as drug-carrier-coated stent and uncoated stents as carrier-coated stent.

Test bacterium: Clinical isolate (from ureteral stent sample) of biofilm-forming strains of *Staphylococcus epidermidis* and *Escherichia coli* obtained from a clinical laboratory was used for the *in vitro* studies. All the strains were cultured to late logarithmic growth phase on blood agar plates at 37°C for 18 h before testing. Bacterial cells were then resuspended in normal saline and adjusted to 2.0×10^5 CFU mL⁻¹ by visual comparison with a 0.5 McFarland standard.

Antibacterial activity

Qualitative test-agar diffusion test: Tests for qualitative antibacterial activity were carried out based on the method described by Bayston *et al.* (1989). Three discs were tested from each preparation (drug-carrier coated stents, carrier coated stents and uncoated stents). Antibacterial activity was expressed as the diameter of the zone of inhibition minus the diameter of the test disc.

Quantitative test-Bacterial adherence studies: Quantitative bacterial adherence studies were carried out by the method described by Gollwitzer *et al.* (2003). In brief, 50 µL aliquot of bacteria 1 suspension (2.5×10^5 CFU mL⁻¹) was added to a test tube containing 1 mL artificial urine (Table 1). To calculate the amount of bacteria added (inoculum dose), 100 µL samples of bacterial suspension were plated on nutrient agar plates after 1:10 serial dilutions. After 3 h of incubation the adherent bacteria were detached with a water-bath sonicator. The amount of detached bacteria was calculated as CFU mL⁻¹ after the 1:10 dilution series were plated onto nutrient agar plates.

Table 1: Composition of artificial urine (A:B = 1:1)

Solution-A (g L ⁻¹)		Solution-B (g L ⁻¹)	
CaCl ₂ ×H ₂ O	1.76	NaHPO ₄ ×H ₂ O	6.80
Na ₂ SO ₄	4.86	NaHPO ₄	0.86
MgSO ₄ ×7H ₂ O	1.14	Na ₂ Cit×2H ₂ O	1.16
KCl	12.13	NaCl	13.4

$$\text{The effect of inoculum dose} = \frac{\text{CFU of detached bacteria}}{\text{CFU of the inoculum dose}}$$

To see whether the drug-carrier-coated stents could inhibit bacterial growth during the 3 h incubation period, remaining bacteria were calculated as CFU mL⁻¹ after a 1:10 dilution series. The ratio of CFU of the remaining bacteria to CFU of the inoculum dose was calculated. Similar set of experiment was done parallel for the carrier-coated stents to check the efficiency of antimicrobial agent.

CFU of the remaining bacteria : CFU of the inoculum dose

In vitro challenge method: The *in vitro* challenge test was described based on the method proposed by Bayston and Barsham (1988). In brief, the test stents were exposed to first challenge dose of test organisms (*Staphylococcus epidermidis* and *Escherichia coli*) kept rotated at 130 rpm. If no colonisation was detected after 1 week incubation, a second challenge dose was added. The dose may be continued till growth of the organisms was visualized.

Statistical analysis: Data from bacterial adherence studies were compared for statistical significance using chi square test with p<0.05 considered significant.

RESULTS

Antibacterial activity

Qualitative test-agar diffusion test: The diffusing ability of the antimicrobial drugs from the coated stent pieces to retard the growth of test cultures seeded on MHA plate was calculated based on the zone of inhibition. In Table 2, the zone of inhibition produced by norfloxacin-metronidazole coated segments measured 24 and 28 mm for *Staphylococcus epidermidis* and *Escherichia coli*. And the zones of inhibition for carrier-coated stents were 3 and 4 mm. The uncoated stents showed no zones of inhibition. The numbers represent measurement of the diameter of zones of inhibition in millimeters, not including the diameter of pieces (5 mm) embedded in the agar (Fig. 1).

Quantitative test-bacterial adherence studies: The bacterial adherence studies were carried out for the isolates, *Staphylococcus epidermidis* and *Escherichia coli* separately. Drug-carrier-coated stents and

Table 2: Agar diffusion test against test cultures (*S. epidermidis* and *E. coli*)

Test cultures	Zone of inhibition (mm)		
	Dccs	Ccs	Uncoated
<i>Staphylococcus epidermidis</i>	24	3	0
<i>Escherichia coli</i>	28	4	0

Dccs: Drug-carrier coated stents, Ccs: Carrier-coated stents

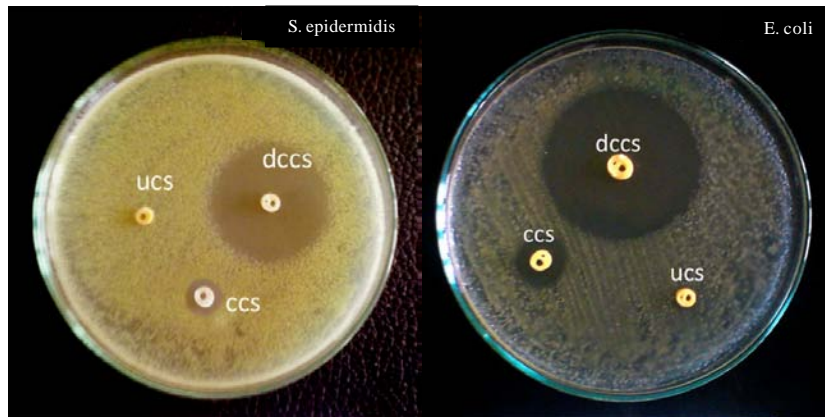


Fig. 1: Agar diffusion test showing zone of inhibition for test cultures, dccs-drug-carrier coated stents, ccs-carrier-coated stents, ucs-uncoated stents. Tocopherol acetate alone coated stents (ccs) showing a small zone are not considered for antimicrobial activity

Table 3: Bacterial adhesion studies of drug-carrier-coated stents and carrier-coated stents

Test isolates	ID (CFU $\times 10^3$)	Drug-carrier-coated stents			Carrier-coated stents		
		DB ^a (CFU $\times 10^3$)	RB ^b (CFU $\times 10^3$)	Chi-square test	DB ^a (CFU $\times 10^3$)	RB ^b (CFU $\times 10^3$)	Chi-square test
<i>S. epidermidis</i>	118	2	12	p<0.05	41	99	p>0.05
<i>E. coli</i>	136	4	19	p<0.05	47	103	p>0.05

Test isolates - *Staphylococcus epidermidis* and *Escherichia coli*, n = 2, length-1cm, dia-5mm, ID-Inoculum dose, DB-detached bacteria, RB-Remaining bacteria^a-Mean data for the adhered bacteria on the stent surface after 3 h incubation ^b-Mean data for the remaining bacteria in artificial urine after 3 h incubation

carrier-coated stents were inoculated with the isolates and adherence was studied *in vitro* based on the effect of inoculum dose. Effect of inoculum dose was calculated by dividing the CFU of detached bacteria by the CFU of inoculum dose for drug-carrier-coated stents and compared simultaneously for carrier-coated stents. Also, to see whether the drug-carrier-coated stents could inhibit bacterial growth during 3 h incubation period, the remaining bacteria was calculated based on the ratio of CFU of remaining bacteria to CFU of the inoculum dose and compared simultaneously for carrier-coated stents.

For *S. epidermidis*, the Table 3 shows that the Detached Bacteria (DB) for drug-carrier-coated stents (dccs) was only 2×10^3 CFU whereas for carrier-coated stents (ccs), it was 41×10^3 CFU. Similarly for *E. coli*, the detached bacteria (DB) for dccs was only 4×10^3 CFU whereas for ccs, it was 47×10^3 CFU. Difference in the number of bacterial counts for dccs and ccs after sonication showed the antimicrobial efficacy of norfloxacin-metronidazole (Fig. 2). Effect of inoculum dose (*Staphylococcus epidermidis* or *Escherichia coli*) for drug-carrier-coated stents when compared with carrier-coated stents showed that the numbers of adhered bacteria in drug-carrier-coated stents (p<0.05) were less than the number of adhered bacteria in carrier-coated stents (p>0.05).

After checking the growth inhibitory action of drug-carrier-coated stents and carrier-coated stents against the remaining bacteria (*Staphylococcus epidermidis* or *Escherichia coli*) during 3 h incubation period, the calculated ratio of CFU of the remaining bacteria to CFU of the inoculum dose showed that the growth of remaining bacteria was inhibited to significant level for drug-carrier-coated stents (p<0.05) than the carrier-coated stents (p>0.05).

In vitro challenge method: Results of *in vitro* challenge of stents which had been processed using a concentration of antimicrobial drugs with TA (10 %), were shown in Table 4. The drug-carrier-coated stents and carrier-coated stents were tested in triplicates against two challenge test isolates, *Staphylococcus epidermidis* and *Escherichia coli*. Resistance to colonization of drug-carrier-coated stents were observed even after 2 consecutive challenge doses, whereas carrier-coated stents were colonized after the first challenge dose of test isolates, hence indicating the absence of antimicrobial coatings on their surface. After second challenge dose the carrier-coated stents were subjected to sonication for the removal of adhered bacteria. The removed bacteria identified as the same challenge isolates indicated that the stents were not contaminated by any other organisms.

Table 4: Ability of challenge test isolates to colonize stents under *in vitro* condition challenge

test isolates	Drug-carrier-coated stents		Carrier-coated stents		Stents tested
	Challenge-1	Challenge-2	Challenge-1	Challenge-2	
<i>S. epidermidis</i>	-	-	+	+	3
<i>E. coli</i>	-	-	+	+	3

-: No colonization, +: Colonization Challenge organisms: *S. epidermidis* and *Escherichia coli*, n = 3, length-1 cm, dia-5mm Challenge dose-1: dccc and ucs flasks were inoculated with challenge dose of bacterial cultures and incubated for 1 week time, dccc inhibits the growth of challenge organism whereas ucs does not inhibit the growth of bacteria. Challenge dose-2: dccc and ucs flasks were inoculated with challenge dose of bacterial cultures and incubated for 1 week time, dccc inhibits the growth of challenge organism whereas ucs does not inhibit the growth of bacteria

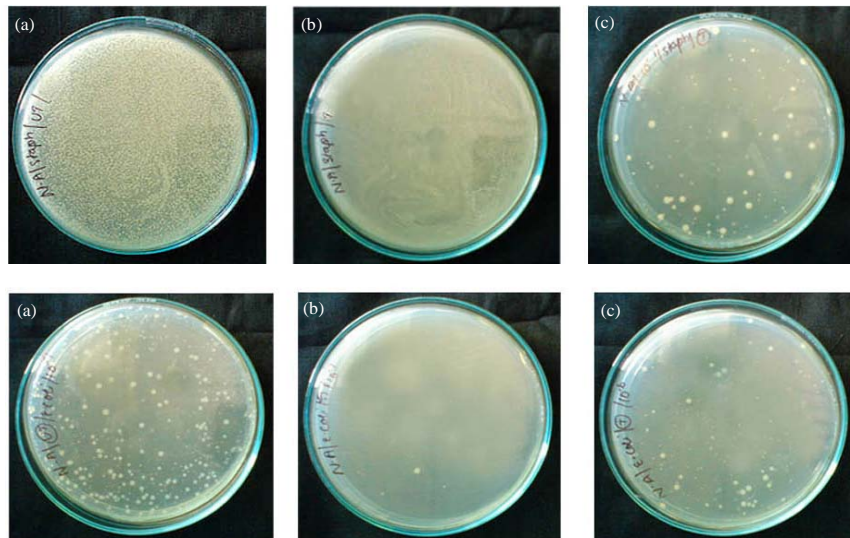


Fig. 2: Viable counts of adhered *Staphylococcus epidermidis* and *Escherichia coli* on coated and uncoated stents, (a) ID-Inoculum dose, (b) DB-detached bacteria and (c) RB-Remaining bacteria

DISCUSSION

Urinary tract infections are the most common nosocomial infections occurring in critically ill patients. Urinary catheter and stent-associated infections are difficult to be treated with antibiotics and there is a need to change catheters. It is also found that the rates of infections associated with reimplanted devices exceed that of first time implants by several folds (Quesada and Light, 1993). Catheters are manufactured from silicone or from latex and then coated in either silicone or hydrogel. These materials provide attractive, unprotected sites for bacterial attachment. In addition, irregular surfaces left by the manufacturing process, particularly around eyeholes, can trap cells from infected urine flowing through the catheter (Stickler *et al.*, 2003). Another problem found was that the biofilms produced by urease-positive bacteria such as *Proteus mirabilis* pose particular threats to the health of catheterized patients. Urease generates ammonia and creates alkaline conditions under which crystalline biofilms develop rapidly and block the urine flow from

bladder resulting in urinary retention, painful distension of bladder, reflux of infected urine to kidneys, pyelonephritis and septicaemia (Kunin, 1997). Also, it was found that heparin coated ureteral stents were free from encrustation and biofilm formation. Heparin-coated ureteral stents have limited use as they are more expensive than standard stents and cost four times the price of uncoated stents (Cauda *et al.*, 2008). The simplest way to prevent biofilm formation is to impregnate catheters with a broad-spectrum antimicrobial agent that elutes into the surrounding environment and attack planktonic bacteria in the vicinity of the device before they colonize the surface and adopt biofilm-resistant phenotype (Danese, 2002). Other factors that may have contributed to the superior efficacy of catheters impregnated with antibiotics include the particular method used to incorporate antimicrobial agents into the catheter material, the resulting concentration and availability of those agents on the catheter surface (Pittet *et al.*, 1994).

Difficulties in delivering effective concentrations of antimicrobial agents from catheters for prolonged periods have limited the usefulness of antimicrobial catheters in patients undergoing longterm catheterization.

Norfloxacin is active against a wide range of urinary tract pathogens and able to inhibit bacterial colonization of urinary catheters *in vitro* (Reid *et al.*, 1994). In our current research, similar results were obtained with norfloxacin when it was used in combination with metronidazole. It is reported that cells growing in the presence of sub-MIC concentration of norfloxacin have lower hydrophobicity and do not adhere to surfaces easily (Cerca *et al.*, 2005). Norfloxacin inhibits the activity of the bacterial enzyme DNA gyrase, an essential conformation for DNA replication in the intact cell (Dollery, 1999). The 5-nitro groups of metronidazole causes a loss of the helical structure of DNA with subsequent DNA strand breakage (Kucers *et al.*, 1997). Since both drug acts on the similar target site (DNA) of bacteria, it proves the character of synergism.

In this study, Qualitative agar diffusion test was carried out as a preliminary experiment to determine the efficacy of drug carrier coated stents against the test cultures *in vitro*. Similar work was done using CSF shunt catheter pieces coated with different antibiotics (gentamicin, clindamycin) dissolved in chloroform (Bayston *et al.*, 1989). Zone of inhibition measured for gentamicin and clindamycin was 16 mm and 18 mm against the test organism *S. epidermidis*. Owlia *et al.* (2007) investigated ceftriaxone-loaded microspheres against *Salmonella typhi*, *Salmonella paratyphi* A and *Salmonella paratyphi* B. The maximum diameter of inhibition zone caused by the microspheres was 19 mm. In the present study, the drug-carrier-coated stents against *S. epidermidis* and *E. coli* showed more inhibitory zones (24 mm and 28 mm) when compared to the results in cited article. The obtained values in our research are in support to the results of the above mentioned reviews.

In the quantitative test, the coverage of a broad spectrum of pathogens was the crucial factor for antibiotics of choice. Norfloxacin was chosen because it is a basic antibiotic for treating implant infections (Dollery, 1999), whereas metronidazole was clinically applied in more-pronounced infections and treatment of many anaerobic bacterial infections since several anaerobic implant infections were reported by Kucers *et al.* (1997). Coatings with incorporated norfloxacin-metronidazole showed significant growth inhibition effect than uncoated stents. Also, bacterial adhesion after 3 h of incubation in a 2.0×10^6 CFU mL⁻¹ *Staphylococcus aureus* suspension

could also be dramatically reduced by drug-carrier-coated stents compared to uncoated stents. Similar results were obtained for *Escherichia coli*. In pathologically relevant bacterial concentrations, as demonstrated by Elek and Conen (1957), the drug-carrier coatings developed, achieved a bacterial eradication rate of 100%. Gollwitzer *et al.* (2003) coated Kirschner-wires (K-wires) by a solvent casting technique under aseptic conditions with and without incorporated antibiotics. Release kinetics of gentamicin and teicoplanin were studied in phosphate-buffered saline. Initial bacterial adhesion of *Staphylococcus epidermidis* on coated and uncoated implants was determined by radiolabelling and counting of detached viable organisms. The incorporated antibiotics showed a continuous release over a period of at least 96 h with an initial peak of release in the first 6 h. Attachment of non-viable microorganisms, detected by radiolabelled bacteria, was increased significantly by the polymer coatings ($p < 0.05$). In contrast, the number of viable bacteria was reduced by the pure polymer ($p < 0.01$) and further by the polymer-antibiotic combinations ($p < 0.05$). Influences of (MPEG-b-PDLL) methoxy poly (ethylene glycol)-b-poly (D, L-lactide) drug ratio and film thickness on ibuprofen-loaded film characteristics and drug release behaviours were investigated by Phromsopha and Baimark (2009). From FTIR and differential scanning calorimetric results indicated that the ibuprofen was well distributed throughout the MPEG-b-PDLL film matrices. The drug release rates increased as the drug ratio increased and the film thickness decreased. The drug release from the films occurred by drug diffusion mechanism. Jun *et al.* (2006) studied controlled-release drug delivery systems for thermo-sensitive polymers such as biodegradation, biocompatibility, Lower Critical Solution Temperature (LCST) and biotoxicity. Surini *et al.* (2009) revealed that Pregelatinized Cassava Starch Succinate (PCSS) microspheres loaded with propranolol hydrochloride have good mucoadhesive property on both of gastric and intestinal mucosa. Hydroxypropylmethyl cellulose and Carbopol 974P used as linker to PCSS hydrophilic matrix significantly extended the drug release. The obtained values in our research are in support to the results of the above mentioned reviews.

In vitro challenge test conducted by Bayston *et al.* (1989) showed an *in vitro* model of CSF shunt catheter colonization using a large challenge dose of *Staphylococcus epidermidis*. In the test, the antimicrobials (combination of rifampicin and fusidate) failed to protect against colonization where as another combination (rifampicin and clindamycin) protected against two challenge doses of test isolate. From their

study they proved that the process was likely to be useful in preventing hydrocephalus shunt infection Bayston *et al.* (1989). In our present research similar results were obtained, no colonization was observed even after two challenge doses of *Staphylococcus epidermidis* and *Escherichia coli*. The obtained values in our research are in support to the results of the above mentioned review.

The antimicrobial drugs (norfloxacin and metronidazole) used in the present study were chosen according to several criteria. The first was that they should be capable of molecular migration through crosslinked silicone elastomer, an index of this being solubility in ethanol (Matl *et al.*, 2008). The second criterion was that they should be active against most strains of urinary tract pathogens and the third was that they should have been administered systemically in humans without known significant risk of hypersensitivity or toxicity (Bayston *et al.*, 1989). The fourth criterion was that they should be sufficiently stable to allow sterilisation by autoclaving. The fifth was that they showed good synergistic activity to inhibit the growth of test isolates at greater level compared when the drugs were used alone. Almost all coagulase-negative *Staphylococci* and other pathogens from urinary tract of humans are susceptible to norfloxacin and some of the anaerobic bacteria (*Bacteroid* sp.) are susceptible to metronidazole (Bharadwaj *et al.*, 2003). When norfloxacin is used alone therapeutically, resistance develops rapidly and this is the main reason for combining it with metronidazole. Many antimicrobials were excluded on one or more of these backgrounds.

CONCLUSION

In this study, the development of a drug delivery system consisting of lipid-based polymer, tocopherol acetate with incorporated norfloxacin and metronidazole in order to release high drug concentrations locally, in the area of implant infection was demonstrated. The present findings are unique and significant in the aspect that, the ureteral stents coated with polymer and antimicrobial drugs inhibits the growth of urinary tract pathogens on the surface of the implants and its surrounding tissues. In addition, anti-infective coated stents showed the highest effect on inhibiting bacterial adherence that resulted in decreasing the risk of device colonization. If these results can be confirmed *in vivo*, these drug delivery systems could be of great interest for temporary ureteral stenting after ureteroscopy to prevent biomaterial centred infections.

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