Research Article

BIOPOLYMER TOCOPHEROL ACETATE AS A DRUG CARRIER TO PREVENT BACTERIAL BIOFILM FORMATION ON SILICONE URETERAL STENTS

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ABSTRACT

The present invention relates to indwelling medical devices, such as ureteral stents made of silicone, the material of which is impregnated with one or more antimicrobial agents (ofloxacin and ornidazole) to inhibit the growth of different bacterial pathogens that colonize the device surface. Coating of silicone ureteral stents by using a drug-delivery system was demonstrated in this study using dip-coating method. The technique was done by impregnating the stent pieces in the polymer mixed antibiotic solution for uniform surface coating (*drug-carrier-coated stents*). After coating, both qualitative and quantitative tests were carried out. Agar diffusion test was performed as qualitative test to find out the sensitivity of coated stents against the clinical stent 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). *In vitro* challenge test showed that the coated stent does not contain any challenge isolates on their surface even after second dose. The development of the biodegradable drug delivery systems described here and the *in vitro* studies of this system, highlights the most important requirements for effective as well as compatible anti-infective coatings of silicone stents. Through continuous local release, high drug levels can be produced at only the targeted area and physiological bacterial proliferation can be completely inhibited.

Keywords: Ureteral stent, silicone, tocopherol acetate, ofloxacin, ornidazole, *in vitro* challenge test.

INTRODUCTION

Infection following implantation of medical implants is a risk factor feared by physicians and patients alike. One such implant-associated infection is urinary tract infection (UTI).Majority of UTI is associated with the use of urinary stents and catheters including transurethral foley, suprapubic and nephrostomy catheters[1]. 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[2]. 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[3]. 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 defense. In order to be successful, stents should be biocompatible and coated with anti-infective agents.

Common methods used to coat anti-infective agents onto the lipophilic surfaces of silicone urinary stents are the use of surfactant-mediated agents, e.g., benzalkonium chloride[4] or tridodecylmethylammoniumchloride[5]. Another method of drug binding is the incorporation of anti-infective agents into biodegradable polymer carriers[6]. The biodegradable polymers provides a slow and sustained release of anti-infective agents to establish a locally acting drug-delivery system. Different types of anti-infective agents have been used in the implants with biodegradable polymers. These polymers are poly D, L-lactic acid, PolyL-lactic acid, ester of diglycerin and tristearin[7]. 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).

Tocopherol acetate 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[8], in the present study it was used as a coating material in silicone implants for sustained release of antimicrobial drugs. The anti-infective agents (ofloxacin and ornidazole) for the study showing good synergistic activity when tested with a normal checker board method were selected[9].

In order to prevent the growth of aerobic and anaerobic bacteria on the surface of stent, a fluoroquinolone compound (ofloxacin) and a nitroimidazole compound (ornidazole) were mixed and coated on the stent surface. 200mg of each drug mixed and tested under *invivo* conditions, shows good bactericidal activity. Ofloxacin inhibits the activity of the bacterial enzyme DNA gyrase, which is responsible for the negative super coiling of DNA, an essential conformation for DNA replication in the intact cell[10]. The 5-nitro group of ornidazole 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[11].Since both drugs acts on the similar target site (DNA) of bacteria, it proves the character of synergism. Based on dip coating method described by Matl in 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 surface of stents. Adhered bacteria were released using waterbath-sonicator and colony forming units were calculated by 1:10 dilution series[12]. 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[13].

With the above background information, as a preliminary study, the main aim of this research is,

- ✓ to make an anti-infective coating of stent using a cheaper drug carrier called tocopherol acetate,
- ✓ to use DNA target antibiotics like ofloxacin and ornidazole as anti-infective agents,
- ✓ to observe the coated drugs using specific topographical analysis-SEM,
- ✓ 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

Medical implants

Commercially available stent was used for the present study. Sterile stent was cut into pieces (n=6, length-1 cm, diameter-2mm) under aseptic conditions in a laminar airflow. The average weight of the stent samples was assessed by weighing each sample three times, because weight standards are more accurate than length measurements.

Anti-infective coating

a) Drug carrier – tocopherol acetate[7]

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.

b) Preparation of anti-infective agents (drugs)[7]

The drugs (ofloxacin and ornidazole) used in the study were checked for their synergistic activity based on the checker board method described by Bharadwaj *et al.*, 2003. Ofloxacin (Merck, India) and ornidazole (Merck, India) were suspended in 99% ethanol (Sigma chemicals) for tocopherol acetate (drug carrier) at a concentration of 20 mg/ml. To decrease the particle size, suspensions were homogenized for 5 min using a hand homogenizer under sterile conditions. The resulting suspension was added to the drug carrier (TA), for building up a drug concentration of 10%.

Coating process[7]

The commercial stent was cut into pieces (n=6, length-1 cm, diameter-5mm) and sterilized in the autoclave at 121°C. Tocopherol acetate as a drug carrier was used for incorporating ofloxacin and ornidazole (drugs) at a concentration of 10%. Sterile stent pieces were coated with the carrier containing the drug by dip-coating method, ensuring a regular polymer coating, referred to below as *drug-carrier-coated stent.* The coating procedure was carried out in sterile sealable glass vials in the presence of a magnetic stir bar on a magnetic stirrer (REMI, India) for 25 min, with a drying period of 15 min between the two coating procedures. All coating steps were carried out under aseptic conditions in a laminar air flow hood. To determine the antibacterial efficiency of *drug-carrier-coated stents,* another set of stent pieces (n=3, length-1 cm, diameter-2mm) were coated in the same way with the drug carriers alone without drugs; these control stents are referred to below 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 2.0×10^5CFU/ml by visual comparison with a 0.5 McFarland standard.

Antibacterial activity

a) Qualitative test - Agar diffusion test[14]

Tests for qualitative antibacterial activity were carried out on silicone stent discs of 5 mm in diameter (cutted from 1 cm coated and uncoated stents). Three discs were tested from each preparation (*drug-carrier coated stents, carrier coated stents and uncoated stents*). The discs were all rinsed twice in PBS before testing to remove any surface accumulation of drug. The discs were placed on the surface of Mueller-Hinton agar plate which had previously been seeded with an overnight broth culture of the test bacteria and incubated at 37°C overnight. Antibacterial activity was expressed as the diameter of the zone of inhibition minus the diameter of the test disc.

b) Quantitative test-Bacterial adherence studies[12]

A 50µl aliquot of bacterial suspension (2.5×10^5 CFU/ml) was added to a test tube containing 1ml artificial urine (Table-1) and two stent pieces. To calculate the amount of bacteria added (inoculum dose), 100µl samples of bacterial suspension were plated on nutrient agar plates after serial 1:10 dilutions of bacterial suspensions. The amount was expressed as colony forming units (CFU) per millilitre. After 3 h of incubation with mild shaking, the stent pieces were removed from the test tube and rinsed four times with phosphate buffered saline (PBS). The adherent bacteria were thereafter detached with a water-bath sonicator. The amount of detached bacteria was calculated as CFU/ml after the 1:10 dilution series were plated onto nutrient agar plates. The effect of inoculum dose was taken into account by dividing the

$$\frac{CFU \ of \ detached \ bacteria}{CFU \ of \ the \ inoculum \ dose}$$

To see whether the *drug-carrier-coated stents* could inhibit bacterial growth during the 3h incubation period, remaining bacteria were calculated as CFU/ml 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

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

Solution-A (g/l)		Solution-B (g/l)	
$CaCl_2 \ X \ H_2O$	1.76	$NaHPO_4 \ X \ H_2O$	6.80
Na_2SO_4	4.86	$NaHPO_4$	0.86
$MgSO_4 \ X \ 7H_2O$	1.14	$Na_3Cit \ X \ 2H_2O$	1.16
KCl	12.13	NaCl	13.4

(Table extracted from dissertation thesis entitled "Effect of nitrate and ofloxacine blended caprolactone-l-lactide treatings on the properties of bioabsorbable self-reinforced polylactideurospirals" published in Tampere University, Finland)[12]

Statistical analysis

Data from bacterial adherence studies were compared for statistical significance using chi square test with $P < 0.05$ considered significant.

In vitro challenge method[13]

The model of stent colonisation was that described previously[13]. Briefly, the test stent was inserted into a controlled-environment chamber (500 ml conical flask) and autoclaved. The chamber was then placed in a shaker incubator (130 rpm) and maintained at 37°C throughout. During incubation the stent was rotated with brain-heart infusion medium (Hi-media, India) at a controlled rate. All procedures were carried out using vigorous aseptic technique to avoid contamination. The challenge organisms were *Staphylococcus epidermidis* and *Escherichia coli*, whose biochemical and other characteristics were known in detail, and which have been isolated from a colonised stent. They were susceptible to the antimicrobials (ofloxacin and ornidazole) used in the process. The challenge dose consisted of 1 ml of an overnight culture of the each organism in brain heart infusion, found by viable counting to contain 2.0×10^5 CFU/ml. After injection of the challenge dose, the stents were kept rotated at 130 rpm. An uncoated stent was used as control to ensure that the challenge organism was capable of colonising. Both coated and uncoated stents were tested in triplicate (n =3, length-1cm, diameter-2mm). The stents were examined visually each day and samples of infusion medium were collected periodically for culture. If no colonisation was detected after 1 week incubation, a second challenge dose was given, with a second control stent. Any stents remaining apparently uncolonised were then removed from the system and examined thoroughly for bacteria using a flushing technique[14]. Any organisms isolated from the stents at any stage were carefully identified and their characteristics compared to those of the challenge organisms in order to discount contamination. They were also tested for susceptibility to the antimicrobials present in the stent from which they had been recovered.

RESULTS AND DISCUSSION

Antibacterial activity

a) 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 were calculated based on the zone of inhibition. Table-2 and Figure-1 shows the results of qualitative tests for ofloxacin-ornidazole coated segments and their efficacies against the tested microorganisms. The numbers represent measurement of the diameter of zones of inhibition, in millimeters, not including the diameter of pieces embedded in the agar (5 mm). Zones

of inhibition produced by ofloxacin-ornidazole coated segments measured 23 and 27 mm for the test cultures.

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[14]. Zone of inhibition measured for gentamicin and clindamycin was 16 mm and 18 mm against the test organism *S. epidermidis*. In the present study, the *drug-coated-carrier stents* against *S. epidermis* and *E.coli* showed more inhibitory zones (24 mm and 28 mm) when compared to the results in cited article.

Figure 1: Agar diffusion test showing zone of inhibition for test cultures
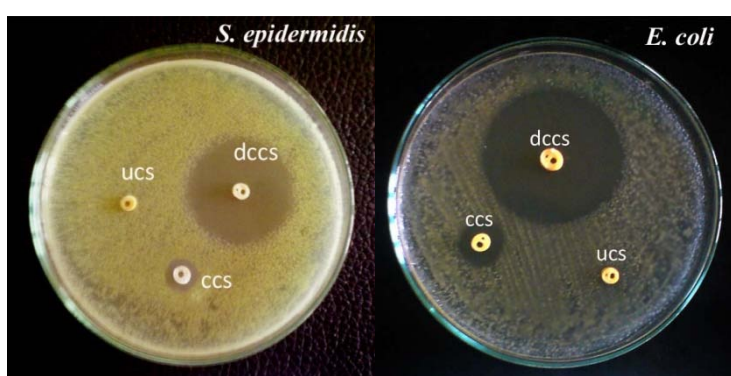
dccs – *drug-carrier coated stents*, ccs – *carrier-coated stents*, ucs – uncoated stents
In the figure, tocopherol acetate alone coated stents (ccs) showing a small zone are not considered for antimicrobial activity

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

Test cultures	Zone of inhibition (in mm)		
	dccs	ccs	uncoated
Staphylococcus epidermidis	23	5	0
Escherichia coli	27	7	0

dccs – *drug-carrier coated stents*, ccs – *carrier-coated stents*

b) Quantitative test - Bacterial adherence studies

The bacterial adherence studies were carried out for the isolates, *Staphylococcus epidermidis* and *Escherichia coli* separately. *Drug-coated-carrier stents* and *carrier-coated stents* were added 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-coated-carrier stents* and compared simultaneously for *carrier-coated stents*. Also, to see whether the *drug-coated-carrier stents* could inhibit bacterial growth during 3 hours 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*. Using the chi square statistical analysis, the results were recorded in Table-3 and Figure-2. Effect of inoculum dose (*Staphylococcus epidermidis* or *Escherichia coli*) for *drug-*

coated-carrier stents when compared with *carrier-coated stents* showed that the numbers of adhered bacteria in drug-coated-carrier stents ($P<0.05$) were less than the number of adhered bacteria in *carrier-coated stents* ($P>0.05$). To check the growth inhibitory action of *drug-coated-carrier stents* and *carrier-coated stents* against the remaining bacteria (*Staphylococcus epidermidis* or *Escherichia coli*) during 3 hours 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-coated-carrier stents* ($P<0.05$) than the *carrier-coated stents* ($P>0.05$).

In the quantitative test, the coverage of a broad spectrum of pathogens was the crucial factor for antibiotics of choice. Ofloxacin was chosen because it is a basic antibiotic for treating implantinfections[10], whereas ornidazole was clinically applied in more-pronounced

infections and treatment of many anaerobic bacterial infections since several anaerobic implant infections was reported[11]. Coatings with incorporated ofloxacin-ornidazole showed significant growth inhibition effect than uncoated stents. Also, bacterial adhesion after 3 h of incubationin a 2.0×10^6 CFU/ml *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[15], the drug-carrier coatings developed achieved a bacterial eradication rate of 100%. In another study conducted by Markku Multanen, 2002 the bacterial adhesion rate was reduced to undetectable levels in the bioabsorbale stents coated with silver nitrate and ofloxacin. Hans Gollwitzer, 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 radio labelling and counts 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 radio labeled 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 ($P < 0.05$).

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

Test isolates	ID (CFU X 10^3)	Drug-coated-carrier stents			Carrier-coated stents		
		DB[a] (CFU X 10^3)	RB[b] (CFU X 10^3)	Chi-square test	DB[a] (CFU X 10^3)	RB[b] (CFU X 10^3)	Chi-square test
S. epidermidis	121	3	11	P<0.05	46	101	P>0.05
E. coli	127	5	17	P<0.05	49	109	P>0.05

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

Figure 2: Viable counts of adhered *Staphylococcus epidermidis* and *Escherichia coli* on coated and uncoated stents

A - ID-Inoculum dose, B - **DB**-detached bacteria, C - **RB**-Remaining bacteria

A - ID-Inoculum dose, **B** - DB-detached bacteria, **C** - RB-Remaining bacteria

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

Challenge test isolates	Drug-coated-carrier stents		Carrier-coated stents		Catheters tested
	Challenge-1	Challenge-2	Challenge-1	Challenge-2	
S. epidermidis	-	-	+	+	3
E. coli	-	-	+	+	3

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

Figure 3: *In vitro* challenge test against *Staphylococcus epidermidis* and *Escherichia coli*

Challenge dose - 1
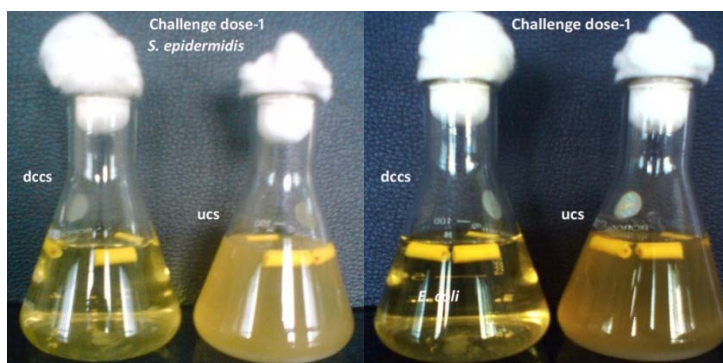
dccs – *drug-carrier coated stents*, ucs – uncoated stents
Figure showing the inhibitory effect of dccs over challenge bacteria after 1 week incubation, while ucs inoculated flask shows (turbidity) growth of challenge bacteria

Challenge dose - 2
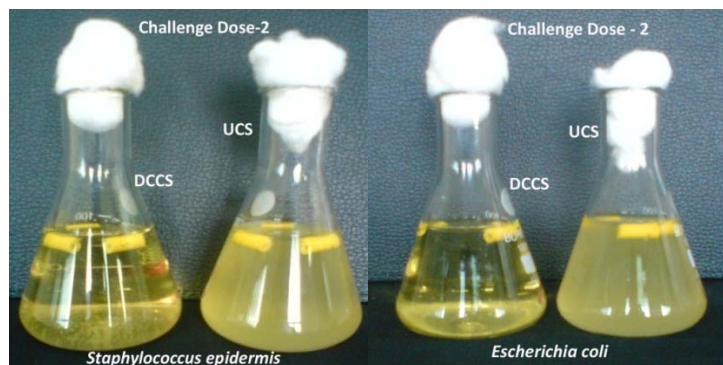
dccs – *drug-carrier coated stents*, ucs – uncoated stents
Figure showing the inhibitory effect of dccs over challenge bacteria after 2 week incubation, while ucs inoculated flask shows (turbidity) growth of challenge bacteria

In vitro challenge method

Results of *in vitro* challenge of catheters which had been processed using a concentration of antimicrobial drugs with TA (10 %) were shown in Table-4 and figure-3. *Drug-coated-carrier stents* and *carrier-coated stents* were tested separately in triplicates against two challenge test isolates, *Staphylococcus epidermidis* and *Escherichia coli.* Resistance to colonization of *Drug-coated-carrier stents* were observed even after 2 consecutive challenge doses (*Staphylococcus epidermidis* and *Escherichia coli*). Whereas *carrier-coated stents* colonized after the first challenge dose of test isolates, indicated 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.

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[14].

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[16]. 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 eye-holes, can trap cells from an infected urine flows through the catheter[17]. Another problem was found that biofilms produced by urease-positive bacteria such as *Proteus mirabilis*, pose particular threats to the health of catheterized patients. As 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[18]. Also, it was found that heparin coated ureteral stents were free from encrustation and biofilm Heparin-coated ureteral stents have limited use as they are more expensive than standard stents and cost four times the price of uncoated stents[19]. 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 plank tonic bacteria in the vicinity of the device before they colonize the surface and adopt biofilm-resistant phenotype[20]. 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[21]. Difficulties in delivering effective concentrations of antimicrobial agents from catheters for prolonged periods have limited the usefulness of antimicrobial catheters in patients undergoing long-term catheterization.

Ofloxacin is active against a wide range of urinary tract pathogens and able to inhibit bacterial colonization of urinary catheters in vitro[22]. It is reported that cells grow in the presence of sub-MIC concentration of ofloxacin have lower hydrophobicity and do not adhere to surfaces easily[23]. Ofloxacin inhibits the activity of the bacterial enzyme DNA gyrase, an essential conformation for DNA replication in the intact cell[10]. The 5-nitro group of ornidazole causes a loss of the helical structure of DNA with subsequent DNA strand breakage[11]. Since both drugs acts on the similar target site (DNA) of bacteria, it proves the character of synergism. The antimicrobial drugs

(ofloxacin and ornidazole) used in the present study were chosen according to several criteria. The first was that they should be capable of molecular migration through cross-linked silicone elastomer, an index of this being solubility in ethanol[7]. 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[14]. 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 where used alone. Almost all coagulase-negative staphylococci and other pathogens from urinary tract of humans are susceptible to ofloxacin, and some of the anaerobic bacteria (*Bacteroid*sps) are susceptible to ornidazole[9]. When ofloxacin is used alone therapeutically, resistance develops rapidly and this is the main reason for combining it with ornidazole. Many antimicrobials were excluded on one or more of these grounds.

CONCLUSION

In this study, the development of a drug delivery system consisting of lipid-based polymer, tocopherol acetate with incorporated ofloxacin and ornidazole in order to release high drug concentrations locally, in the area of implant infection was demonstrated. Instead of taking oral drugs against urinary tract infections due to catheterization or stent implantation, in our research we suggest, the antibiotic coated ureteral stents may inhibits the colonization of urinary pathogens (Escherichia coli, staphylococcus epidermis). The antibiotic combination may also inhibit other pathogens causing similar infection (*Proteus* sp, *citrobacter* sp and *Pseudomonas aeruginosa* - not tested in this research). Oral antibiotic drugs also gains resistance when it was used continuously. But the polymer, tocopherol acetate coated stents release the antibiotic at a sustained rate so that organisms may fail to gain resistance. 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. The chance of tissue reactions to the polymer and antimicrobial drugs are very low. Also, the polymers are highly biodegradable in nature. Due to its degrading ability in body tissues and fluids, it releases the antimicrobial drugs at a constant and sustained rate. In conclusion, stents impregnated in ofloxacin-ornidazole using dip coating method, were shown to have broad spectrum, prolonged antimicrobial durability and high efficacy. In addition, anti-infective coated catheters showed the highest effect on inhibiting bacterial adherence that result 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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