Study on the Effect of Cephalosporin - Quinolone Composite Drug Coatings on Urinary Catheters to Prevent Catheter Associated Biofilms

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Abstract

The present research work was designed with the objective of developing antibacterial urinary catheter coatings using cephalosporin+quinolone drug composites to prevent catheter associated biofilms. In this present study the surface colonizing ability of test bacteria on the urinary catheter sample materials was investigated using exit-site challenge test. Synergistic activity of Cefixime + Ciprofloxacin was determined against all the test organisms. Cefixime and Ciprofloxacin coated Urinary catheter (UC_{CC}) sample materials were further subjected to antibacterial activity. High biofilm producing, *Staphylococcus aureus*and *Staphylococcus epidermidis* exhibited 18.3±1.80mm and 19.3±1.04mm of inhibitory zones respectively. Persistence Test results emphasized that carriers had played a major role in retaining the drugs on the catheter materials till 4^{th} Day for UC_{CC} coated samples. Topographical analysis of UC_{CC} coated samples revealed the presence of crystallized drug particles on the catheter surface; exhibiting adherence to the greatest possible extent. Topographic analysis of UC_{MM} coated catheters showed homogenous coating of crystallized drug particles with parallelogram shapes; thus preventing biofilm formation of bacteria on the catheter surface. FTIR spectrum of coated silicone catheter proved that the significant functional groups were not altered. The antibacterial activity, FESEM, FTIR characterizations and biocompatibility test results revealed that UC_{CC}coated catheters are biocompatible and could be used for urinary catheterized patients.

Keywords: Cefixime, Ciprofloxacin, Urinary catheters, Biofilm, Biocompatible

Introduction

Bacterial cells that adhere on a surface and grow in multicellular aggregates to form complex community are termed as biofilms (Branda et al., 2005). Biofilms are micro-colonies of bacterial cells exist in the form of extracellular polymeric substances called glycocalyx (Saini et al., 2011). Commonly, biofilms on internal and external sites of urinary catheters causes indwelling CAUTI - catheter associated urinary tract infections (Alves et al., 2014).

Biofilm formation is regulated by the expression of polysaccharide intracellular adhesion property in bacterial species like *Staphylococcus aureus* and *Staphylococcus epidermidis* (Atshann et al., 2012) and *Pseudomonas aeruginosa* (Fereshteh et al., 2012). In *Pseudomonas aeruginosa* gene clusters, termed *cup* (chaperone-usher pathways) involved in the assembly of novel fimbrial structures was identified. The *cup* gene cluster contains *cupA*, *cupB*and*cupC* genes, where *cupA*is found to be required for initiation of biofilm formation and adhesion to inert surfaces of catheters (Isabelle et al., 2004). As these genes were reported to be highly responsible for the biofilm formation in the indwelling medical devices like urinary catheters, and uretal stents, early detection of biofilm expressing genes in the target organism shall be considered as the essential approach towards the prevention and management of catheter-associated infections. The gene identification approach would aid in selecting an appropriate antibacterial drug to prevent and treat the catheter associated infections.

Catheter associated infection also results in ascending infection of bladder, ureter and kidney (Bereket et al., 2012). The ascending infection has a high risk of causing cross infections because the pathogen acts as a reservoir of resistant to antibiotics. Oral prophylactic measure to treat CAUTI also leads to antibiotic resistance (Nicolle, 2014).

Hence, need for surface coating of catheters with antibacterial agents considered significant. Previous research works on antibacterial catheters signifies the need for surface modified catheters in post-operated patients admitted in intense care units (Koon et al., 2017). To make the antibacterial catheters more successful, the catheters should be coated with synergistic antibacterial drugs and carriers. Cefixime and Ciprofloxacin was selected as synergistic drugs and tocopherol acetate as a drug carrier. The different origins of these drugs inhibit the ability of organisms in gaining resistance. Mode of action of the drugs was targeting the DNA Gyrase of the organisms; so that the replication of DNA was inhibited (Katie et al., 2014).

The capability to improve the biocompatibility of catheters by coating with synergistic drugs was evaluated using a cheaper biodegradable carrier 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 (Sharma et al., 2010). 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 (Liu et al., 2003), in the present study it was used as a coating material in urinary catheters for sustained release of synergistic antibacterial drugs.

Taking into consideration of the above facts, the present research work was designed with the objective of developing antibacterial urinary catheter coatings using cephalosporin+quinolone drug composites to prevent catheter associated biofilms. Cefixime was selected as cephalosporin drug and ciprofloxacin was selected as quinolone drug. The study also aimed to determine the effect of the cross-linking of antimicrobial agent to the catheter surface on the antimicrobial efficacy, durability, persistence and compatible properties.

Materials and Methods

The present study was carried out in Department of Microbiology, PG and Research Laboratory, RVS College of Arts and Science, Sulur, Coimbatore, India.

Determining the surface colonizing capability of test bacteria on Urinary catheter material

The surface colonizing capability of test bacteria on Urinary catheter material (Silicone) was determined using standard preliminary (Exit-Site challenge test) and confirmatory tests (Microtitre plate biofilm assay).

***Preliminary Exit-site challenge test* (Bayston *et al.*, 2009)**

Exit-site challenge test was performed as preliminary test. This test was used to identify the ability of specific test organism to grow on a type of biomedical materials used in the study. In this method, three-quarter strength Iso-sensitest semi solid Agar was poured into a sterile boiling tube and allowed to solidify. The surface of the agar was then inoculated with 18h cultures of test organisms [*Staphylococcus epidermidis, Staphylococcus aureus, Escherichia coli, Proteus mirabilis* and *Pseudomonas aeruginosa*]. Urinary catheter samples were partially inserted into the semi-solid medium through the inoculated area and incubated at 37°C. Migrating ability of the test bacteria from the ‘‘exit site’’ down the material track i.e., outside of the materials were assessed visually up to 24-48 hours.

***Microtitre plate biofilm assay* (Christensen *et al.*, 1985)**

Bacterial attachment to an abiotic surface is assessed by measuring the stain taken up by adherent biomass in a 96-well plate format by means of microtitre biofilm assay. The test organisms were grown in 96-well microtitre plate for 48 hours. The anaerobes were grown inside McIntosh Field jar simultaneously. The wells were washed to remove any unbound test bacteria. Cells remaining adhered to the wells were subsequently stained with a dye that allowed visualization of the attachment pattern. Each of the test organisms was inoculated in a 5 ml culture broth and grown to stationary phase. Cultures were diluted at 1:100. Following this, 100 µl of each diluted cultures was pipetted into eight wells in a fresh microtitre plate. The plate was covered and incubated at optimal growth temperature for 24-48 hours. Four small trays were set up in a series and 1 to 2 inches of tap water was added to the last three. The first tray was used to collect waste, while the others were used to wash the assay plates. Unbound bacteria if any were removed from each microtitre dish by briskly shaking the dish out over the waste tray.

About 125 µl of 0.1% crystal violet solution was added to each well. Staining was done for 10 min at room temperature. The crystal violet solution was removed by shaking each microtitre dish out over the waste tray. The dishes were washed successively in each of the next two water trays and as much liquid as possible was shaken out after each wash. To remove any excess liquid, each microtitre dish was inverted and vigorously tapped on paper towels. The plates were allowed to air-dry. Added 200 µl of 95% ethanol to each stained well. The plates were covered to allow solubilisation by incubating for 10 to 15 min at room temperature. The contents of each well were briefly mixed by pipetting. Following this, 125 µl of the crystal violet-ethanol solution was transferred from each well to a separate well in an optically clear flat-bottom 96-well plate. The optical density (OD) of each of these 125µl samples was measured at a wavelength of 500 to 600 nm. Optical density (OD) of stained adherent bacteria was determined with a micro ELISA auto reader. The OD values were considered as an index of bacteria adhering to surface and forming biofilms. Based on the OD value the adherence of organism in the plate can be classified as below (Table-A).

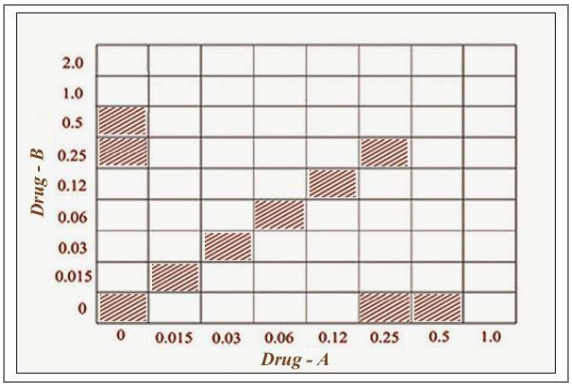
Checking the synergistic activity of antibacterial drugs against test bacteria (Qasiasgar and Kermanshahi, 2009)

The synergistic activity of Cefixime - Ciprofloxacin on all the test bacteria was determined by the standard checker board titration method. To determine the inhibitory concentrations of each drug separately and in combinations, the minimal inhibitory concentrations (MIC) was simultaneously identified in this method. The fractional inhibitory concentrations (FIC) of the drugs were calculated from MIC values to determine the synergism between the two drug combinations (Cefixime - Ciprofloxacin).

To assess antimicrobial combinations *in vitro* the checkerboard method was selected. In this technique the predetermined concentrations (µg/ml) were used ranging 0.015, 0.03, 0.06, 0.12, 0.25, 0.5, 1.0 and 2.0. According to Plate-A, the wells in the Microtitre plate of left hand column were used for the predetermined concentrations of Drug-A (Cefixime), the wells in the microtitre plate of bottom row were used for Drug-B (Ciprofloxacin) and the

wells in the 45 degree line were used for mixed drug combinations (Cefixime + Ciprofloxacin). In all the selected wells 10µl of test bacteria (1 X 10^4 CFU/ml) was inoculated. After inoculation, the selected wells were added with selected concentration of respective drugs as per given in Fig. 1. The plates were incubated for 16-20h at 37 ºC. The MIC values were recorded based on the evidence of growth on respective concentrations. For confirmation, about one loop of sample from each well was inoculated onto Nutrient Agar plates and observed for growth or growth inhibition of respective MIC values selected.

Plate-A: Checkerboard model to determine synergism of two drugs



(The picture was adapted from Qaziasgar and Kermanshahi, 2008)
Bottom row: To determine MIC of [Drug-A: Cefixime],
Left column: to determine MIC of [Drug-B: Ciprofloxacin],
Centre: MIC of (Drugs) - Cefixime + Ciprofloxacin

***Evaluating the synergism between antibiotic drug combinations* (Bharadwaj*et al.*, 2003)**

Fractional inhibitory concentration index (FICI) was calculated by using the following equation.

FIC *index* = FICA + FICB

Formula to determine synergy

$$FIC_{index} = FIC_A + FIC_B$$

$$FIC_A = \frac{MIC_A \text{ in combination}}{MIC_A}$$

$$FIC_B = \frac{MIC_B \text{ in combination}}{MIC_B}$$

where, A was the minimal inhibitory concentration (MIC) of Drug-A in a plate that was the lowest inhibitory concentration in its row, and B was the MIC of Drug-B in a plate that was the lowest inhibitory concentration in its column. MIC_{AB} was the lowest inhibitory concentration of Drug A and B in combination in the 45 degree line. With this method, synergism has traditionally been defined as an FIC index of 0.5 or less and partial synergy as a FIC index of >0.5 - ≤1.0; antagonism has been defined as a FIC index of ≥2.0.

Interpretation:

Mean FICI ≤ 0.5 → Synergy, (p< 0.5),

Mean FICI >0.5 - ≤1.0→ Partial synergy, (p> 0.5)

Mean FICI ≥ 2.0→ Antagonism

Urinary catheters and surface coatings

After analysing the synergistic activity of different Cefixime and Ciprofloxacin, the drug combination mixtures were added with drug-carrier to fabricate the urinary catheter (silicone). The catheter samples were fabricated by imparting antibacterial drugs and carriers using a standard two-dip-coating method. Following were the protocols of antibacterial drug preparations, dip-coating the materials, antibacterial activity of the drug coated materials and its durability determinations.

Preparation of Synergistic drug concentrations for coating Urinary catheter samples (Gollwitzer et al., 2003)

The antibacterial drugs for coating the catheter sampleswere prepared using a solvent-casting technique. The carrier (tocopherol acetate) was mixed with each of the synergistic drugs (Cefixime and Ciprofloxacin) by the following procedure. All the carriers were selected based on their biological properties like; enhancement of antibacterial activity; provides sustained and constant release of drugs, food and medical grade polymers.

Tocopherol acetate + synergistic drugs

Carrier, 650 mg of tocopherol acetate was added in 5 ml of 0.1N NaOH at a concentration of 130 mg/ml. To the solvent carrier mixture, Cefixime and Ciprofloxacin were added to attain a final concentration of 1%.

Coating the materials with antibacterial drugs and carriers using standard Two Dip-coating method (Matl*et al*., 2008)

All the catheter samples were subjected for coatings in 3 different groups [Group-A, B and C]. The coated materials in Group-A, B and C were referred below as *drug-carrier coated* (*dcc*), *carrier-coated* (*cc*) and *Uncoated* (*uc*) catheter samples.

Seeding Catheter surface with lyophilized cefixime + Ciprofloxacin and a carrier (Tocopherol acetate)

Process is composed of two steps (seeding and crystallization). For seeding, 50 mg of Cefixime + Ciprofloxacin was weighed, grinded for 3 min and 1.6 mg of grinded drugs was transferred into 5ml glass vial. 4ml of n-Hexane (Sigma-Aldrich) was added to this vial and sonicated (amplitude 60 for 15min and then for 2-5min at amplitude 100) until homogeneous dispersion of drugs in hexane was formed (Fig. 3). After sonication, catheter pieces were mounted on shrinkable tube placed on needle and needle loaded with samples was placed at the centre of the vial containing the dispersed drugs in hexane (one sample per trial). These vials were then placed in the ultrasonic bath (Shimadzu) for 10 min at 30°C to form seeding layer. Samples were gently taken out of the vial and allowed to dry at room temperature. These dried and seeded samples were preceded to the next crystallization step.

Crystallization of lyophilized synergistic drugs on seeded Catheter surface

For crystallization, 50mg of Synergistic drugs and 1% of Vitamin-E (alpha tocopherol) as drug carrier was weighed and dissolved in 3 ml of ethyl acetate. This solution was transferred to 100 ml glass tube and this tube was filled drop wise with 65ml n-hexane to form homogenous solution. Catheter were placed in this solution at 25°C/5 min for crystallization of Synergistic drugs on seeding layer to form crystals carpet formation, and then dried overnight. Finally, after coating the catheter with both drugs, it is referred as Cefixime and Ciprofloxacin coated Urinary catheter (UC_{CC}).

Antibacterial activity of Cefixime and Ciprofloxacin coated Urinary catheter (UC_{CC})against the test organisms (El-rehewy*et al*., 2009)

Qualitative Analysis

The method was performed for analysing the antibacterial activity of Catheter samples materials after dip-coating with synergistic antimicrobial drugs and carriers. In this qualitative method the pre-measured size of all sterilized Catheter samples were tested from each preparation [*drug-carrier coated* (*dcc*), *carrier-coated* (*cc*) and *uncoated* (*uc*) Catheter samples]. The materials were all rinsed twice in phosphate buffered saline before testing to

remove any surface accumulation of drug. All Catheter samples were placed on the surface of Mueller-Hinton agar plate which had previously been seeded with an overnight broth culture of the test organisms and incubated at 37°C for 24 to 48 hours. Antibacterial activity was expressed as the diameter of the zone of inhibition.

Quantitative Analysis

Antibacterial activity for synergistic coated catheters were quantitatively analysed using bacterial adherence test. The effect of Cefixime and Ciprofloxacin coated Urinary catheter (UC_{CC}) was tested against the test organism using a standard bacterial adherence test. The materials were placed separately in a tube with 5 ml of each of the test bacteria and incubated at 37 °C for 18 h. During the incubation period the bacterial cells adhere on the surface of the Catheter samples. After incubation, the numbers of viable adherent cells were determined as follow: The sample materials were collected aseptically and washed in a sterile test tube of 10 ml normal saline to remove the non-adherent cells. The washed pieces were transferred each in 10 ml fresh sterile saline, and sonicated for 30 seconds to dislodge the sessile adherent cells using an ultra-sonicator. After sonication, serial dilution of the sonicated saline was made and the number of sessile bacteria which indicates degree of adherence was determined by viable count technique. Similar experimental set up was run in parallel for *uncoated* (*uc*) materials. The difference in number of adhered cells on drug coated UC_{CC}materials and *uncoated* (*uc*) materials were determined statistically using chi square analysis.

The percentage reduction of adhered organisms on the *drug-carrier coated* materials was determined using a standard percentage reduction formula.

$$\text{Bacterial reduction (\%)} = A - B/A \times 100$$

Where,

A = number of adhered organisms (in CFU) obtained from the *uc*materials
B = number of adhered organisms (in CFU) obtained from the UC_{CC} materials

Persistence Test: Antibacterial durability of UC_{CC} (Baston*et al*., 2009)

Persistence test is the continuation process of qualitative antibacterial activity test. The test was performed to determine the durability of antibacterial coatings of the drug coated and drug-carrier coated mesh materials.

This test was performed for all the samples till no zone was evident on the MHA plates seeded with the test bacteria. The results obtained in qualitative analysis (Section 3.5) were taken as Day-1 results. From Day-2 the test was performed and the antibacterial inhibitory zones were recorded and tabulated for each test organisms for each day separately till no significant inhibitory zones were observed.

Physical Characterization Studies

The surface properties of synergistic drug coated catheter samples before and after coating with antibacterial drugs were characterized using two standard parameters, Fourier transform infra-red spectroscopy (FTIR) and scanning electron microscopy (SEM).

***Scanning Electron Microscopic analysis of UC_{CC}* (Matl et al., 2008)**

The surface coatings of the drugs and carriers on catheter samples were observed using Scanning electron microscopy (SEM). SEM evaluation was also used to know the uniformity of coating of finishing chemicals over the specimen. The topographic analysis of coated and uncoated test materials was prepared for SEM using a suitable accelerating voltage (10 KV), vacuum (below 5 Pa) and magnification (X 3500). Metal coating was used as the conducting material to analyse the sample.

***Fourier Transform Infra-Red spectroscopic analysis of UC_{CC}* (Coates, 2000)**

The alteration in the functional groups of test materials (silicone catheter samples) due to the addition of drugs and carriers was determined chemically using FTIR spectroscopy. The FTIR absorption spectra of the

UC_{CC}samples were recorded in the range of 400-4000 cm^{-1} by KBr disc method. FTIR spectra of the samples were determined using Shimadzu FTIR spectrophotometer. All the samples were prepared in KBr discs with a hydrostatic press at a force of 5.2 τ cm^{-2} for 3 minutes to reduce the moisture content on the disc surface. Each disc was dried under radiation to remove excess moisture content. FTIR analysis was done separately for drugs, carriers, uncoated silicone catheters and *drug-carrier coated* silicone catheters (UC_{CC}).

Biocompatibility of the UC_{CC} by MTT assay (Budman*et al.*, 2012)

Cytotoxicity assay is often used to evaluate the *in-vitro* cytotoxicity of polymeric components as it is a quick, effective method for testing mitochondrial impairment and correlates quite well with cell proliferation. It is based on the use of tetrazolium salt 3-[4,5-dimethylthiazolyl-2]-2,5-diphenyl tetrazolium bromide (MTT), which can be converted to an insoluble blue formazan product by mitochondrial enzymes in viable cells. L_{929} fibroblast cell line is often used to evaluate cytotoxicity of synergistic-drugs + carriers.

The fibroblast cell lines were cultivated in 12-well-microtitre plates to reach confluence growth. The synergistic-drugs + carriersamples were applied directly to the developed fibroblast monolayer. Before cell seeding, the specimens were pre-wetted in 70 % aqueous ethanol solution for 48 h, rinsed twice with ultrapure water and immersed in 1ml DMEM fibroblast medium in 24-well plates for 2h in an incubator at 37°C. The specimens were then seeded with L929 fibroblast cell line at 10,000 cells per well according to routine cell-culture methods. The plates were incubated at 37°C and 5% CO_2 for fifteen days. A well without drugs (control-1) and another well without cells (control-2) were included in this study. The effect of synergistic-drugs + carrieron the biocompatibility of fibroblast was evaluated using the photometric MTT assay.

At each time point, samples were taken from the 24-well plates and transferred into new plates for the MTT study. The MTT solution was prepared by dissolving the powder in phosphate buffered saline at a concentration 1 mg/ml. After 1 hr of incubation, the purple crystals were dissolved by adding sodium dodecylsulphate (SDS) in a 1:1 mixture of water and dimethyl formamide (DMF) at a concentration of 20% w/v. After adding 1ml of MTT medium (0.0005mg/ml) to each well, the plates were incubated for 3h, rinsed and desorbed in 100ul of 70% isopropanol. After being agitated rapidly at 400rpm/min for 40min, the dyed medium was transferred to 96-well plate, and read at 550nm. The biocompatibility or cell viability is expressed as a percentage of the control sample (100%).

Results and Discussion

Determining the biofilm forming abilities of test organism

Exit-site challenge test

In this present study the surface colonizing ability of test bacteria on the urinary catheter sample materials was investigated using exit-site challenge test. All the test organisms used in the research colonized the material surfaces between 24 to 48 hours. Among the test organisms *Staphylococcus epidermidis* and *Pseudomonas aeruginosa* colonized with in 24hours; *Escherichia coli*, *Klebsiella pneumoniae* and *Staphylococcus aureus* colonized the catheter surface after 48 hours (Fig. 1).

Microtitre plate assay

Staphylococcus epidermidis and *Staphylococcus aureus* showed OD values >0.240. Moderate biofilm formation was evident for *Pseudomonas aeruginosa* (OD-0.160), *Klebsiella pneumoniae* (OD-0.186) and *Escherichia coli* (OD-0.192) (Table-1).

Synergistic activity of Cefixime + Ciprofloxacin combinations using Checker Board titration method

Synergistic activity of Cefixime + Ciprofloxacin was determined against all the test organisms. All the five test organisms showed complete synergistic effect for Cefixime + Ciprofloxacin combination. Most significantly, *Staphylococcus epidermidis* and*E. coli* showed complete synergy with the mean MIC_{AB} value 0.06µg/ml respectively. Other significant organisms *Staphylococcus aureus* also showed complete synergy with the mean MIC value of 0.12µg/ml. *Pseudomonas aeruginosa and Klebsiella pneumoniae* showed complete synergy with the mean MIC value of 0.25µg/ml and 0.5µg/ml respectively (Fig. 2, Table-2).

Urinary catheter coating with Cefixime + Ciprofloxacin – UC_{CC}

Cefixime and Ciprofloxacin coated Urinary catheter (UC_{CC}) sample materials were further subjected to antibacterial activity. The results of the same were described below separately.

Antibacterial activity of UC_{CC} against the test organisms

Qualitative Analysis

The diffusing ability of the antibacterial synergistic drugs from UC_{CC} coated samples to retard the growth of test bacteria was calculated based on the zone of inhibition. High biofilm producing, *Staphylococcus aureus*and *Staphylococcus epidermidis* exhibited 18.3±1.80mm and 19.3±1.04mm of inhibitory zones respectively. Other moderate biofilm producers, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Escherichia coli*also showed significant inhibitory zones measuring 18.6±0.57mm, 18.9±0.57mm and 19.3±1.04mm respectively (Fig. 4, Table-3).

Quantitative Analysis

The quantitative antibacterial activity of UC_{CC} coated samples was determined using bacterial reduction percentage against the test organisms. Maximum bacterial reduction percentage was observed for biofilm producing *Staphylococcus aureus* (96.5±1.80%). Other cultures also showed significant reduction percentage. *Escherichia coli* and *Pseudomonas aeruginosa* were reduced upto95.6±1.04% and 93.6±1.04% respectively (Fig. 5, Table-4).

Persistence Test: Antibacterial durability of UC_{CC}

Persistence of drugs on the UC_{CC} coated samples were determined based on the antibacterial activity. During the test, the coated samples showed significant inhibitory zones on Day-1 (Table-3). In Table-4, from 2^{nd} Day to 4^{th} Day, inhibitory zones were found to be reduced gradually when tested against all the test organisms was shown. On 5^{th} Day no inhibitory zones were observed indicating that drugs were persistent on the coated materials till 4^{th} day. The obtained results emphasized that carriers had played a major role in retaining the drugs on the catheter materials till 4^{th} Day for UC_{CC} coated samples (Table-5). In contrast, drug alone coated materials showed inhibitory zones only till Day-1. No inhibitory zones were evident from 2^{nd} Day of analysis.

Physical Characterization Studies

Scanning Electron Microscopic analysis of UC_{CC}

Topographical analysis of UC_{CC} coated samples was carried out using FESEM analysis. The analysis revealed the presence of crystallized drug particles on the catheter surface; exhibiting adherence to the greatest possible extent. Drug coatings as large uniform and continuous layer of parallelogram shapes on the catheters were observed after seeding and crystallization steps. Parallelogram shaped drugs in the crystal form was evident clearly from the given images below (Fig. 6). FESEM analysis of the coated samples also evidenced that the homogenous coating of drug+carrier mixture does not provide any surface space on the catheter for the bacterial adhesion or biofilm deposition. This was mainly by reducing the depressions on the material surfaces by the crystallized deposition of drugs on the catheters.

Fourier Transform Infra-Red spectroscopic analysis of UC_{CC}

FTIR spectrum of the *drug-carrier* (synergistic drugs and carrier) coated silicone was analysed for detecting the chemical interactions among them. From the spectrum it was observed that addition of drugs and carriers did not altered the functional group of catheter material (silicone). In the present research, the spectrum for each drugs, carrier, silicone and drug-carrier coated silicone material was presented separately. All the peaks assigned for each functional group of the compounds was presented in Fig. 7.

Biocompatibility of the UC_{CC} by MTT assay

Biocompatibility of UC_{CC} coated samples was evaluated by *in-vitro* cell culture cytotoxicity (MTT) assay. All the drug coated catheter samplesexhibited no effect on cell viability during the assay. The viable cell count of L_{929} fibroblast cells was not altered during the assay period (24 hours). This was evident when compared to the control samples. As the cell viability percentage (95.6±1.04%) was found almost similar to that of the control samples (>99.0±0%), the UC_{CC} samples are confirmed to be biocompatible (Fig. 8, Table-6).

Discussion

Urinary catheter associated infection in hospitalized post-surgery patients is mainly due to microbial colonization on the exit-site of catheters. Effective antibacterial modification of urinary catheter surface that can prevent device colonization was selected as a primary objective. Antibacterial activity results exhibited significant bacterial reduction percentage qualitatively and quantitatively against all the test bacteria. Antibacterial durability test emphasized that carriers had played a major role in retaining the drugs on the catheter materials till 4th Day of analysis.

Antibacterial activity results obtained in the present research was mainly due to the mode of action of each drugs that acts on the bacterial cell components to kill the causative organism. This was illustrated from the literature survey described by researchers separately for ciprofloxacin and Cefixime.Brogden, RN and Campoli-Richards, earlier in 1989 explained that, cefixime has the ability to bind specifically on penicillin-binding proteins located inside the bacterial cell wall. This leads to inhibition of the third stage of bacterial cell wall synthesis which leads to celllysis. Cell lysis was mediated by bacterial cell wall autolytic enzymes such as autolysins. In another study, Danni Ramdhani et al., (2021) revealed that cephalosporins exert bactericidal activity by interfering with bacterial cell wall synthesis and inhibiting cross-linking of the peptidoglycan. The cephalosporins are also thought to play a role in the activation of bacterial cell autolysins which may contribute to bacterial cell lysis.

Another drug, Ciprofloxacin (quinolones) are among the most widely used antibiotics on account of their broad spectrum of activity that encompasses many pathogenic Gram-negative and Gram-positive bacteria (Trucksis*et* al., 1991). Ciprofloxacin has the ability to reduce structural integrity of bacterial DNA due to scission of phosphor-di-ester backbones leads to strand breakage. According to Edwards *et al*, (1980) the confirmatory evidence for strand breakage comes from studies which show a drug-induced increased single-strand content of DNA, its decreased molecular length and weight, and direct measurement of single and double - strand breaks by sucrose gradient sedimentation. The overall mechanism of action of ciprofloxacin therefore is to decrease the stability and integrity of DNA by strand breakage.The quinolone drugs target the bacterial type II DNA topoisomerase, DNA gyrase and DNA topoisomerase IV. These hetero-tetrameric enzymes manipulate DNA topology by introduction of transient double-stranded breaks in bound DNA (G-segment) through which a second DNA fragment (T-segment) may be passed (Maxwell and Gellert, 1986). Binding, by intercalation (Laponogov*et al*., 2010), of quinolone antibiotics to the complex of enzyme and the cut G-segment stabilizes this so-called cleavage complex, leading to accumulation, and eventual release, of double-stranded DNA breaks that are ultimately lethal to the cell (Drlica and Zhao, 1997). Clearly, because of this molecular basis of action of cefixime and ciprofloxacin, bacterial resistance could not occur by way of an altered DNA or enzymes involved in DNA biosynthesis.

Topographic analysis of UC_{MM} coated catheters showed homogenous coating of crystallized drug particles with parallelogram shapes; thus preventing biofilm formation of bacteria on the catheter surface. Scanning electron

microscope determines the difference between the surface of coated silicone and uncoated silicone. Coated silicone was expected to be ultra-smooth when compared to that of uncoated material. The advantage of smoothness on the material surface was reviewed by the following researchers. It has been found that the irregularities of polymeric surfaces promote bacterial adhesion and biofilm deposition whereas the ultra-smooth surface does not favour bacterial adhesion and biofilm deposition (Scheuerman*et al.*, 1998). This may happen since a rough surface has a greater surface area and the depressions in the roughened surfaces provide more favourable sites for colonization (Taylor *et al.*, 1998). Boyd *et al.*, 2002 showed that an increase in surface roughness on stainless steel, from 0.04 μm for polished stainless steel to 0.30 μm for abraded, increased bacterial adhesion strength more than a larger increase in surface roughness from 0.04 μm to 0.96μm for unpolished stainless steel. The drug-carriers used in the present study aided in making ultra-smooth surface of the test materials; so that the bacterial adhesion or biofilm deposition was inhibited by reducing the depressions in the material surfaces. SEM analysis of coated and uncoated silicone materials presented in the pictures of plate-8.2(a) proved this factor. In the picture, the coated silicone surface was observed to be smoother than the uncoated silicone surfaces. Similar observations were recorded by Mat*et al.*, (2008) for the *drug-carrier coated* PTFE vascular stents. They observed stable and regular coatings with ultra-smoothness on the material surface after dip-coating.

FTIR spectrum of coated silicone catheter proved that the significant functional groups were not altered when compared with the uncoated materials. C-H vibration for methyl groups of silicone, terminal C-H stretch of Ciprofloxacin and asymmetric methyl groups of tocopherol acetate was evident from a common peak obtained at 2931.47cm^{-1}. The obtained peak showed that the functional group of silicone was not altered. Other significant functional group of silicone corresponding to silicon-oxy compounds (Si-O-Si) was also not altered. This was evident from the peaks obtained at 1062.52cm^{-1} and 1034.41cm^{-1}. Common peak at 3116.28cm^{-1} represented the hydroxyl groups of synergistic drugs, Cefixime and Ciprofloxacin. The significant quinolone (1605.32cm^{-1}) and NO_2 (1575.13, 1251.26cm^{-1})groups of synergistic drugs evident on UC_{CC} coated silicone indicated no chemical interactions after coating. MTT assay did not inhibit the cell growth and morphology of fibroblast cells; indicating the biocompatibility of UC_{CC} coated materials.

Conclusion

Antibacterial activity results exhibited significant bacterial reduction percentage qualitatively and quantitatively against all the test bacteria. FTIR spectrum proved that all significant functional groups attributed to ciprofloxacin, and Cefixime, was present on coated materials. MTT assay results did not showed cytotoxic activity on L_{929} cells. Hence, the antibacterial activity, FESEM, FTIR characterizations and biocompatibility test results revealed that UC_{CC}coated catheters are biocompatible and could be used for urinary catheterized patients. In future, the comparative drug release from the coated catheters and detection of biofilm expressing genes in the causative pathogen need to be studied extensively to prove the significance of this research work in the field of pharma and medical sciences.

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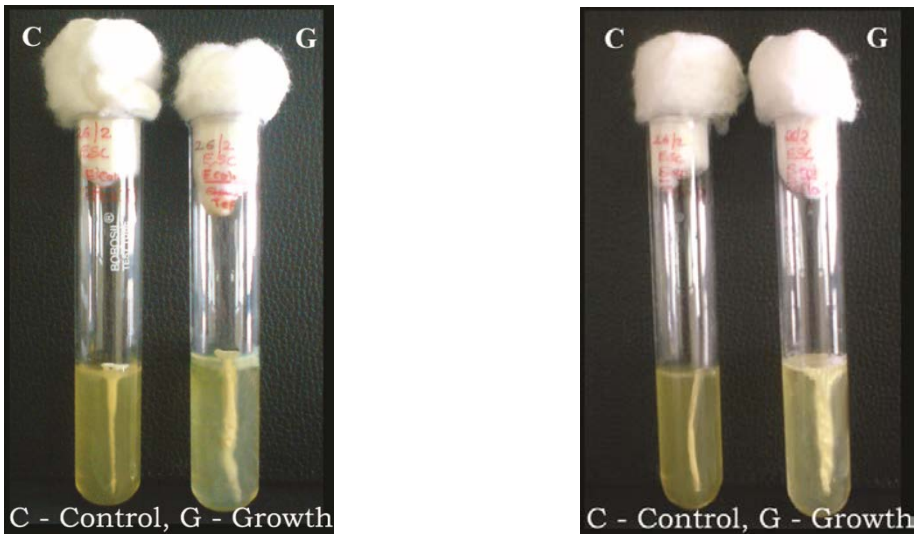
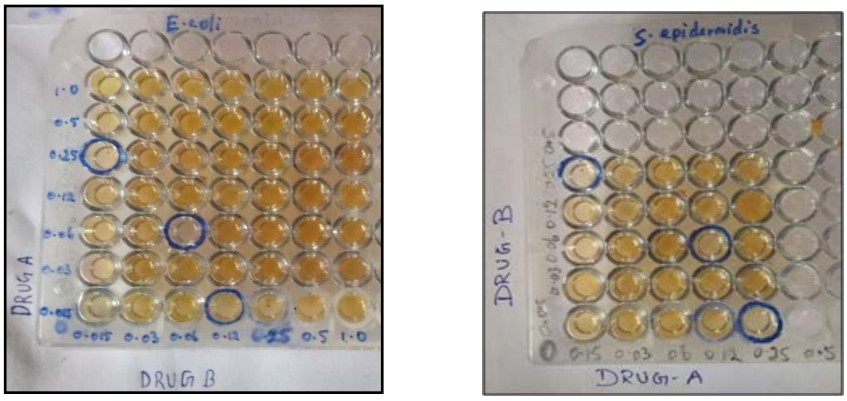
Fig. 1: Exit-site challenge test



Escherichia coli *Staphylococcus epidermidis*

Fig.2: Simplified Checker Board method of Cefixime-Ciprofloxacin against biofilm producing Test Organisms



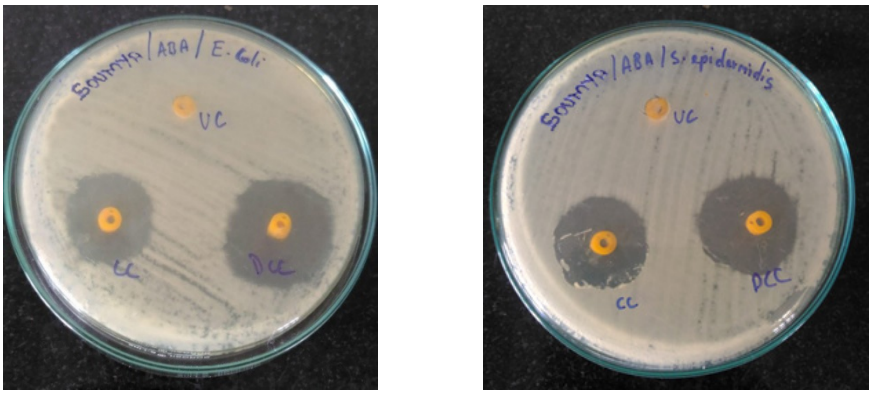
MIC_A – 0.25, MIC_B – 0.12, MIC_{AB} – 0.06 Test Bacteria: *E. coli*

MIC_A – 0.25, MIC_B – 0.25, MIC_{AB} – 0.06 Test Bacteria: *S. epidermidis*

Fig. 3: Preparation and Coating catheters with synergistic drugs

Catheter samples coating in synergistic drugs

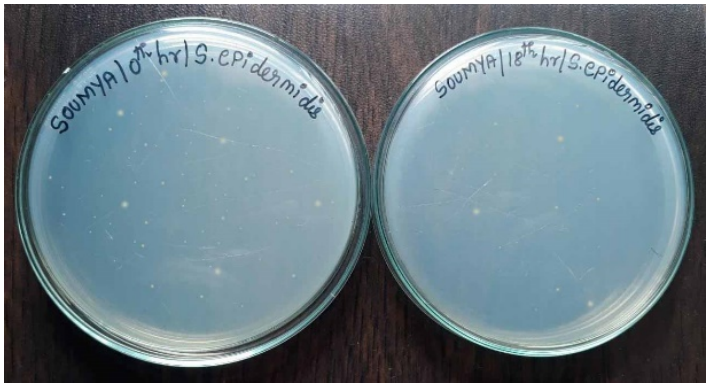
Fig. 4: QualitativeAntibacterial activity of UC_{CC} against the test organisms



Escherichia coli *Staphylococcus epidermidis*

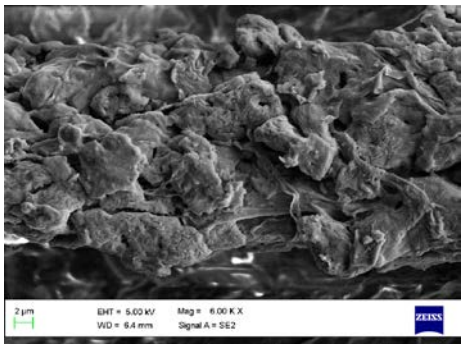
Fig. 5: QuantitativeAntibacterial activity of UC_{CC} against the test organisms

Escherichia coli

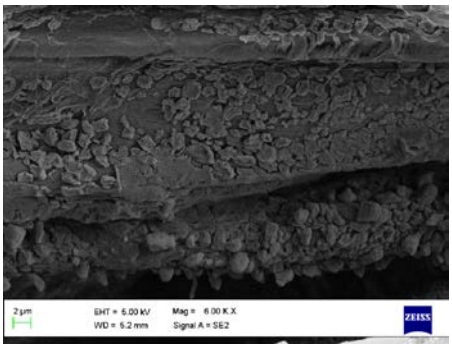


Staphylococcus epidermidis

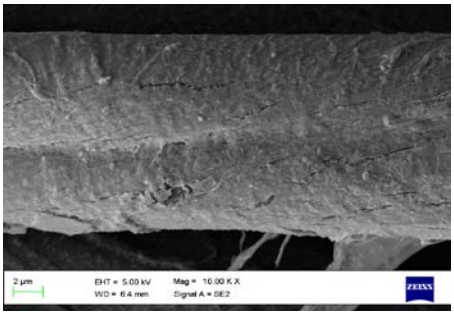
Fig. 6: Scanning Electron Microscopic analysis of Catheter samples



Coated – Seeding



Coated – Crystallization



Uncoated sample

Fig. 7:*Fourier Transform Infra-Red spectroscopic analysis of UC_{CC}*

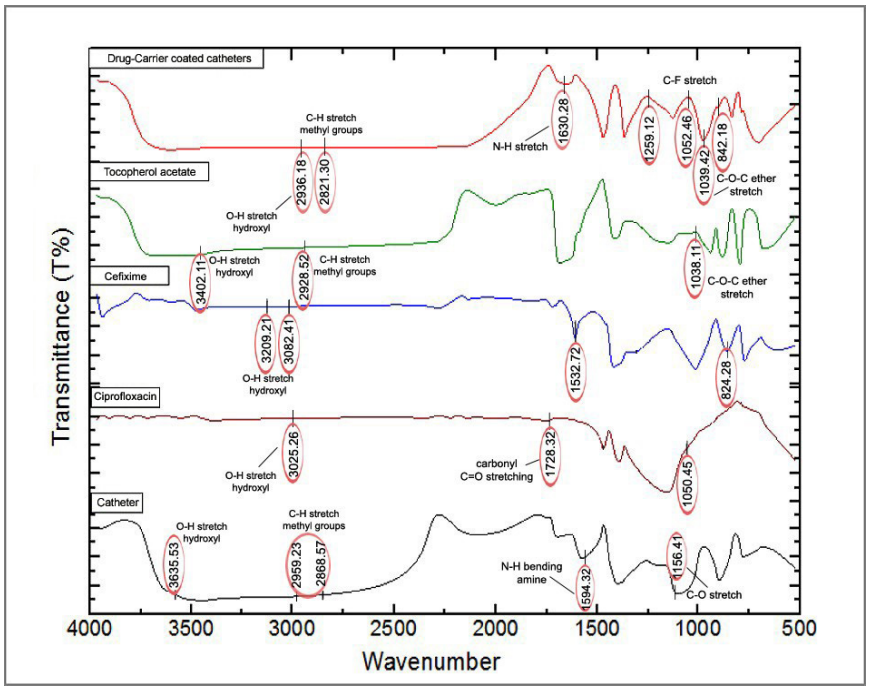
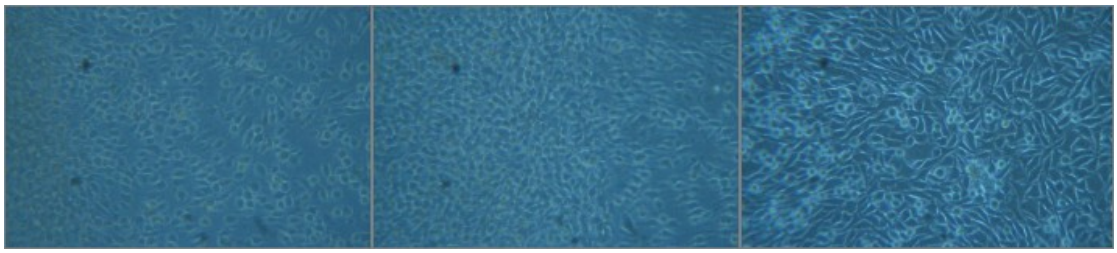


Fig. 8: Biocompatibility of the UC_{CC}



5µg 15µg Control

(Phase contrast microscopic analysis of L_{929} fibroblast cell line morphology)

Table-A: Classification of biofilm formation

Mean OD values	Biofilm formation	Biofilm index
<0.120	Nil	Non / weak
0.120-0.240	Moderately	Moderate
>0.240	Strong	High

Table adapted from Mathur*et al*., (2006)

Table-1: Microtitre plate assay

Test bacteria	Biofilm formation (OD 570nm)	Biofilm index
Staphylococcus epidermidis	0.244	High
Escherichia coli	0.192	Moderate
Staphylococcus aureus	0.242	High
Klebsiella pneumoniae	0.186	Moderate
Pseudomonas aeruginosa	0.160	Moderate

Table-2: Synergistic activity of Cefixime + Ciprofloxacinagainst test bacteria

Test Bacteria	MIC_A	MIC_B	MIC_{AB}	Index
Staphylococcus epidermidis	0.25	0.25	0.06	S
Staphylococcus aureus	0.25	0.25	0.12	S
Escherichia coli	0.25	0.12	0.06	S
Klebsiella pneumoniae	1.0	0.5	0.5	S
Pseudomonas aeruginosa	0.5	1.0	0.25	S

A – Cefixime, B - Ciprofloxacin, AB- Combined concentration of Cefixime-Ciprofloxacin
S – Synergy

Table-3: QualitativeAntibacterial activity of UC_{CC} against the test organisms

Sample	Organisms	Zone of Inhibition (mm)		
		dcc **urinary catheters**	***cc*** **Carrier coated catheters**	***uc*** **urinary catheters**
Urinary catheter	*Staphylococcus epidermidis*	18.3±1.80	11.6±0.57	0
	Escherichia coli	19.3±1.04	10.3±0.75	0
	Staphylococcus aureus	18.6±0.57	12.3±1.25	0
	Klebsiella pneumoniae	18.9±0.57	11.6±1.04	0
	Pseudomonas aeruginosa	19.3±1.04	11.3±1.80	0

dcc – drug and carrier coated, cc – carrier coated, uc - uncoated

Table-4: Quantitative antibacterial activity of UC_{CC} against the test organisms

S. No	Test organism	Bacterial reduction (%)	
		Uncoated catheters	UC_{CC} (coated catheters)
1.	*Escherichia coli*	0	95.6±1.04
2.	*Staphylococcus aureus*	0	96.5±1.80
3.	*Pseudomonas aeruginosa*	0	93.6±1.04
4.	*Staphylococcus epidermidis*	0	92.5±1.25
5.	*Klebsiella pneumoniae*	0	93.0±0.57

Table - 5: Persistence Test: Antibacterial durability of UC_{CC}

S. No	Test organism	Zone of inhibition (mm)									
		Day-1		Day-2		Day-3		Day-4		Day-5	
		dcc	***cc***	***dcc***	***cc***	***dcc***	***cc***	***dcc***	***cc***	***dcc***	***cc***
1	*Staphylococcus epidermidis*	18.3±1.80	11.6±0.57	14.6±1.15	9.1±2.02	8.6±0.76	7.1±1.15	7.8±1.25	0	0	0
2	*Escherichia coli*	19.3±1.04	10.3±0.75	15.3±1.52	10.8±1.44	9.6±0.76	0	8.1±0.76	0	0	0
3	*Staphylococcus aureus*	18.6±0.57	12.3±1.25	13.6±1.15	10.6±1.15	9.8±1.25	0	7.1±0.28	0	0	0
4	*Klebsiella pneumoniae*	18.9±0.57	11.6±1.04	14.6±0.57	11.1±2.02	8.6±1.32	0	8.8±1.25	0	0	0
5	*Pseudomonas aeruginosa*	19.3±1.04	11.3±1.80	15.1±1.25	9.5±0.83	8.3±2.46	7.5±0.57	7.1±1.15	0	0	0

dcc – drug and carrier coated, cc – carrier coated

Table - 6: Biocompatibility of the UC_{CC} – Cell viability test

S. No.	Fibroblast cell lines – MTT Assay			Cytotoxic reactivity (Biocompatible)
	Concentration (µg)	*Cytotoxicity (%)	*Cell viability (%)	
1	5	7.1±0.76	93.6±1.04	Biocompatible
2	15	5.1±0.57	95.6±1.04	Biocompatible
3	Control	0	>99±0.0	Biocompatible

*Mean ± Standard deviation