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DRUG ELUTING STENTS COATED WITH RAPAMYCIN CRYSTALS FOR THE PREVENTION OF RESTENOSIS AND BIOFILM FORMATION

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ABSTRACT

Restenosis and biofilm formation during coronary stent implantation were considered to increase the complications of cardiovascular disease. To prevent these dual problems, drug-eluting stents coated with crystallized drug-polymer mixtures (Rapamycin-Polyvinyl pyrrolidone) were developed and its efficacy was investigated in the present study. Single layer coating using seeding and crystallization of drug-polymer mixture on the stent surface were carried out for effective drug delivery at the target site. Efficacy of rapamycin release concentration from the coated drug-eluting stents for the prevention of restenosis using High performance liquid chromatography was determined. Antibacterial activity of the drug-eluting stents against biofilm organisms was simultaneously studied. High performance liquid chromatography established the efficiency of modified coating technique and controlled release of rapamycin from drug-polymer mixture. Antibacterial activity of drug-eluting stents showed maximum inhibitory zone of 31.3mm against *Staphylococcus aureus* for 2X crystallized rapamycin coated stents. SEM images of rapamycin coatings on the stents exhibits extremely large uniform and continuous layer of parallelogram shaped rapamycin crystals in drug loaded stents. The sustained release of drugs fulfilled the primary aim of developing an anti-infective method for post-operative infection caused by biofilm organisms and also the prevention of restenosis.

Key words Restenosis, Crystallization, Rapamycin, Polymer, Stents, Biofilm

INTRODUCTION

Implantation of coronary stents in atherosclerosis cases is considered as a common method with different complications depending on several factors[1]. Stent implantation leads to arterial injury which further activates vascular smooth muscle cells. These cells migrate and proliferate with extracellular-matrix formation which results in the development of neointimal tissue. Thus developed extreme neointimal hyperplasia leads to restenosis within the treated segment with ischemia, requiring repeat revascularization[2]. Certain growth factors with cellular or a cellular elements and the interaction of cytokines was considered as the pathophysiology of restenosis[3]. Failing to prevent the occurrence of these factors was considered insufficient to inhibit restenosis. About 35% of even successfully treated atherosclerotic lesion was reported to re-occlude within 6 months. This additional revascularization process, bypass surgeries and atherectomy was significantly considered to be the cost increasing factors [4].

Different pharmacological approaches to reduce and prevent restenosis reported were failed due to lack of essential drug concentrations at the target site[5]. Alternative to systemic therapy, delivering drugs offers the advantages of allowing high concentrations of drug at the stent implanted site[6]. The first-generation of drug-eluting stents (DES) with controlled release of sirolimus or paclitaxel drugs from degradable polymers were considered as a novel alternate to greatly reduce clinical measures of restenosis[7,8,9]. But it was reported that restenosis still occurs, and very late stent thrombosis is more common with first-generation DES due to delayed healing and re-endothelialisation. Although re-endothelialisation is multifactorial in cause, degradable polymer surface coatings can play a part[10]. There was also an equal need for reasonable stent coatings that can degrade gradually and be absorbed slowly by the body without creating the afore-mentioned adverse side effects. The polymer earmarked for these biodegradable coating is poly vinyl pyrrolidone (PVA) because of it biocompatibility and high rate of biodegradation[11].

Another major risk associated with surgical placement of medical stents such as coronary stents and/or vascular prostheses is their high infection rate due to pathogens mostly like *Staphylococcus aureus* and *Staphylococcus epidermidis*. Both biofilm producing organisms have the capability to colonize the implants in patients by adhering on their own proteins[12]. In the field of medical implants, the formation of biofilms on different types of materials like catheters and stents was considered to be a challenging complication[13]. Bacteria in biofilm are considered to be protected very well from the immune defense of host cells. An increase in antibiotic resistance is the consequence[14]; even high drug concentrations locally do not completely eradicate bacteria in biofilms[15]. It is therefore of great importance to prevent bacterial adhesion on vascular stents[16].

Polymers like poly lactic acid (PLA) and poly vinyl pyrrolidone are biocompatible and biodegradable usually used as a carrier molecule for stable and sustained drug delivery at the target site[17]. These polymers are mixed with drugs in desired proportion; and the drug-polymer coating was applied by dipping or spraying onto the stent surface continuously to establish desired coating layer thickness. Even though dip-coating procedures were found convincing under *in vitro* conditions, the coating solution may get entrapped in the device structure typically causes bridging, in other words forming of a

film across the open space between structural members of the stents[18]. The problem with straightforward antimicrobial loading into an implantable medical device by coating or immersion was the generation of resistance. Release of standard antibacterial drugs from the biomedical device surface is not coordinated with exposure to bacteria. This may results in leaching of sub-inhibitory levels of the antimicrobial agents; in sufficient to prevent infection but increases the risk for selecting antimicrobial resistant strains[19]. Hence, the focus is now on the development of novel DESs with biodegradable drug carriers from which drug can be dispensed in a modulated manner.

In this present research, a preliminary attempt was made whether the drug-polymer (Rapamycin-Polyvinyl pyrrolidone) mixtures could retard the dual problems like restenosis and biofilm formation simultaneously. This work highlights the use of Crystallized-Rapamycin loaded PVA polymer for cardiovascular/coronary stent coating. The mechanism of drug release from the polymer matrix, aimed at preventing restenosis and inflammation. To achieve this, a novel two step surface modifications of the stents were implied. Single layer coating using seeding and crystallization of drug-polymer (Rapamycin-PVP) mixture on the stent surface were carried out for effective drug delivery at the target site. In dip-coating procedures multiple layer coating occurs on the stent surface. Unlike the multiple layer coating, single layer coated stents offer constant and sustained drug release profile; the release concentration of drugs from the surface being enough for the dual problems of restenosis and biofilm formation.

MATERIALS AND METHODS

The present research work was carried out in the Department of Microbiology, Sree Narayana Guru College, Coimbatore, Tamil Nadu, India. About 5 different bacterial test cultures isolated from the biological specimens were procured from a diagnostic laboratory at Coimbatore, Tamil Nadu, India. All the dehydrated biological media used in the research were commercially procured from HiMedia, Mumbai, India. All the other chemicals were procured from Sigma-Aldrich, India. Scanning Electron Microscope (Carl Zeiss microscopy Ltd, UK & SIGMA) analysis of the samples was carried out in SITRA, South India Textile Research Association, Coimbatore, India. HPLC analysis of drug release profile was carried out in Dalmia Research Centre, Coimbatore, India. The entire research work was carried out from November 2015 to October 2016.

Surface colonizing ability of test organisms: Exit-site challenge test[20]

Exit-site challenge test was performed to investigate the ability of the organism to grow both external and inner surface of implants or stents. In this method, Iso-sensitest semi solid Agar was used for this analysis. Media surface was inoculated with 100µl of test organisms [*Staphylococcus aureus* (ATCC 29213), *Escherichia coli* (ATCC 43894) and *Pseudomonas aeruginosa* (ATCC 700829)]. Each braided stents with pre-measure size of 15mm in length was spliced under sterile conditions. All the stent samples are sterilized and inserted onto the semi-solid media with half of the portion projecting outside. The stents should be inserted through the inoculated area and incubated at 37°C. The migration ability of each test bacteria from the exit site down the material track or in other words, outside of the materials were observed for 24 hours.

Biofilm forming ability of test organisms: Microtitre plate biofilm assay [21]

Biofilm forming ability of each test organism is evaluated using the microtitre plate method described by Christensen et al[21]. In brief, all the test organisms [*Staphylococcus aureus* (ATCC 29213), *Escherichia coli* (ATCC 43894) and *Pseudomonas aeruginosa* (ATCC 700829)] were allowed to grow in the 96 well titre plate for 48 hours. All the wells are washed with double distilled water and added crystal violet dye to evaluate the remaining adhered organisms. These adhered organisms were considered to be as the biofilm growth on the inner surface of plate. Added About 100µl of 90% ethanol was added to each stained well. After incubating the plates, the stains in ethanol layer are measured to each stained well at a wavelength of 600nm. Optical density of test organisms considered as biofilm producers were read using a ELISA reader (Braun, Germany). Based on the OD value, the adherence of organism in the silicate tubes and titre plates were classified as mentioned in Table 1.

Preparation of crystallized Rapamycin (RM)[22]

Polyester fiber was procured commercially form a yarn unit at tirupur and its physical parameters were recorded as per manufactures standards. The yarn was braided into a typical hollow structure to form a textile based stent. This braiding fabrication was carried out in such a way which exactly looks like a stent used regularly in biomedical applications (Figure 1). This fabricated textile stent is coated with rapamycin for the further studies.

Seeding of stents with rapamycin

Process is composed of two steps (seeding and crystallization). For seeding, 50 mg of rapamycin was weighed, grinded for 3 min and 1.6 mg of grinded rapamycin was transferred into 5 ml 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 Rapamycin in hexane was formed. After sonication, stents were mounted on shrinkable tube placed on needle and needle loaded with stent was placed at the centre of the vial containing the dispersed Rapamycin in hexane (one stent per trial). These vials were then placed in the ultrasonic bath (Shimadzu,) for 10 min at 30°C to form seeding layer. Stents were gently taken out of the vial and allowed to dry at room temperature. These dried and seeded stents were preceded to the next crystallization step.

Crystallization of stents with rapamycin

For crystallization, 50mg of Rapamycin 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. Stents were placed in this solution at 25°C/5 min for crystallization of Rapamycin on seeding layer to form crystals carpet formation, and then dried overnight. Similarly seeding and crystallization was carried out for after 2 concentration of rapamycin as mentioned below in Table 2.

SEM analysis of stents coated with crystallized rapamycin

The surface coatings of the drugs on stent materials were observed using Scanning electron microscopy. 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 analyze the sample.

Assessing the qualitative antibacterial activity of drug-eluting stents[23]

Qualitative antibacterial activity of drug-eluting stents was performed as per the method described by El Rehewy et al[23]. This method was also called as slurry dip-coating technique. The stents are coated with rapamycin under controlled sterile conditions. The pre-measured size of 5mm in length was selected for coated and uncoated preparations [drug and carrier coated at different concentration (1X, 2X) and uncoated materials]. All the materials were washed in phosphate buffered saline (PBS) to remove any surface accumulation of drug. All test materials were placed on the surface of Nutrient agar (Hi Media) plate which was seeded with overnight broth culture of each test organisms [*Staphylococcus aureus* (ATCC 29213), *Escherichia coli* (ATCC 43894) and *Pseudomonas aeruginosa* (ATCC 700829)]. All the plates were incubated at 37°C for 24h to observe the zone of inhibition around the coated stents. The antibacterial activity of each coated stents was expressed as the diameter of the zone of inhibition.

***In vitro* analysis of efficacy of rapamycin release concentration for the prevention of restenosis using High performance liquid chromatography**[24]

The efficacy of rapamycin concentration released from the drug-eluting stents was analyzed using High performance liquid chromatography (HPLC) with a known standard. The release profile of rapamycin drug from the biodegradable polymer matrix for a period of 120hours was studied under simulated biological conditions. Standard solutions were prepared by dissolving 10 mg of rapamycin drug in 10ml of mobile phase. This mobile phase was then diluted upto 100 ml. Diluted 20 µl standard was injected in the HPLC column and standard chromatogram for this standardized solution was obtained. For *in-vitro* rapamycin kinetics study, stents were incubated in 50 ml of phosphate buffer saline (PBS) solution at 37°C with constant agitation at 300 rpm in a thermo mixer. Each drug coated stents were removed at 30 min, 1, 2, 4, 8, 12, 24, 48, 72, 96 120 hours from their release vials and analyzed for amount of rapamycin release in PBS. Rapamycin was extracted using DCM which was later evaporated using dry nitrogen gas. Mobile phase was added to this and the resultant supernatant was analyzed for rapamycin content by High performance liquid chromatography (HPLC), and the rapamycin released at regular interval from each stent was calculated.

RESULTS

Surface colonizing ability of test organisms: Exit-site challenge test

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

Biofilm forming ability of test organisms: Microtitre plate biofilm assay

In Table 3, the optical density (OD) values and biofilm index of the test organisms were presented based on the biofilm classification described by Christensen *et al*., (1985). All the three test organisms showed significant biofilm index value indicating as strong biofilm producers. All the three organisms showed OD values >0.240 [*S. aureus* (0.290), *E. coli* (0.270) and *Pseudomonas aeruginosa* (0.260)]. The differences in OD values observed during the analysis were due to the amount of crystal-violet (dye) absorbed by the test organisms in the microtitre well. Appropriate control was maintained throughout the test.

SEM analysis of stents coated with crystallized rapamycin

This study describes a novel crystalline coating methodology for therapeutic agents using a drug, rapamycin. The whole process was broadly divided into seeding and surface crystallization of rapamycin. Stents were coated with rapamycin crystals and topographical analysis was carried out using SEM. The outer surface of the stent was found fully covered with RM seeds. Representative SEM images of rapamycin coatings on the stents exhibits extremely large uniform and continuous layer of parallelogram shaped RM crystals in drug loaded stents. The difference in the morphology of uncoated and coated stents was clearly evident in Figure 2a and 2b respectively. This crystal coating process was effective for both low (1X) and high (2X) drug loading and provides a continuous crystals coating on the stent surface however with difference in particle size. This was mainly due to typical coating morphology achieved due to polymer mixture with the drug rapamycin.

Assessing the qualitative antibacterial activity of drug-eluting stents

The diffusing ability of the rapamycin from the drug-eluting stents to retard the growth of test bacteria seeded on MHA plate was calculated based on the zone of inhibition. The zone of inhibition measured in millimetres for each drug-eluting stents (tested in triplicates) was calculated to obtain the mean value. In Table 4, revealed the antibacterial activity of drug-eluting stents for all the test organisms. No inhibitory zones were observed for uncoated materials.

***In vitro* analysis of efficacy of rapamycin release concentration for the prevention of restenosis using High performance liquid chromatography**

In vitro release study was conducted on developed rapamycin-PVP crystallized stents loaded with 1X and 2X strength of drug. Release concentration of rapamycin in PBS at specific temperature was determined. The release study was conducted for 120hours in PBS at 37°C with three stents from each type (1X and 2X).

Stent materials coated with rapamycin when released from crystalline rapamycin-PVP mixture indicated that the rate of drug release was exponentially related to the release time. In Figure 4, the lag phase exhibited initial burst effect from 0.5 h to 4 hours (49µg, 49µg, 49µg and 64µg). Followed this lag phase, increase in drug concentration was observed from 8 hours to 24 hours (79µg, 86µg and 99µg). In PBS at pH 7.0, the hydrophilic polymer, PVP undergoes degradation during the log phase. Due to the rate of polymer degradation, the release of drugs was facilitated at higher rate than the initial burst level concentration.

During this phase the mean concentration release of rapamycin was remained almost constant (98µg, 100µg, 102µg and 102µg) from 48 hours to 120 hours, indicating the sustained rate of drugs from the coated stents. Almost similar release profile of rapamycin was observed for the 2X rapamycin coated stents (38µg, 38µg, 38µg and 52µg, 64µg, 75µg and 89µg, 94µg, 98µg, 101µg, 102µg) from 0.5 hours to 120 hours (Figure 5).

DISCUSSION

The biofilm forming ability of each test organisms was determined using Exit-site challenge test in the present study. Among the three-high biofilm forming organisms, the skin associated *Staphylococcus aureus* colonized with in 24h. This quick surface colonization ability of the organisms indicates that their ability to cause stent associated infections during coronary stent implantation procedures. This property was emphasized by Sweda Sreekumar et al[25] during their research work. The authors have stated that the migration or growth of biofilm producers around the stent materials was indicated by tracking of bacteria along the abluminal surface. In another study by Bayston et al[20] this statement was well inaccordance with the present research work. The inoculated site was considered to be as skin or tissue exit-site and migration of the organisms along the media surface was considered to be as the tissue tunnel and its surroundings.

The obtained results in exit-site challenge test were well in accordance with the other preliminary experiments conducted in this research work. Hence, the results obtained in Exit-site challenge test by the test organism *Staphylococcus aureus*, maximum biofilm index value of 0.290 OD during Microtitre plate biofilm assay was observed. The obtained results indicated the ability of *Staphylococcus aureus* to produce biofilm associated or stent associated infections. According to Mathur et al[26] the current method is the sensitive and accurate method for evaluating the biofilm forming ability of any organims[26]. The method is also considered as the suitable quantitative model to study the adherent properties of organism on any medical implants, prostheses and stents.

The topographical analysis of coated stents revealed the presence of crystallized drug particles on the stent surface; exhibiting adherence to the greatest possible extent. SEM analysis of the coated stent also evidenced that the homogenous coating of drug-polymer mixture does not provide any surface space on the stent for the bacterial adhesion or biofilm deposition. This was mainly by reducing the depressions on the material surfaces by the crystallized deposition of rapamycin on the stent. The obtained results were found supportive when compared to the results of Olena Rzhepishevska et al[27]. The researchers found that the surface charge of antibacterial coatings was more efficient in reducing bacterial adhesion and biofilm formation.

Antibacterial activity of developed drug-eluting stents coated with 1X and 2X strength rapamycin showed significant inhibitory zones against the biofilm producing test organisms. The inhibitory zones ranged from 18.9mm to 31.3mm during the analysis. Interestingly, maximum inhibitory zone of 31.3mm against high biofilm producing *Staphylococcus aureus* were observed for 2X crystallized rapamycin coated stents. Other biofilm producers like *Escherichia coli* and *Pseudomonas aeruginosa* also showed significant inhibitory zones measuring 25.6mm and 21.9mm respectively. The obtained inhibitory zones for 2X coated drug-eluting stents were more when compared to that of stents coated with 1X concentration. This is evident from the Table-4 and figures presented. The polymer PVP on the stent surface assisted the elution of drug from the stents after placing on the agar surface. Degradation of polymer occurs at a sustained rate when the stent was exposed on the moistened agar surface. This in turn the polymer makes the drug to release at constant rate from the agar surface to inhibit the biofilm producing organisms. In Figure 3a, 3b and 3c, the clear inhibitory zones around the drug-eluting stents against the biofilm producing test cultures were presented. The rate of degradation of the polymer in the PBS was considered to be directly proportional to the rate of release of drugs[28]. When the rate of degradation was high, then the release concentration was also found out to be high. Similar condition was experienced by Matl et al[29] during their research when they analysed the release of gentamicin and teicoplanin from PTFE vascular stents grafted with carriers like DL-lactic acid, tocopherol acetate and dynasan. Zarida et al[30] reported that the rate of release of tobramycin and gentamicin was due to the rate of degradation of the drug containing calcium phosphate beads. Comparative analysis was found supportive during the analysis.

A great diversity of antibiotics used for reasonable graft protection were described in the literature, including, gentamicin and teicoplanin. Forster et al[31] in their study carried out to assess the potential of gentamicin coated polyurethane sleeves to inhibit bacterial colonization on external fixation pins and wires. The initial burst release was characterized by predicted pin tract gentamicin concentrations of >80 µg/ml at the 2 h and 1day elution time points. Similarly, in the present research >35 µg of rapamycin drug was eluted as the initial burst concentration between 0.5 hours and 4 hours from the drug-eluting stents. This concentration of antibacterial drugs which may deliver directly to the stent implanted tract, are far beyond those that could be achieved via oral or intravenous administration i.e., below the pathologically relevant concentrations (systemic antibiotic prophylaxis - 100 to 200mg/day). Furthermore, the expected concentration of the test drugs at the implantable tract remained above the National Committee for Clinical Laboratory Standards (NCCLS) MIC breakpoint of 4µg/ml[32]. Thus the release concentrations of the drugs more than the MIC value could prevent the growth of organisms in the surrounding tissues of the material implanted. Holt et al[33] investigated the reduction of bacterial colonization in external fixation pins via nitric oxide release coatings. The researchers concluded that the application of nitric oxide releasing xerogel coatings can inhibit bacterial colonization of external fixation pins both during the initial postsurgical period and up to 48 days post-implantation.

This drug releasing phenomenon aided by polymer PVP for the prevention of biofilm formation was also described for its ability to prevent the formation of restenosis in the next subsequent section.

In vitro analysis of efficacy of rapamycin release concentration for the prevention of restenosis using High performance liquid chromatography was studied. During the preparation of drug-polymer mixture, the drugs were not dissolved completely in the dissolving agents used, so the samples were coated by drug-polymer suspensions. As a result, coatings consisted of rapamycin particles got incorporated into the polymer. An initial drug burst in 30 minutes of elution was the consequence, since drug particles from the surface of the coating dissolved rapidly after contact with elution buffer (PBS). Particles located deeper inside the polymer was released only after their degradation. The use of poly vinyl pyrrolidone as a polymer offers the possibility to place hydrophilic drugs on the surfaces of hydrophobic stents and implants, building up a slow-release drug delivery system independent of the drug charge. This

statement was well coincided with the literature review of Puranik et al[34]. The researchers cited the types and significant properties of polymer coated implants, biodegradable implants and drug-carrier stents. This slow-release drug delivery system containing crystallized rapamycin-PVP mixtures from stent surface could have a cytostatic effect on the neointimal growth, which is desirable in the later stages of vascular healing. Protective PVP in the drug-polymer mixture being hydrophilic would degrade within a short span of time in vascular environment. Crystallized rapamycin from the coated stents slowly gets diffused into the surrounding tissues thus preventing restenosis[35].

The dual role of drug-eluting stents in preventing biofilm associated infection and restenosis in coronary implanted cases were thus investigated in this study based on this polymer assisted drug releasing phenomenon.

Table 1: 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 2: Rapamycin concentrations for coating onto stent materials

Strength of rapamycin	Drug-polymer mixture	Weight of the stents after coating
Bare stent (Uncoated)	Nil	18.1mg
1X	1.6mg of rapamycin with biodegradable polymer mixture dissolved in 4ml of n-hexane	18.8mg
2X	3.2mg of rapamycin with biodegradable polymer mixture dissolved in 4ml of n-hexane	19.1mg

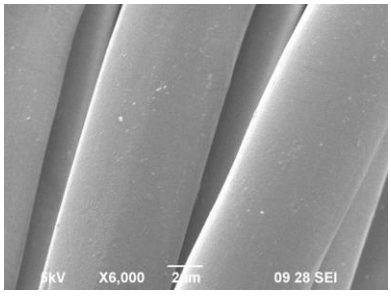
Table 3: Assessing the biofilm forming capability of test bacteria using standard Microtitre plate biofilm assay

Test Bacteria	Biofilm formation (OD 570_{nm})	Biofilm index
Control C_2 (Nutrient broth)	0.04	weak
Escherichia coli	0.27	High
Staphylococcus aureus	0.29	High
Pseudomonas aeruginosa	0.26	High

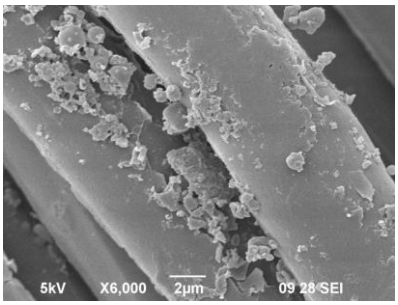
Table 4: Assessing the qualitative antibacterial activity of drug-eluting stents

S. No	Drug eluting stents (DES)	Zone of inhibition (mm)		
		Staphylococcus aureus	***Escherichia coli***	***Pseudomonas aeruginosa***
1	1X Rapamycin coated DES	29.3	23.9	18.9
2	2X Rapamycin coated DES	31.3	25.6	21.9

Figure 1: Textile braided stents as coronary drug-eluting stents

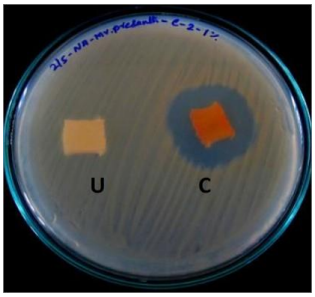


2(a) Uncoated stent surface

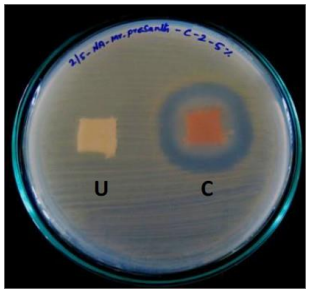


2(b) Stent coated with crystallized rapamycin in irregualr size and parallelogram structures

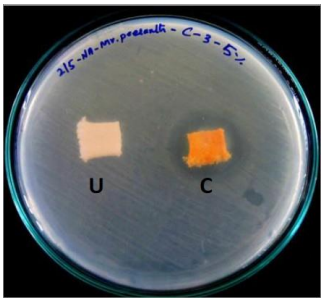
Figure 2: SEM analysis of stents coated with crystallized rapamycin



3(a) *Staphylococcus aureus*



3(b) *Escherichia coli*



3(c) *Pseudomonas aeruginosa*

Figure 3: Assessing the qualitative antibacterial activity of drug-eluting stents

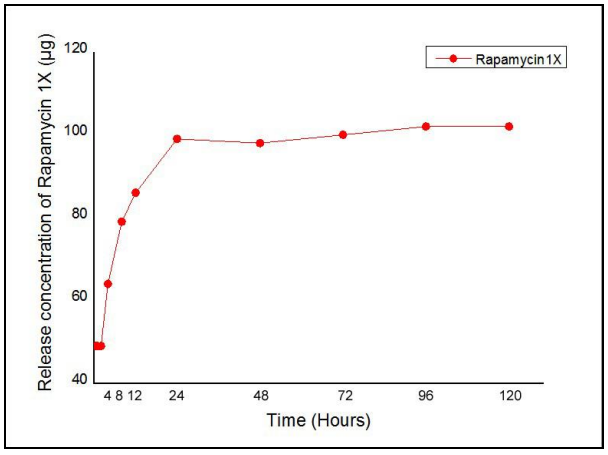


Figure 4: Release profile of Rapamycin (1X) from drug eluting stents

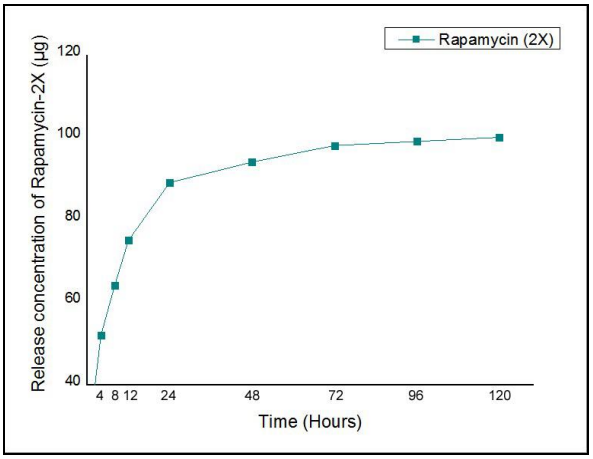


Figure 5: Release profile of Rapamycin (2X) from drug eluting stents

CONCLUSION

In this research, rapamycin-PVP mixtures was crystallized and coated onto the stent materials to retard restenosis and biofilm formation simultaneously. Two different concentrations of drug-polymer mixtures were used for coating the stent materials (1X and 2X). During the study, it was found significant that the drug-eluting stents coated with 2X drug-polymer concentrate could able to retard the growth of biofilm forming organisms and restenosis better than the 1X concentrate when tested using standard assay protocols. The developed drug-eluting stents in the present study revealed the significance of the coronary implantation for patients in preventing critical cardiovascular disease due to biofilm formation and restenosis. The sustained release of drugs from drug eluting stents fulfilled the primary aim of developing an anti-infective method for post-operative infection caused by biofilm organisms and also the prevention of restenosis. The research work is appropriate for the field of Pharmacology because, the developed drug-eluting stents is considered as a novel biomedical product with a combination of drug and biodegradable polymer mixtures.

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