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Coronary Implants Coated with Rapamycin and Heparin to Retard Bacterial Adhesions and Restenosis–An Experimental Study

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ABSTRACT

Background: Atherosclerosis is a common type of coronary artery disease caused by occlusion of coronary arteries. Plaque narrows and hardens the arteries. Stent implantation is considered to be the most successful treatment for atherosclerosis. Hyperplasia and bacterial adhesion are complications found among stent implanted patients.

Methods: The present research investigates solving the dual problems of restenosis and bacterial adhesion using a rapamycin-heparin mixture impregnated with cyclodextrin as a carrier. Drug discharge analysis using HPLC, the bacterial adhesion ability of organisms, and the inhibitory effect of drug-coated stents were evaluated. The uniform coating of drugs on the stents can be observed under scanning electron microscopy.

Results: The discharge of drugs from a coated stent surface was analysed for a maximum of 144h. Polymer degradation occurred and the mean concentration release remained almost constant from 72h to 144h, indicating the sustained release of drugs from the coated stents. Strong bacterial adhesion-producing organisms, Staphylococcus aureus (0.28) and Escherichia coli (0.27), showed a 29.4 ± 1.7mm and 23.2 ± 0.8mm zone of inhibition.

Conclusion: The dual role of stents in preventing bacterial adhesion-associated infection and restenosis was achieved. Thus, a rapamycin-heparin coated drug with a cyclodextrin carrier can be considered as a novel product in the biomedical industry, with respect to its constant drug releasing ability.

Key words: Atherosclerosis, Coronary stent, Bacterial adhesion, Rapamycin, Heparin, Cyclodextrin

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INTRODUCTION

Atherosclerosis, which leads to coronary artery disease, is caused by the occlusion of coronary arteries. Coronary blood vessels become occluded by the deposition of cholesterol, fat, calcium and other cellular components from blood, called plaque, beneath the intima of blood vessels. Plaque causes arteries to narrow and harden. This develops gradually over a number of years and can cause significant blockage of arteries, which results in a condition known as atherosclerosis [1].

Stent implantation is the most successful treatment for atherosclerosis. The blockage is removed, and the stent enables the constant flow

of blood through the arteries. Stent implantation can be an immediate treatment after a heart attack; however, it has some disadvantages. Hyperplasia occurs at the site of injured arterial blood vessels [2], which results in restenosis. About 35% of patients treated for atherosclerosis had re-occlusion within a period of six months. Other treatments, such as bypass surgery, atherectomy and the revascularization process are considered to be higher-cost alternatives [3]. Drug-eluting stents were developed to reduce the formation of neointimal tissues, which leads to restenosis, and has a wide range of clinical applications [4].

The drugs are coated on the stent and released after the implant into the coronary vessels. The dip-coating method for coating the stent with a drug has been found to be effective, and the drug is entrapped in the stent and binds to the open spaces. A lower concentration of drugs coated on stents at the target site is the main complication for the prevention of restenosis [5,6]. Pendyala et al. [7] developed the first generation of drug-coated stents. They coated sirolimus drugs on stent surfaces and compared this with plain stents. The synthesis of drug-coated stents that are dissoluble and absorbed by the body gradually, without the formation of hyperplasia, is considered as a novel product [5].

Rapamycin is a macrolide that has antiproliferative properties. At low doses, rapamycin acts as a strong inhibitor of inflammation, with no toxic effects on cells. Encapsulation of rapamycin with a polymeric carrier in stents was used to prevent restenosis. Rapamycin has immunosuppressant functions, which can be used to prevent rejection of kidneys and organs during transplantation. Rapamycin crystals have a low dissolution property. Thus, coating leads to the controlled release of drugs from stents, without the coating of the carrier controlling the release of rapamycin. A crystalline form of rapamycin is chemically more stable and has a longer shelf life than its amorphous form [8,9]. Heparin is commonly used as a blood thinner (anticoagulant). Due to its antithrombotic and antiproliferative properties, heparin is used to treat deep vein thrombosis (a blood clot in a vein) and arterial thromboembolism (a blood clot in an artery). The anticoagulant property of heparin makes it useful for the treatment of atherosclerotic artery disease. Heparin can be directly injected into veins. The antiproliferative and antithrombotic effect of heparin was analysed and compared with dipyridamole and aspirin among femoropopliteal bypass graft patients [10]. Patients who were given heparin had a higher graft survival of 12 months, compared with patients given dipyridamole and aspirin. Stents containing heparin and rapamycin, or curcumin regulated a stable and gradual drug release.

The carriers are polymers mixed with an effective drug that enables a stable and sustained regulation of the drug. Cyclodextrin is a polysaccharide that is externally hydrophilic in nature. Cyclodextrin have complexation ability and versatile properties that make it efficient in drug delivery mechanisms. Cyclodextrin enhances the stability, solubility, and bioavailability of impregnated drug molecules, which makes it an effective drug carrier in the pharmaceutical industry [11].

Infection in coronary stents and catheters is one of the devastating complications among implanted patients. These are difficult to treat and the mortality rate is high.S. aureus and P. aeruginosa are the pathogens mostly involved in stent infections. These pathogens colonize the implants, which results in bacterial adhesion. The infection-causing organisms develop and gradually become resistant to antibiotics [12]. Therefore, preventing bacterial adhesion on vascular stents is considered highly significant. The antibacterial activity of heparin was examined by Rosett and Hodges [13]. They concluded that heparin inhibited the growth of eight isolates of test organisms. Most of the gram-positive organisms are susceptible and resistant to heparin.

The present research investigates solving the dual problems of restenosis and bacterial adhesion using a rapamycin-heparin mixture impregnated with cyclodextrin as a carrier. The crystallized rapamycin and heparin were coated on stents. This drug release mechanism aims at preventing restenosis and antimicrobial resistance.

METHODS

Procurement of SS mesh for the fabrication of coronary stents

In the present study, the metal stent-like implant

was fabricated using stainless steel (SS) mesh. The fabricated mesh was cut into separate pieces to a standard size (1cm).

Coating of coronary stents with drugs and carriers

The stents were subjected to the process of coating with a drug (rapamycin), an anti-coagulant (heparin) and a drug-carrier (cyclodextrin). Two different phases of coating were carried out for the effective and constant release of drugs from the stent surface. The initial coating process was seeding, followed by crystallization of drugs and carriers on the surface of the stent.

Seeding of stents

A seeding solution was prepared by blending 1000µg of rapamycin and 800µg of heparin in a 5ml solution of hexane (HiMedia). The seeding solution was sonicated until the rapamycin-heparin mixture dispersed completely in n-hexane using an ultrasonic bath (Shimadzu) for a duration of about 10min at 30°C. The stent was kept inside the drug-carrier mixtures and a seeding layer could form on the stent surface. After seeding, the stents were dried at room temperature (37°C).

Crystallization of stents

A secondary layer using a rapamycin-heparin mixture with a drug carrier (cyclodextrin) was coated as crystals on the surface of the seeded layer. The mixture was added to an ethyl acetate solution, and n-hexane was added dropwise into the mixture to form a homogenous solution. The stents were slowly placed into the mixture and incubated for 15 minutes at 20°C for the formation of carpet crystals on the seeding layer of the stents. After crystallization, the stents were dried overnight at room temperature (37°C).

FESEM analysis of drug and carrier-coated coronary stents

The crystallized drug-carrier mixture, coated on the coronary stent surface, was observed using Field-Emission Scanning Electron Microscopy (FESEM). Topographic analysis was also used to confirm the uniform coating of the drug-carrier mixture on the surface of the stent. The crystals of the drug-carrier coating on the stent were observed under a magnification of 6000X.

Release concentration of rapamycin

High performance liquid chromatography was used to analyse the discharge of the drug from the coated stents using the method illustrated by Levy et al.

Bacterial adhesion assay

A microtitre plate assay was used to evaluate the microorganisms involved in the bacterial adhesion. This method was executed as described by others.

Antibacterial activity

The inhibitory effect of the coated stent against the growth of microorganisms was evaluated by antibacterial activity, as illustrated by John et al. The inhibitory zones of stents against test organisms were expressed in diameter.

RESULTS

FESEM analysis of drug and carrier-coated coronary stents

The seeded and crystallized drug-carrier mixture was observed topographically after being surface coated onto the stents. From the FESEM images, it was evident that the external surface of the coated stent was covered with rapamycin-heparin in the form of crystals (Figure 1). The homogenous, crystallized drug-carrier mixture uniformly coated the stent surface, and thus signifies the method of seeding and crystallization protocols.

Release concentration of rapamycin

The drug discharged from the stents was determined in phosphate buffer saline at different time intervals (60min to 144h); the drug discharged from the cyclodextrin (carrier) indicated an exponential discharge of drugs. This was also commensurate with the time of discharge. The same was observed in the graphical representation shown in Figure 2. The rate of discharge that occurred between 60minutes

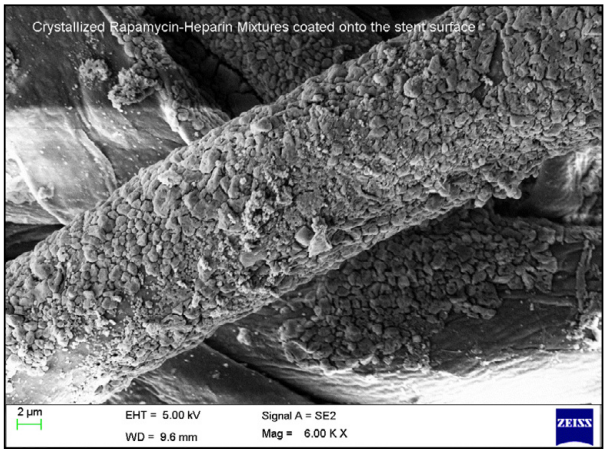

Figure 1: FESEM analysis of drug and carrier-coated coronary stents.

to 4 hours (35µg, 50µg and 60µg) was studied during the lag phase. During this time, a sudden drug release was evident due to unconditional exposure of stents to the buffer solution. The condition would be remarkably similar under in vivo conditions. This is termed as an initial drug-burst effect. In the subsequent phase, an increase in the drug discharge concentration was observed. In 8th, 12th, 24th and 48th hour, the drug discharge concentration was at a rate of 70µg, 85µg, 90µg and 110µg, respectively. The release in these higher concentrations may be due to dissolution of the carrier, cyclodextrin. Despite its hydrophilic properties, the drug release behaviour was influenced during this analysis. From 72h to 144h, the drug-carrier influenced the stable discharge of drugs (120µg, 120µg, 130µg and 125µg).

Bacterial adhesion assay

In this assay, the index of all bacterial adhesion-forming organisms (Table 1) and its respective optical density values were studied. The optical density values of each organism, with respect to the bacterial adhesion index of the test organisms, was compared and presented (Table 2). All organisms showed OD values greater than 0.24 (>0.24) *Staphylococcus aureus* (0.28), *Escherichia coli* (0.27), *Proteus sp* (0.26), *Staphylococcus epidermidis* (0.24) and *Enterobacter aerogens* (0.25). The variation in OD values is due to the difference in the amount of absorbed crystal violet dye by the bacterial adhesion-producing test organisms.

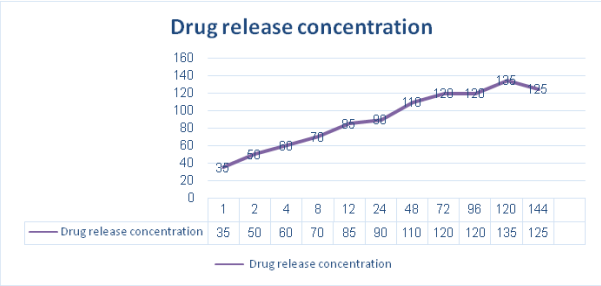

Figure 2: Release concentration of rapamycin.

Table 1: Bacterial adhesion index.

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

Table 2: Bacterial adhesion assay.

S. No	Test Bacteria`	Bacterial adhesion (OD 570nm)	Bacterial adhesion index
1	*Staphylococcus aureus*	0.28	High
2	*Escherichia coli*	0.27	High
3	*Proteus sp*	0.26	High
4	*Staphylococcus epidermidis*	0.24	Moderate
5	*Enterobacter aerogens*	0.25	High

Table 3: Antibacterial activity.

S. No	Test organisms	Zone of inhibition (Mean ± SD)
1	*Staphylococcus aureus*	29.4 ± 1.7
2	*Escherichia coli*	23.2 ± 0.8
3	*Proteus sp*	24.4 ± 1.1
4	*Staphylococcus epidermidis*	21.3 ± 1.5
5	*Enterobacter aerogens*	19.9 ± 1.8

Antibacterial activity

All the coated stents exhibited inhibitory zones ranging from 19.9 ± 1.8mm to 29.4 ± 1.7mm when tested against the respective test organisms (Table 3). Maximum inhibitory zones were obtained against Staphylococcus aureus (29.4 ± 1.7mm of inhibitory zone) and a minimum inhibitory zone of 19.9 ± 1.8mm was observed for *Enterobacter aerogens*. In Figure 3 and Figure 4, the inhibitory zones obtained against the five bacterial adhesion producers were clear. Following *Enterobacter aerogens*, the other test organisms (*Proteus sp, Escherichia coli* and *Staphylococcus epidermidis*) exhibited inhibitory zones in the order of 24.4 ± 1.1mm, 23.2 ± 0.8mm and 21.3 ± 1.5 mm respectively.

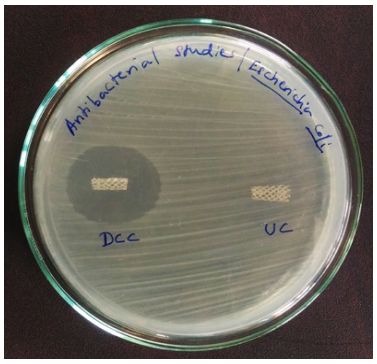

Figure 3: Antibacterial activity *(Escherichia coli).*

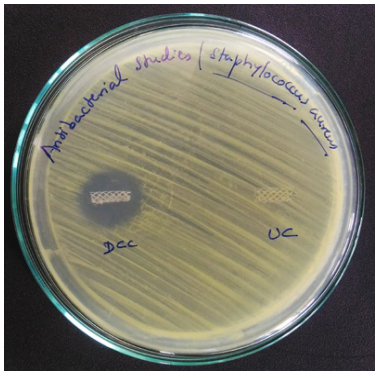

Figure 4: Antibacterial activity *(Staphylococcus aureus).*

DISCUSSION

The efficiency of rapamycin in a cyclodextrin carrier release concentration to prevent restenosis was investigated using HPLC. The stents were impregnated with rapamycin-cyclodextrin and the preliminary drug burst occurred in 60 minutes. The remaining particles present were discharged after the dissolution of the polymer. The constant discharge of the carrier drug was presumed to be the drug attached to the stent surfaces. On a study conducted by Wu and Jin [14], the properties and function of different types of implants were investigated and they concluded that a slow, sustained release of drugs retarded the growth of smooth muscle cells on stents. Similarly, in the present research the carrier cyclodextrin, being hydrophilic, degraded significantly which enabled a constant release of the drug. Gidwani and Vyas [15] analyzed and reported that cyclodextrin can be used in effective drug delivery mechanisms. The property of bonding with drugs has proved that cyclodextrin can serve as a potential carrier for effective and constant drug delivery at the targeted site. Hence, in the present research, the properties of cyclodextrin revealed that the crystallized rapamycin-heparin from the drug-carrier mixture slowly diffused into coronary blood vessels and retarded the formation of neointimal muscle cells.

The release of rapamycin and heparin from the stent surface to prevent restenosis and thrombosis was investigated by Bae et al. [16], who observed that about 67.3% of the rapamycin-heparin drug was discharged within one week. This release of rapamycin significantly inhibited the growth of smooth muscle cells and platelet adhesion in the heparin-coated group, compared with the uncoated group; thus proving the prevention of restenosis and stent thrombosis.

The presence of drug particles on the stent was confirmed by observation under scanning electron microscopy. The uniform coating of the drug-polymer mixture provides no space for the attachment of bacteria. The results obtained were like the observation of Basalus et al. [17]. Coating the stent surface was found to be more effective in reducing bacterial adhesion.

Stents coated with a rapamycin-heparin mixture showed good antibacterial activity against all test organisms. The observed inhibitory zones ranged from 19.9 ± 1.8mm to 29.4 ± 1.7mm. Zone of inhibition of antibacterial analysis was synergistically facilitated by both rapamycin and heparin complex. A study conducted by Rosett et al. [13] proved that heparin can inhibit the growth of stent-associated organisms. The heparin inhibited the growth of five clinical isolates during their analysis. The carrier, cyclodextrin, starts degrading when exposed to a moist surface on plates, and regulates a sustained drug release to prevent the growth of pathogenic producers. Szymanska et al. [18], found that the dissolution percentage of a carrier in phosphate buffer saline, and the discharge rate of the drug was high, denoting a direct relationship between dissolution and drug discharge.

Similarly, Elayarajah et al. [19], investigated the prevention of bacterial adhesion on urinary stents. The norfloxacin-metronidazole drug mixture and polymer tocopherol acetate were impregnated on the stent surface. The antibacterial inhibitory effect of drug-coated stents was evaluated using the agar diffusion test against Escherichia coli and Staphylococcus epidermidis. The drug mixture inhibited the growth of both the test organisms. The researchers observed that the adherence of bacteria on the coated stent surface was significantly reduced. The degradation of the tocopherol acetate facilitated the release of norfloxacin-metronidazole from the coated stents. The release of drugs from the drug-carrier mixture would thus aid in preventing the adherence of bacteria on the stent surface.

The present research similarly evaluated stents coated with a drug-carrier mixture. The antibacterial activity of the drug-carrier mixture prevents bacterial adhesion, and the sustained drug release prevents the growth of neointimal tissues on surface of the stent.

CONCLUSION

A drug-carrier mixture was impregnated onto the stent surfaces to achieve microbial resistance and prevent the growth of neointimal tissue, which leads to restenosis. The significance of stent implantation with a drug-carrier mixture to prohibit atherosclerosis and stent infection was discussed in the present research. The primary objective of synthesizing a drug-coated

stent, which acts as anti-infective agent for post-operative coronary artery stent infection, was achieved by sustained drug release, and the growth of smooth muscle cells was also prevented. The stents impregnated with different drug combinations and a dissoluble carrier is considered as a novel product in biomedical industry.

CONFLICT OF INTEREST

The authors declare no interests.

CONTRIBUTIONS/AUTHORSHIP

All the authors contributed equally.

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