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A stent for co-delivering paclitaxel and nitric oxide from abluminal and luminal surfaces: Preparation, surface characterization, and *in vitro* drug release studies

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ABSTRACT

Most drug-eluting stents currently available are coated with anti-proliferative drugs on both abluminal (toward blood vessel wall) and luminal (toward lumen) surfaces to prevent neointimal hyperplasia. While the abluminal delivery of anti-proliferative drugs is useful for controlling neointimal hyperplasia, the luminal delivery of such drugs impairs or prevents endothelialization which causes late stent thrombosis. This research is focused on developing a bidirectional dual drug-eluting stent to co-deliver an anti-proliferative agent (paclitaxel – PAT) and an endothelial cell promoting agent (nitric oxide – NO) from abluminal and luminal surfaces of the stent, respectively. Phosphonoacetic acid, a polymer-free drug delivery platform, was initially coated on the stents. Then, the PAT and NO donor drugs were co-coated on the abluminal and luminal stent surfaces, respectively. The co-coating of drugs was collectively confirmed by the surface characterization techniques such as Fourier transform infrared spectroscopy, scanning electron microscopy (SEM), 3D optical surface profilometry, and contact angle goniometry. SEM showed that the integrity of the co-coating of drugs was maintained without delamination or cracks formation occurring during the stent expansion experiments. *In vitro* drug release studies showed that the PAT was released from the abluminal stent surfaces in a biphasic manner, which is an initial burst followed by a slow and sustained release. The NO was burst released from the luminal stent surfaces. Thus, this study demonstrated the co-delivery of PAT and NO from abluminal and luminal stent surfaces, respectively. The stent developed in this study has potential applications in inhibiting neointimal hyperplasia as well as encouraging luminal endothelialization to prevent late stent thrombosis.

1. Introduction

Coronary artery disease (CAD) is the leading cause of death for both men and women throughout the world [1]. CAD is the narrowing of blood vessels that supply blood and oxygen to the heart. A balloon angioplasty is carried out to open up the narrowed artery so that the blood flow to the heart can be restored [2,3]. However, the artery renarrows (restenosis) in 30–40% of the patients within 6 months after balloon angioplasty [4,5]. Currently, metallic stents are implanted followed by angioplasty to keep the artery open for a longer period of time. The implantation of bare metal stents has significantly reduced the restenosis rate to 20–30% [4,5]. However, the arterial injury that occurs during stent implantation causes a cascade of biological events resulting in neointimal hyperplasia (NH) [6]. NH is a natural wound healing response characterized by the growth and migration of smooth muscle cells followed by the deposition of extracellular matrix inside the arterial lumen [7]. This results in re-occlusion of artery even after the implantation of stents, which is called in-stent restenosis. Drug-eluting stents (DES) are currently implanted to treat NH by releasing anti-proliferative drugs which can inhibit the growth of smooth muscle cells inside the lumen [8,9]. Although the restenosis rate has been reduced to <10% after the implantation of DES, the occurrence of late stent thrombosis (LST) is the major safety concern of DES at present [10]. LST is an adverse clinical event characterized by the formation of blood clots in the arteries after months or years of DES implantation and results in heart attack or death [11–13]. The primary cause of LST is believed to be the delayed or impaired endothelial cell growth on DES [11–13].

Most DES release anti-proliferative drugs in abluminal (toward blood vessel wall) as well as luminal (toward lumen – the inner open space or cavity of a blood vessel is called lumen) directions [10,14]. While the abluminal release of anti-proliferative drugs is highly beneficial in controlling the growth of smooth muscle cells and thereby inhibiting neointimal hyperplasia, the luminal release of such drugs impedes re-endothelialization [12,15–17].

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The re-endothelialization of luminal stent surfaces is of paramount importance because the complete endothelial cell lining prevents the adhesion, aggregation, and activation of blood platelets [18] and thereby inhibits LST. Hence, there is a need to co-deliver therapeutic agents from abluminal and luminal stent surfaces to inhibit the growth of smooth muscle cells and to encourage the growth of endothelial cells, respectively. The research goal of this study is to develop a bidirectional dual drug-eluting coronary stent which can co-deliver an antiproliferative agent (paclitaxel) and an endothelial cell promoting agent (nitric oxide) from abluminal and luminal surfaces, respectively.

Polymeric carriers are commonly used to deliver drugs from stents. Some (not all) polymers used in DES can cause inflammatory and hypersensitive reactions [19–22]. Hence, the research in the area of drug delivery platforms for stents can be broadly classified under the following two categories: (a) developing more biocompatible polymer platforms; (b) developing totally polymer-free platforms. In this study, a polymer-free platform using phosphonoacetic acid based molecular coatings was used to co-deliver the drugs from stents. The reasons for choosing paclitaxel (PAT) and nitric oxide (NO) to be co-delivered from abluminal and luminal stent surfaces, respectively are as follows. The effect of PAT on inhibiting the growth of smooth muscle cells and preventing neointimal hyperplasia has been well reported in the literature [23–25]. Also, PAT has been used in U.S. Food and Drug Administration (FDA) approved stents [25]. NO is well known for enhancing endothelialization of different material surfaces [26–28]. In addition, NO has been shown to inhibit neointimal hyperplasia [27,29,30] and thrombosis [26,27,31].

In this study, five different groups of stents were used. They are (a) chemically cleaned control stents; (b) stents coated with only phosphonoacetic acid with no drugs on either side; (c) stents with PAT coating on the abluminal surface and no nitric oxide donor drug coating on the luminal surface; (d) stents with a nitric oxide donor drug coating on the luminal surface and no PAT coating on the abluminal surface; (e) stents co-coated with PAT and nitric oxide donor drug on the abluminal and luminal stent surfaces, respectively. The reasons for using the single drug coated stents (groups – c and d) in this study are that these stents are not only the right controls for the co-coated stent but also to make sure that no differences arise during the co-coating process.

2. Materials and methods

2.1. Materials

Cobalt–chromium (Co–Cr) alloy bare metal stents were purchased from Fortimedix B.V. (Netherlands). These stents were 11.2 mm in length with strut dimensions of 0.083 mm × 0.092 mm (width × thickness). Absolute ethanol (200 proof), acetone, methanol, dimethyl sulfoxide (DMSO), phosphonoacetic acid (PAA), and phosphate-buffered saline with 0.05% tween-20 (PBS/T-20, pH = 7.4) were obtained from Sigma–Aldrich (USA). HPLC-grade water and acetonitrile were also purchased from Sigma–Aldrich. Nitric oxide donor drug diethylenetriamine diazeniumdiolate (DETA NONOate; formula name: (Z)-1-[N-(2-aminoethyl)-N-(2-ammonioethyl)amino]diazen-1-ium-1,2-diolate) and nitrate/nitrite colorimetric assay kit were purchased from Cayman Chemical (Ann Arbor, MI). Paclitaxel (PAT) was purchased from ChemieTek (Indianapolis, IN). All chemicals were used as received.

2.2. Chemical cleaning of stents

Co–Cr alloy stents were chemically cleaned by sonicating in ethanol, acetone, and methanol twice for 10 min each with fresh solvents used each time. The stents were then dried using N_2 gas. The chemically cleaned stents are referred to here as control stents # 1 (CS#1).

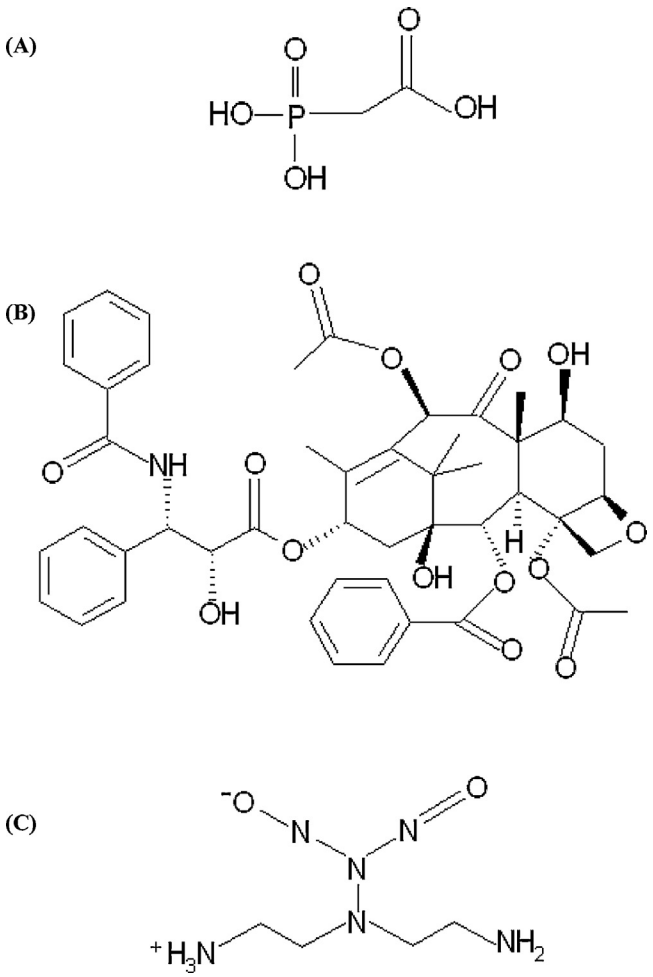

Fig. 1. Chemical structure of (A) phosphonoacetic acid (PAA), (B) paclitaxel (PAT) and (C) nitric oxide donor drug diethylenetriamine diazeniumdiolate (DETA NONOate).

2.3. Phosphonoacetic acid coating on stents

The chemically cleaned stents were immersed in 3 mL of 1 mM solution of phosphonoacetic acid (PAA, chemical structure shown in Fig. 1A) in de-ionized water (DI-H_2O) for 24 h. The stents were then taken out of the solution and transferred to an oven without rinsing. In the oven, the stents were heat treated in air at 120 °C for 18 h. The stents were then cleaned by sonication in DI-H_2O for 1 min followed by N_2 gas drying. Thus prepared PAA coated stents are referred to here as control stents # 2 (CS#2).

2.4. Paclitaxel coating on the abluminal surface of the stent

Paclitaxel (PAT, chemical structure shown in Fig. 1B) was spray coated on the stents. For coating PAT on the abluminal stent surface, initially, the stent was placed on a mandrel in which the luminal stent surface was in close contact with the mandrel. This was done to prevent any PAT leaking onto the luminal stent surface during spray coating. A solution of PAT at a concentration of 1 mg/mL was prepared in a solvent mixture containing 75% ethanol and 25% DMSO. The PAT solution was sprayed on the stents using a Medi-Coat stent coating system (Sono-Tek Corporation, Milton, NY). The