In vitro study of poly(ethylene carbonate) as a drug-eluting stent coating

Huijuan Bian\textsuperscript{a,b}, Shaoxiong Zhou\textsuperscript{a,b,*}, Xinjie Liang\textsuperscript{a,b}, Qiang Li\textsuperscript{a,b}, Wei Han\textsuperscript{a}

\textsuperscript{a}Iron & Steel Research Institute, China Iron & Steel Research Group, No. 76 Xueyuan Nanlu, Haidian, Beijing 100081, China
\textsuperscript{b}Research Center of Biomaterials, Advanced Technology & Materials Co., Ltd., No. 76 Xueyuan Nanlu, Haidian, Beijing 100081, China

Received 27 February 2012; accepted 27 April 2012

**KEYWORDS**

Poly(ethylene carbonate); Poly(lactic-co-glycolic acid); Sirolimus; Stent coating; Drug release

**Abstract**

An in vitro feasibility study of the use of poly(ethylene carbonate) (PEC) as a biodegradable coating material for drug-eluting stents is reported, and the performance of PEC is compared with that of poly(lactic-co-glycolic acid) (PLGA). Scanning electron microscopy (SEM) images of PEC and PLGA discs after treatment with an alkaline KO\textsubscript{2} solution as a superoxide source showed that the PEC maintained its integrity whereas holes and small particles appeared during the treatment of PLGA. Sirolimus and paclitaxel were loaded into PEC and PLGA in order to study drug release performance. Attenuated total reflectance-infrared (ATR–FTIR) spectroscopy of sirolimus, PEC and the sirolimus-loaded PEC coating showed that no chemical reaction occurred between sirolimus and PEC. The results of atomic force microscopy (AFM) revealed that the mean roughness (Ra) values of the bare metal stent (BMS) and the drug-eluting stent (DES) were 2.3 nm and 1.0 nm, respectively. After balloon expansion experiments, no delamination or destruction of the PEC coating was observed. The drug release profile of sirolimus was different from that of paclitaxel when PEC was employed as the drug carrier, and the release...
1. Introduction

Percutaneous coronary intervention (PCI) has become the favored method to treat patients with coronary artery disease. In the past thirty years there have been three revolutionary advances in PCI. In the first decade of its use (1977–87), interventional cardiologists experimented with angioplasty balloons. Although an artery can be opened successfully using a balloon, in a small percentage of cases it was found that the artery collapsed when the balloon was deflated. A second problem soon became evident as well, in that 30–40% of all coronary arteries closed up again after balloon angioplasty. Over the following decade, several generations of bare metal stents (BMS) were developed. The use of a BMS virtually eliminated many of the complications of abrupt artery closure, but some restenosis persisted—the percentage of restenosis of treated arteries decreased from 30% to 60% for balloon angioplasty to 10–30% for BMS [1]. The first-generation of drug-eluting stents (DES) with controlled release of sirolimus (Cypher™) or paclitaxel (Taxus™) from durable polymers began a new era, and greatly reduced angiographic and clinical measures of restenosis compared with BMS [2–4].

It is acknowledged that all medical devices and therapies have limitations, but those associated with this innovative technology were quickly recognized [5]. Restenosis still occurs, and very late stent thrombosis is more common with first-generation DES than with BMS due to delayed healing and re-endothelialisation. Although re-endothelialisation is multifactorial in cause, durable polymer surface coatings can play a part [3,6,7]. To limit the exposure of the artery to the polymer, DES programs now aim to either greatly reduce the amount of polymer on the stent or use biodegradable polymers [8]. Biodegradable polymer DES offers controlled elution of the active drug from the stent backbone by means of a biocompatible polymer coating which, after completion of its useful function, slowly degrades to inert organic monomers, thereby eliminating the risk associated with the long-term presence of a durable polymer in the coronary vessel wall. Although Byrne et al. [9] found that there was no significant difference between biodegradable polymer and permanent polymer DES in terms of three-year outcomes, the possibility of biodegradable polymers improving the long-term safety of drug-eluting stents has been noted by Holmes et al. [10]. Nevertheless, the potential benefits of biodegradable polymers should be evaluated with longer follow-up [11].

PEC is a rubber-like polymer which has been shown to have good biodegradability and biocompatibility in vivo [12]. The use of PEC as a stent coating giving controlled release of paclitaxel in vitro was first demonstrated [13] in 2007, and it was concluded that PEC is a promising stent-coating material due to its good biological, mechanical and biodegradation properties. Here we report a further in vitro feasibility study of the utility of PEC as a coating material for drug-eluting stents. Bare 316 L stainless stents were covered directly by sirolimus-incorporated PEC without using a non-biodegradable polymer, such as poly(p-xylylene) (PPX), as a basecoat. Sirolimus was chosen as the incorporated drug since it is known to be effective in prevent the excessive proliferation of smooth muscle cells in vessel walls.

2. Experimental

2.1. Materials

PEC was kindly provided by the Changchun Institute of Applied Chemistry, Chinese Academy of Sciences (Jilin, China). PLGA was purchased from Jinan Dai Gang Biological Engineering Co., Ltd (Shandong, China). Sirolimus was obtained from Hangzhou Eastpro Pharmaceutical Technology Co., Ltd. (Zhejiang, China), and had a purity of over 96%. Paclitaxel was purchased from Kunming Wan Bao Biological Technology Co., Ltd. (Yunnan, China). 316 L stainless steel stents (15 mm in length, 2 mm in diameter) were provided by Advanced Technology & Materials Co., Ltd. (Beijing, China). Dichloromethane and acetonitrile were of analytical grade. The properties of PEC and PLGA polymers used in this study are summarized in Table 1.

2.2. Preparation

4 g of PEC or PLGA was completely dissolved in 20 mL of dichloromethane, and the solution poured into a petri dish. In order to prevent the formation of voids within the polymer
film, a glass cover was put on the dish to ensure slow dichloromethane evaporation. After 72 h of solvent evaporation, 10 mm x 10 mm x 0.2 mm discs were die-cut from the thick polymer film. The discs were dried for three days in a drying chamber under reduced pressure at room temperature until constant weight was obtained.

A solution of the polymer (PEC or PLGA) in dichloromethane (1% w/v) containing the drug (sirolimus or paclitaxel) with a specified concentration was sprayed onto the 316 L stainless steel stent struts using an automatic spray coating method (SONO-TEK Stent Spraying System, USA) to form a polymer matrix. In some cases, the matrix was subsequently covered with a second polymer layer without the drug. The mass of drug loaded and the thickness of the coating were determined by the concentration of the solution and the spraying time. Finally, the coated stent was dried under vacuum for 2 day at room temperature.

2.3. Characterization

Molecular weights (Mw=weight average molecular weight; Mn=number average molecular weight) and molecular weight distribution (PDI=polydispersity index) were determined by gel permeation chromatography (Waters 515-410, Waters, USA). The ratio of ethylene carbonate units to ether groups in PEC was determined by nuclear magnetic resonance (NMR) spectroscopy (Bruker AM 400, Bruker Corp., Germany), with deuterated chloroform as a solvent. The glass transition temperature (Tg) was characterized using a differential scanning calorimeter (SDT 2960, TA Corp., USA). Polymer samples were sealed in aluminum pans and heated twice in a nitrogen atmosphere. Thermograms covering the range from −40 to 190 °C were recorded at a heating and a cooling rate of 10 °C per minute. The second run was used for measurement of Tg. Tensile measurements were performed using an extensometer (Model 1185, Instron, USA) with an oven at 37 °C.

The morphologies of the coated stents and the surface of the polymer discs were investigated by SEM (JEOL JSM-6390 SEM, JEOL Co., Ltd., Japan). Prior to SEM examination, samples were sputter-coated with a gold layer under vacuum for 30 s (SBC-2, KYKY Technology Development Ltd., China). The morphology and the mean roughness (Ra) of the coatings were characterized by atomic force microscopy (AFM) (Nanoscope IIIa, Digital Instruments, Veeco Metrology Group, America). The PEC/sirolimus blend was also characterized by attenuated total reflectance–Fourier transform infrared (ATR–FTIR) spectroscopy (Model 750, Nicolet, USA).

For in vitro drug release tests, a drug-loaded stent was incubated in 2 mL of normal phosphate buffered saline (PBS, 0.05 M, pH 7.4), which was used to simulate an in vivo environment. The specimens were kept in a low speed self-balancing centrifuge (BFX4-80, Buiyang Centrifuge Plant, China) at a speed of 72 rpm at 37 °C. At defined time intervals the buffer was withdrawn and replaced by 2 mL of fresh medium. The 2 mL release protocol was mixed with 3 mL of acetonitrile. The mixed solution was centrifuged at 4000 rpm for 10 min, then 3 mL of supernatant was taken out and filtered through a 0.45 μm filter membrane, and finally the amount of drug released was quantified by UV–vis spectrophotometry (UV-2102 PCS, Unico, USA). All measurements were performed in triplicate to get average values.

3. Results and discussion

3.1. 1H NMR spectroscopy

To determine whether PEC meet the demands for a pharmaceutical application or not, it is important to identify the structural components affecting its biodegradability behavior. PEC with a large molecular weight (278 kDa) was employed, since previous studies [14] have shown that PEC with molecular weight smaller than 130 kDa is resistant to biodegradation in vivo.

Fig. 1 shows the 1H NMR spectrum of the PEC. The relative intensities of the signal at 4.37 ppm (Integral Is) from the ethylene carbonate units (ethylene units between two carbonate functions) (–OOC–O–CH2–CH2–O–COO–), those at 4.29 and 3.73 ppm (Integrals Ic and Ia) from ethylene units between one carbonate and one ether function (–OOC–O–CH2–CH2–O–) and that at ca. 3.65 ppm (Integral Id) from ether units between two ether functions (–O–CH2–CH2–O–) were determined. The ratio of ethylene carbonate units (ECU) to ether functions (EF) and the IEF (incorporated ether function) value can be calculated from the following formulae [14]:

\[
\% \text{ of ECU} = 100 \times \frac{I_a}{I_a + I_b + I_c + I_d} = 82.54\%
\]

\[
\% \text{ of EF} = 100 \times \frac{I_c + I_d}{I_a + I_b + I_c + I_d} = 14.18\%
\]

\[
\% \text{ of IEF} = 100 \times \frac{I_c + I_d}{I_a + I_b + I_c + I_d} = 13.98\%
\]

The degradation time of PEC is usually less than 30 day in vivo [12,15], whereas a longer time of degradation and drug release is desirable for clinical application because coronary restenosis and vascular smooth muscle cell proliferation and migration often occur within 3–6 months after PCI [16,17]. In our case the IEF value of 13.98% is so large that the degradation of PEC will have a lag time, which will effectively lengthen the degradation time. In general, drug release from a conventional non-degradable matrix involves an initial burst with a fast rate, followed by a period of slower release. The influence of the lag time may mean, however, that the rate of

![Fig. 1](image-url)
release of a drug from the PEC film over time is more uniform than for a typical matrix. This is because during the lag time the drug release results only from dissipation, but an increase in drug release after the onset of degradation should compensate for the decrease in the rate of dissipation after the initial burst.

3.2. Degradation of the PEC film

Biodegradation can be characterized by two surface-related phenomena: bulk erosion and surface erosion. In the case of bulk erosion, biodegradation proceeds throughout the polymer matrix. As for the surface erosion, the biodegradation

Fig. 2 SEM micrographs of PLGA and PEC discs: 100 times magnification views of (a) PLGA and (b) PEC before degradation; (c) 27, (e) 200, and (g) 500 times magnification views of PLGA after degradation for three weeks; (d) 100, (f) 200, and (h) 500 times magnification views of PEC after degradation for three weeks.
proceeds exclusively at the surface. Biodegradation of PLGA involves the former, whilst that of PEC involves the latter. To understand the surface morphology, PLGA and PEC discs were incubated in PBS (pH = 12) containing potassium superoxide, KO₂ (170 mmol), because it has been shown that PEC is degraded by superoxide radical anions [12,18,19], which are mostly produced in vivo by inflammatory cells. The PBS was replaced by fresh solution every day. After three weeks of incubation, the discs were taken out and evacuated to constant weight.

SEM micrographs of the PLGA and PEC discs before and after incubation are shown in Fig. 2. In both cases, the initial smooth surface was found to be porous after incubation for three weeks. But holes and small particles appeared during the treatment of PLGA, whereas the PEC disc maintained its integrity better. It has been shown that particle shedding from a stent coating causes increased vascular inflammation [20].

Thus, the PEC coating should be less likely to induce inflammation and the formation of a thrombus than PLGA. Furthermore, PEC is first degraded into ethylene carbonate (EC) which is not stable and decomposes, ultimately giving harmless CO₂ and H₂O [18]. Both the degradation process itself, and the degradation products of PEC, should have no adverse affects when it is used as a coating material. Therefore, the mechanism of surface degradation-controlled release of PEC may provide the basis for “on demand” drug-eluting stent coatings.

3.3. ATR–FTIR spectra of sirolimus-loaded PEC coating

The ATR–FTIR spectra of sirolimus, PEC, and sirolimus-incorporated PEC are shown in Fig. 3. The spectrum of sirolimus (Fig. 3(a)) shows peaks at 3400, 2930, 1718 and 1634 cm⁻¹ which can be assigned to the stretching vibrations of O–H, C–H, C=O and C=C respectively. The peaks at 1443 and 1376 cm⁻¹ are the asymmetric and symmetric C–H bending vibration peaks, the peak at 1091 cm⁻¹ is characteristic of C–N, and the peaks at 995 and 756 cm⁻¹ are the out-of-plane vibration peaks of C=C and C–H, respectively. The spectrum of PEC (Fig. 3(b)) shows strong peaks at 1740 and 1218 cm⁻¹, which can be assigned to the C=O and C=O, stretching vibrations, respectively. The spectrum of the sirolimus-loaded PEC coating (Fig. 3(c)) is a superposition of the spectra of PEC and sirolimus showing that both are incorporated in the blend, with no chemical reaction occurring between them.

3.4. Morphology of PEC-coated and bare metal stents

The surface morphologies of a bare metal stent (BMS) and a PEC-coated stent (DES) were characterized by SEM and AFM, as illustrated in Fig. 4. SEM images showed that the
surfaces of both BMS and DES were relatively smooth. The values of the mean roughness (Ra) of BMS and DES, as measured by AFM, were 2.3 nm and 1.0 nm respectively. The smoother surface of the DES suggests that the pits and scratches on the surface of BMS were filled up by the sirolimus-incorporated PEC coating. For implants in direct contact with blood, the surface roughness is an important factor affecting the blood compatibility, since rough surfaces increase the likelihood of blood clotting, whereas smooth surfaces have a small area in contact with the blood, giving better blood compatibility [21]. Thus, DES has a more compatible surface than BMS.

3.5. Balloon dilatation experiments with DES

It is known that the stent exists in an expanded state after implantation in the coronary artery. Polymeric coatings should be resistant during implantation and expansion of the stent. It is important that the integrity of the coating is retained during stent crimping and implanting using balloon catheters, because cracking or peeling of the coating may cause severe (sub)acute thrombotic and inflammatory events, even including abrupt vessel closure [22].

Balloon dilatation experiments were carried out with a pressure of 15 MPa, which is greater than the clinical distending pressure of 10 MPa, to study the mechanical properties of the PEC stent coating. SEM images of the sirolimus-loaded PEC coated stent after dilatation (Fig. 5) show that the surface remains smooth without any signs of disintegration or delamination. The coating keeps integrated even near the bend of the stent struts where are the mechanically stressed sites [23]. There are two factors which may contribute to the excellent mechanical properties of the PEC coating. It is known that – OH groups are formed on stainless steel substrates after pickling [24] and these can form hydrogen bonds with the carbonyl oxygen atom of the polycarbonate structure, strengthening the binding between the polymer and the metal. In addition, the glass transition temperature ($T_g$) of the polymer has an important effect on the flexibility of the stent coating. The $T_g$ of PEC is 19°C. At 37°C, PEC is in a rubbery state with a tensile elongation of over 700%, which is an important material property [25] and ensures that PEC will not fracture during balloon dilatation experiments. Thus, the present results indicate that the PEC coating can meet clinical dilatation requirements.

3.6. Drug release kinetics curves

Sirolimus is the most prominent member of the group of inhibitors of the mammalian target of rapamycin (mToR) [26]. mToR inhibitors, such as sirolimus, also have antiproliferative and antimigratory effects on vascular cell lines. Clinical evidence suggests that cytostatic drugs, such as sirolimus, have a superior outcome in terms of efficacy and safety compared with cytotoxic drugs, such as paclitaxel, when employed on a DES platform [27].

In the present study, the influence of sirolimus- and paclitaxel-loaded PEC coatings on drug release profiles in PBS was studied. The release curve in Fig. 6(a) illustrates that the initial sirolimus release rate from a simple PEC carrier is extremely fast. It is, therefore, necessary to coat a second layer of drug-free polymer on top of the sirolimus-incorporated PEC film. The ratio of the thicknesses of the drug-loaded polymer to the drug-free polymer was 2:1. The drug-free PEC coating functioned as a diffusion barrier that provided a controlled release of 60% of the drug within a time period of 30 day (Fig. 6(b)). The release profiles in Fig. 6(c) and (d) shows that the drug release of paclitaxel was not significantly affected by adding a drug-free polymer film (the mass of drug loaded and the thickness of both drug-loaded polymer and drug-free polymer films in (c) and (d) were the same as in (a) and (b), respectively), and the rate of release was much slower than for sirolimus. It is widely accepted that the preliminary stage after stent implantation is critical as far as prevention of vascular intimal injury and inflammation are concerned. Thus, a sufficient amount of sirolimus can be released in this...
period, whereas the amount of paclitaxel is likely to be insufficient.

The influence of different polymers on the release behavior of sirolimus was also examined. Fig. 7 shows drug release kinetics for PEC and PLGA. A biphasic release profile was observed for PEC, with ca. 70% of the drug released rapidly within the first 20 day, followed by a much slower rate of release. In the case of PLGA, an initial phase of rapid drug release in the first three days was followed, first by a slow, and finally a more rapid release, leading to a sigmoid liberation profile for sirolimus. The rate of drug release therefore clearly depends on the polymer drug carrier. Thus, a sufficient amount of sirolimus can be released from the PEC coating to meet clinical requirements.

4. Conclusions

(1) SEM images of PEC and PLGA after treatment with an alkaline KO\textsubscript{2} solution as a superoxide source show that PEC retains its structural integrity while PLGA undergoes significant degradation. Thus, the surface erosion characteristics of PEC are superior to the bulk erosion characteristics of PLGA as a coating material. After balloon expansion experiments up to a pressure of 15 MPa, no delamination or destruction of the PEC coating was observed by SEM, which indicates that the coating resists mechanical stress during stent expansion and meets the clinical requirements for dilatation.

(2) ATR–FTIR spectra of sirolimus, PEC and the sirolimus-loaded PEC coating show that there is no chemical reaction between sirolimus and PEC and the coating is simply a blend of sirolimus and PEC. AFM shows that the sirolimus-incorporated PEC coated stent has a more biocompatible surface than a bare metal stent, as it is much smoother. The drug release profiles of drug-eluting stents depend on both the drug and its carrier. However, the drug release behavior of each DES studied in this work can extend for more than 50 day.

(3) Apart from its controlled and site-specific drug elution, good biocompatibility and resistance to degradation, the PEC matrix has excellent mechanical properties in terms of flexibility and long lasting adherence to the stent surface. This feasibility study under in vitro conditions demonstrates that PEC has good application prospects as a coating material for drug-eluting stents and merits further investigation in vivo.

Acknowledgments

The authors wish to thank the financial support of the National High-tech Research and Development Program of China (2011AA030103). We also gratefully thank Yanjun Xie for the selfless help on SEM tests and Tianyang Song for the helpful assistance in the experiment from Advanced Technology & Materials Co., Ltd.

References


