In vivo biocompatibility of a plasma-activated, coronary stent coating

Anna Waterhouse [a,e,1], Steven G. Wise [a,e], Yongbai Yin [b], Buchu Wu [c], Barbara James [d], Hala Zreiqat [d], David R. McKenzie [b], Shisan Bao [c], Anthony S. Weiss [a], Martin K.C. Ng [e], Marcela M.M. Bilek [b,*]

[a] *School of Molecular Bioscience, University of Sydney, NSW 2006, Australia*
[b] *Applied & Plasma Physics Group, Physics School, University of Sydney, NSW 2006, Australia*
[c] *Discipline of Pathology, School of Medical Sciences, University of Sydney, NSW 2006, Australia*
[d] *School of Aerospace, Mechanical and Mechatronic Engineering, University of Sydney, NSW 2006, Australia*
[e] *Heart Research Institute, Sydney, NSW 2042, Australia*

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ABSTRACT

Bare metal and drug-eluting coronary stents suffer an inherent lack of vascular cell and blood compatibility resulting in adverse patient responses. We have developed a plasma-activated coating (PAC) for metallic coronary stents that is durable, withstands crimping and expansion, has low thrombogenicity and can covalently bind proteins, linker-free. This has been shown to enhance endothelial cell interactions *in vitro* and has the potential to promote biointegration of stents. Using the rabbit denuded iliac artery model, we show for the first time that PAC is a feasible coating for coronary stents *in vivo*. The coating integrity of PAC was maintained following implantation and expansion. The rate of endothelialization, strut coverage, neointimal response and the initial immune response were equivalent to bare metal stents. Furthermore, the initial thrombogenicity caused by the PAC stents showed a reduced trend compared to bare metal stents. This work demonstrates a robust, durable, non-cytotoxic plasma-based coating technology that has the ability to covalently immobilize bioactive molecules for surface modification of coronary stents. Improvements in the clinical performance of implantable cardiovascular devices could be achieved by the immobilization of proteins or peptides that trigger desirable cellular responses.

1. Introduction

Metallic coronary stents now dominate in percutaneous coronary interventions [1]. However, the clinical performance of both bare metal stents (BMS) and drug eluting stents (DES) is less than ideal and causes significant adverse patient outcomes. BMS are prone to high rates of vessel renarrowing known as restenosis [2], while DES suffer from an ongoing risk of late stent thrombosis [3], polymer hypersensitivity [4], polymer delamination [5] and delayed re-endothelialization [6].

New generation DES aimed at reducing these adverse effects are being developed and although promising, reliance on dual anti-platelet therapy and safety outcomes following their cessation remain issues [7]. Additionally, polymer instability is a persistent problem, with thick polymer coatings delaminating and exposing thrombogenic bare metal stent struts to the vasculature [5]. Similarly, newly developed bioresorbable stents could potentially provide benefits by degrading over time, however these are yet to be fully developed. Their clinical applicability is likely to be limited by intrinsic problems, such as poor deliverability, flexibility and radial strength. The thicker struts required to compensate for reduced radial strength of the materials [8] are known to cause increased restenosis and thrombosis [9,10]. There remains a need for robust, biomimetic coatings for metallic stents.

We have developed a plasma-activated coating (PAC) for surface modification of metallic alloys using plasma enhanced chemical vapour deposition [11]. PAC is smooth and strongly adheres to the underlying metal by an energetic ion stitching deposition process. The coating is wear resistant under pulsatile flow and is able to withstand crimping and expansion without delaminating *in vitro* [11,12]. PAC has strikingly low thrombogenicity compared to 316L stainless steel in static adhesion and flow loops *in vitro* using whole human blood [12,13]. Furthermore, PAC allows linker-free covalent immobilization of functional biomolecules to the surface with the potential to allow improved biointegration of a range of biomedical implants [11,13].

In this proof of principle study, we have evaluated the acute response to PAC stents compared to bare metal stainless steel stents in a well-established animal model [14]. In a rabbit denuded

* Corresponding author. Tel.: +61 2 93516079; fax: +61 2 9351 7725.
 E-mail address: marcela.bilek@sydney.edu.au (M.M.M. Bilek).
[1] Present address: Wyss Institute, Harvard, Boston MA 02115, USA.

bilateral iliac artery model we analysed the feasibility of PAC as a stent coating. We evaluated coating integrity, the rate of endothelialization, the initial immune response and thrombogenicity caused by the stent.

2. Methods

2.1. Stent design and treatment

The stent design was based on the dimensions of commercially available stents. The stents were laser cut from slotted tubes of 316L vacuum melted stainless steel and electropolished to remove surface contaminants (Laserage, Waukegan, Illinois, U.S.A). The PAC was deposited without active heating or cooling of the stents. A graded interface was created using reactive magnetron sputtering from a cathode of 316L stainless steel. The substrate holder was rotated to expose all surfaces of the stents to the sputtered flux. Argon and acetylene were injected into the chamber through a distributed gas line. To form the graded interface, a pure stainless steel coating was first deposited onto the stainless steel stents, followed by a coating that contained gradually increasing fractions of plasma polymer deposited by plasma enhanced chemical vapour deposition. To achieve this, the acetylene follow rate was increased from zero until a pure plasma polymer layer was formed. The initial sputtering voltage during the deposition of pure metal was 800 V while the cathode current was maintained at 3 A. Increasing the flow rate of acetylene while keeping the cathode current constant eventually results in the deposition of a pure plasma polymer material when the cathode is fully covered by a plasma polymer layer deposited on the cathode surface. In this way, the stainless steel cathode can be fully protected from sputtering, resulting in a pure PAC layer at the surface. The exposure to highly energetic reactive species in the plasma ensures that the coated stents are sterile. Transfer of the stents to storage vials was performed under sterile conditions.

### 2.2. Energy dispersive X-ray microanalysis (EDS)

Samples were imaged with a Philips XL 30 CP scanning electron microscope using an acceleration voltage of 10 kV. EDS analysis was carried out using an EDAX detector and EDX control software at a working distance of 11 mm. The EDS was operated in area mode with an accumulated live time of 150 s at 5 kV, producing a penetration depth of 375 nm.

2.3. Animal care and surgery

Study approval was obtained from Sydney South West Area Health Animal Ethics Committee (protocol number 2008/036) and the University of Sydney Animal Ethics Committee (protocol number K00/12-2009/3/5243). Experiments were conducted in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purpose. Adult male New Zealand White Rabbits were housed in individual cages in a temperature and light controlled room and given standard rabbit chow ad libitum with free access to sterilized drinking water.

A total of 14 animals received surgery, $n = 4$ controls (denuded on 1 iliac artery and no manipulation of the bilateral artery) and $n = 10$ BMS vs PAC. Rabbits weighing 3–4 kg were sedated with a subcutaneous injection of acetylpromazine (0.5 mg/kg) and anaesthetized with isofluorane (2%) and oxygen (2 l/min) via a face mask. A 5 F sheath was inserted into the right femoral artery by cut down. Endothelial denudation of the iliac arteries was carried out using a 3.25 mm × 10 mm angioplasty balloon catheter (Sprinter® Legend RX) passed over a 0.014″ guide wire to the aorta, inflated to nominal pressure (6 atm with 50% (v/v) contrast/saline) and withdrawn retrograde to the external iliac artery three times. The four control animals received no further manipulation. A BMS or PAC stent was hand crimped onto a 3.25 mm balloon catheter, advanced to the common iliac artery and expanded for 15 s (6 atm) to a diameter of 3.1 mm, giving a stent to artery ratio of 1.2:1. The location and patency of the stented iliac arteries and surrounding vasculature was confirmed by angiography. The procedure was repeated for the left iliac artery. The order and side in which each type of stent was implanted was randomized. Rabbits were anti-coagulated with a single dose of heparin at the time of surgery (100 U/kg) and aspirin (40 mg/day) orally 24 h before surgery and maintained throughout the in-life phase of the study [15,16]. Stented vessels were explanted after 7 days. Anaesthetized animals were exanguinated via left ventricular puncture, perfused with lactated Ringer's solution and given a lethal injection of lethabarb (130 mg/kg). Stents were explanted and flushed with lactated Ringer's solution before fixation.
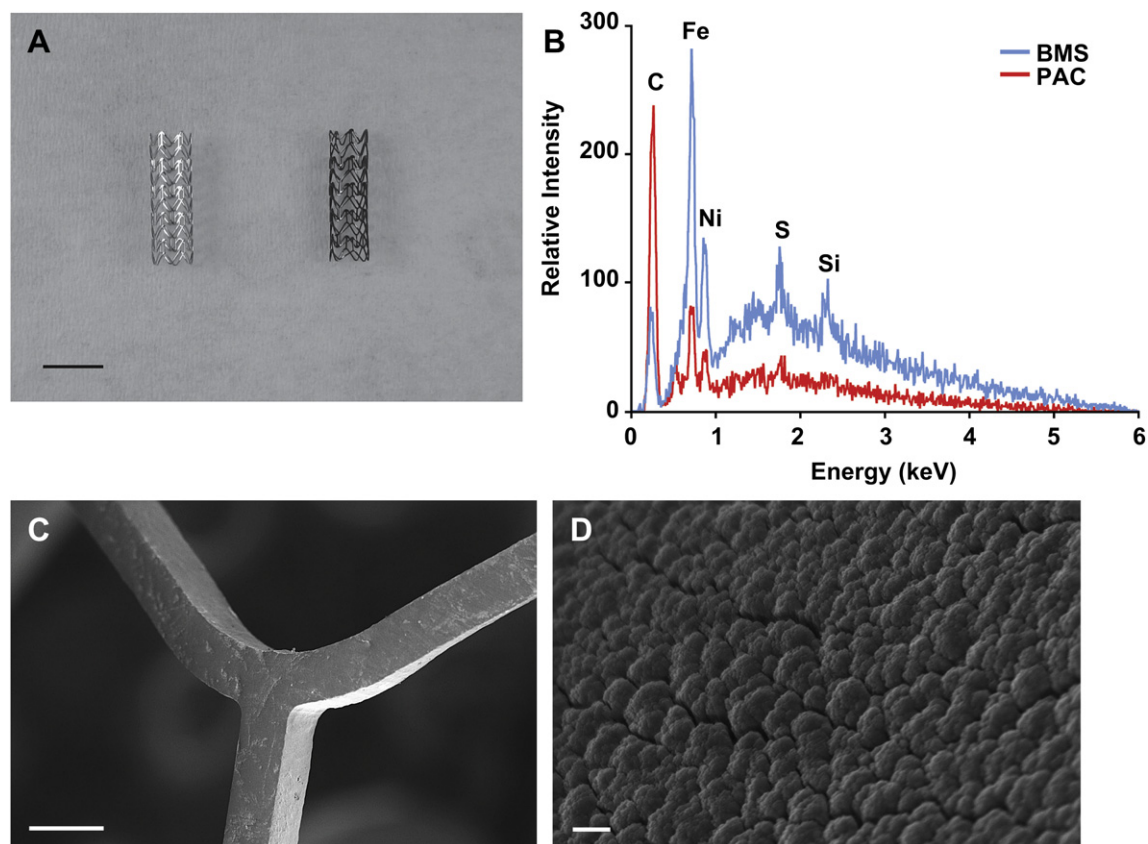
Fig. 1. PAC stent. (A) Photograph of a BMS (left) and a PAC treated stent via the modified deposition protocol (right). Scale bar indicates 5 mm. (B) Elemental composition of a BMS (blue) and PAC (red) stent detected by EDS. The SEM was operated in spot mode at an acceleration voltage of 5 kV (penetration depth of 357 nm) with an accumulated live time of 150 s (C and D) SEM image of a PAC stent surface after crimping and expansion. (C) Scale bar indicates 100 μm. (D) The stress accommodation mechanism through the boundaries of the nanoscale columnar structures is visible. Scale bar indicates 400 nm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)