Delivery of Vitamin-C (L-Ascorbic Acid) from Coronary Stent Material Surfaces

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Statement of Purpose: Anti-proliferative drugs are currently delivered from stents to inhibit neointimal hyperplasia (i.e.) the growth of smooth muscle cells (SMCs) inside the arterial lumen [1]. However, the anti-proliferative drugs not only inhibit the growth of SMCs but also endothelial cells (ECs) [1]. The delayed or impaired endothelialization of stents causes late stent thrombosis [1], which results in heart attack or death. Hence, there is a great need to deliver drugs which can inhibit the growth of SMCs and simultaneously encourage the EC growth. Vitamin-C (L-ascorbic acid, L-AA) has been shown to inhibit SMC growth as well as promote EC growth when systemically administered [2]. The research goal of this study is to deliver L-AA from cobalt-chromium alloy surfaces and to study the interaction of ECs with L-AA coated alloy surfaces for potential use in drug-eluting stents. A phosphoric acid (PA) molecular coating was used to deliver L-AA from the alloy surfaces.

Methods: The chemically cleaned Co-Cr alloy (1cm x 1cm) specimens were immersed in a 100 mM solution of PA in water for 24 h. The specimens were then heat treated in air at 120 °C for 19 h to stabilize the coating. The PA coated specimens were cleaned by sonication in water for 1 min followed by N_2 gas drying. L-AA was deposited on PA coated specimens by the following procedure. A solution of L-AA was prepared in ethanol at a concentration of 12 mg/mL. A 25 µl of the prepared solution was placed on the specimens and allowed the ethanol to evaporate at 37 °C for 24 h leaving a thin film of L-AA on alloy surfaces. An extensive hydrogen bonding is expected to occur between the –OH groups of PA and L-AA. All the specimens (control, PA coated and L-AA deposited Co-Cr alloy) used in this study were characterized using Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), 3D optical surface profilometry (OSP) and contact angle goniometry (CAG). For drug elution studies, the L-AA coated specimens were immersed in phosphate buffered saline (PBS, pH 7.4) at 37 °C for up to 48 hours. The PBS solutions collected at different time points were analyzed for the amount of L-AA released using high performance liquid chromatography. A density of 30×10^3 human aortic endothelial cells (HAECs) was seeded on L-AA coated surfaces. The growth and morphology of ECs were investigated using SEM.

Results: The FTIR spectrum of L-AA coated Co-Cr alloy showed strong peaks for the four –OH groups of L-AA: C(2)–OH at 3210 cm^{-1}; C(5)–OH at 3315 cm^{-1}; C(3)–OH at 3410 cm^{-1}; C(6)–OH at 3525 cm^{-1}. The peaks for C=O (1754 cm^{-1}), C=C (1644 cm^{-1}), and the finger print region of L-AA were also present. Thus FTIR strongly confirmed the L-AA coating on alloy surfaces (Fig 1). SEM image showed the feather shaped L-AA crystals on the alloy surfaces (Fig 2A). OSP topography images showed the uniform distribution of L-AA on the alloy surfaces (Fig 2B). The average roughness of control, PA, and L-AA coated alloy surfaces determined by OSP were 0.015 ± 0.005, 0.008 ± 0.001, and 0.132 ± 0.012 µm respectively. The contact angles of control, PA, and L-AA coated alloy surfaces were 50.6 ± 4.4°, 16.2 ± 8.7°, and 14 ± 3.5°, respectively. *In vitro* drug release studies showed that L-AA was burst released from the alloy surfaces by hour-1 (Fig 3). SEM images taken after 3 days of culture showed the excellent growth of ECs on L-AA coated surfaces (Fig 4A). The spreading of ECs with typical polygonal shape indicated that the L-AA coated surfaces are conducive to endothelialization (Fig 4B).

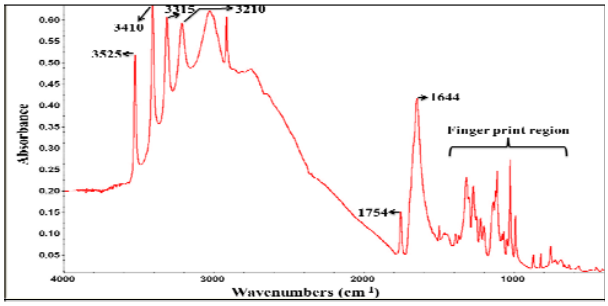

Fig 1. FTIR spectrum of L-AA coated Co-Cr alloy

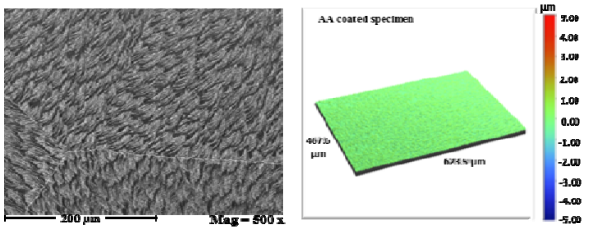

Fig 2: SEM (A) and AFM (B) images of L-AA on Co-Cr

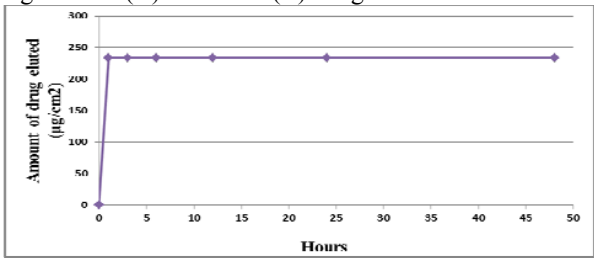

Fig 3. *In vitro* release profile of L-AA from Co-Cr alloy

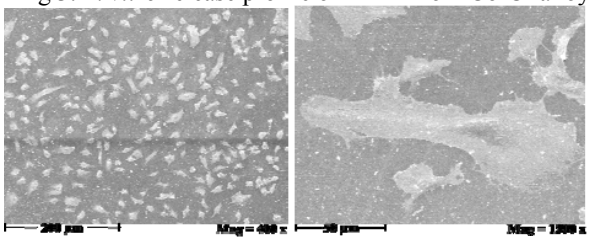

Fig 4. Growth (A) and morphology (B) of ECs on L-AA

Conclusions: L-AA was successfully delivered from Co-Cr alloy surfaces. Also, these surfaces favored the growth of ECs. Thus, this study showed L-AA as a promising drug for delivering from coronary stents.

References: (1) Mani G. Biomaterials 2007; 28: 1689; (2) Aguirre R. Pharmacol Ther 2008; 119: 96-103.