Co-Delivery of Paclitaxel and Nitric Oxide from Abluminal and Luminal Surfaces of a Coronary Stent

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Statement of Purpose: Coronary Artery Disease was treated with bare-metal stents (BMS), but following deployment BMS results in neointimal hyperplasia (NH).[1] NH is the growth and migration of smooth muscle cells (SMCs) inside the lumen, which re-narrows the artery after stent implantation. Drug-eluting stents (DES) inhibit the SMC growth by being coated on both the abluminal and luminal stent surfaces with anti-proliferative drugs. The release of anti-proliferative drugs from the abluminal stent surface inhibits the growth of SMC and prevents NH, but the release of these drugs from the luminal surface inhibits endothelial cell (EC) growth.[2] Delayed endothelialization of the luminal stent surface results in late stent thrombosis. The main objective of this study is to co-deliver paclitaxel (PAT, anti-proliferative agent) and nitric oxide (NO, EC promoting agent) from the abluminal and luminal stent surfaces, respectively.

Methods: Co-Cr alloy stents were chemically cleaned and then immersed in solution of PAA in deionized (DI) H_2O, heated for 18h at 120^oC. PAT was spray coated on the abluminal surface of the stent. A nitric oxide donor drug, diethylenetriamine diazeniumdiolate (DETA NONOate), was coated on the luminal stent surfaces by a dip coating method. This transferred the DETA NONOate from the mandrel to the stent surface. The co-coated stents were characterized using scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FTIR), 3D optical surface profilometry (OP), and contact angle goniometry (CA). The co-coated stents were immersed in PBS/Tween-20 at 37°C for up to 28 days. The PBS/T-20 solutions collected at different points were used to determine the amount of PAT and NO released using high performance liquid chromatography and Griess reagent, respectively.

Results: FTIR spectra confirmed the co-coating of PAT and DETA NONOate on the abluminal (Fig 1A) and luminal (Fig 1B) stent surfaces, respectively. SEM showed that the PAT coating was present only on the abluminal stent surface (Fig 2A) while the DETA NONOate was present only on the luminal stent surface (Fig 2B). The OP images (Fig 3A and B) were also in excellent agreement with the results of FTIR and SEM. PAT and DETA NONOate are hydrophobic and hydrophilic drugs, respectively. Hence, the CAG showed hydrophobic abluminal and hydrophilic luminal surfaces. *In vitro* drug release studies showed that PAT was released from the abluminal stent surfaces in a biphasic manner (Fig 4A) while the NO was released from the luminal stent surfaces in a burst manner (Fig 4B).

Conclusions: A dual drug-eluting coronary stent was successfully prepared to co-elute PAT and NO from abluminal and luminal stent surfaces, respectively. This stent has potential applications in inhibiting neointimal hyperplasia as well as encouraging endothelialization to prevent late stent thrombosis.

References: (1) Mani G. Biomaterials 2007; 28: 1689-1710; (2) Finn AV. Circulation 2007; 115: 2435-41;

Acknowledgements: This study was supported by the South Dakota board of Regents (SDBOR).

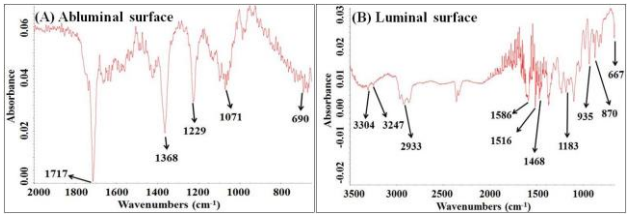

Fig 1. FTIR spectra of (A) PAT and (B) DETA NONOate

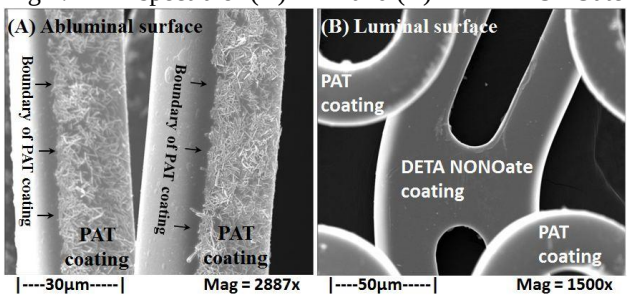

Fig 2. SEM images of (A) abluminal, (B) luminal surfaces

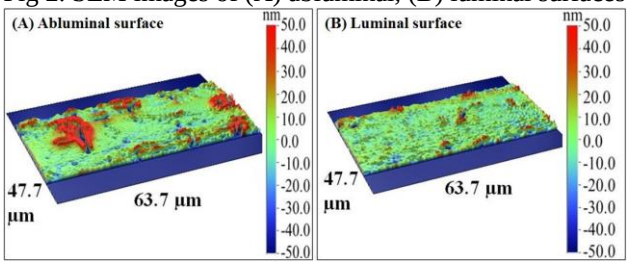

Fig 3. OSP of (A) abluminal and (B) luminal surfaces

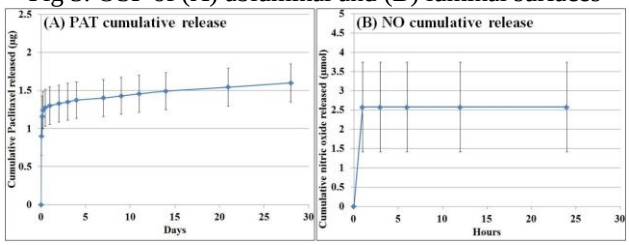

Fig 4. Release profiles of (A) PAT and (B) NO

Synthesis of 1-Butyl-3-Methylimidazolium Derivatives

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Statement of Purpose: This work sought to develop a reliable "green" method of quaternizing imidazole systems using dimethyl carbonate (DMC) to include anionic citrate. The product of this synthesis was designed in order to suspend and stabilize lanthanide nanoparticles for their fluorescence characterization.

Methods: Using the 1:10:10 (amine:DMC:methanol) mole ratio optimized in our labs for production of quaternary alkylammonium methylcarbonate compounds (quats), 1-butyl-3-methylimidazole was synthesized. Reaction took place at 135 ℃, for 20 hours in a Parr pressure reactor, via O_2 removal by N_2 sparge. Removal of methylcarbonate anion took place via anion exchange with citric acid and was refluxed with crude reaction mixture from the Parr under N_2 for 2 hours. The mass of citrate was determined by a 2.5:1 molar ratio of product to acid to warrant production of half the bis quat and half the tris quat to ensure the pH of the product to be around citrate's neutral pK_2. Solvent was removed by roto-evaporation for 3 hours at 80 ℃ via water bath and then lyophilized for 1 hour.

Results: The imidazolium product was converted to bis-1-butyl-3-methylimidazolium citrate. NMR revealed consistent purity > 95% for initial synthesis and anion exchange of the bis/tris product. HPLC suggests presence of small amount of zwitterion byproduct after initial synthesis.

Conclusions: Tris 1-butyl-3-methylimidazolium citrate was synthesized by a green method of quaternizing butylimidazole with dimethyl carbonate in a Parr reactor and anion exchanging the product with citric acid under reflux. The synthesized intermediate was confirmed by NMR and observed by HPLC to consist of 1-butyl-3-methylimidazium methylcarbonate. Initial synthesis may have produced small amount of 2-carboxylate zwitterion. NMR and HPLC confirm citrate quat with no zwitterions or starting material. We believe the anion exchange with citrate allows the elimination of the zwitterions via decarboxylation, due to observation of bubbling anion exchange.

References:

[1]Earl, G. W.;Weisshaar, D. E.; Wineinger, D.; Moeckly, S.; Hanson, M.; Uilk, J.; Rekken, B.; Villa, E.; Zierke, J. P. Quaternary Methyl Carbonates: Novel Agents For Fabric Conditioning. Journal of Surfactants and Detergents **2004**, 8(4), 325-29.

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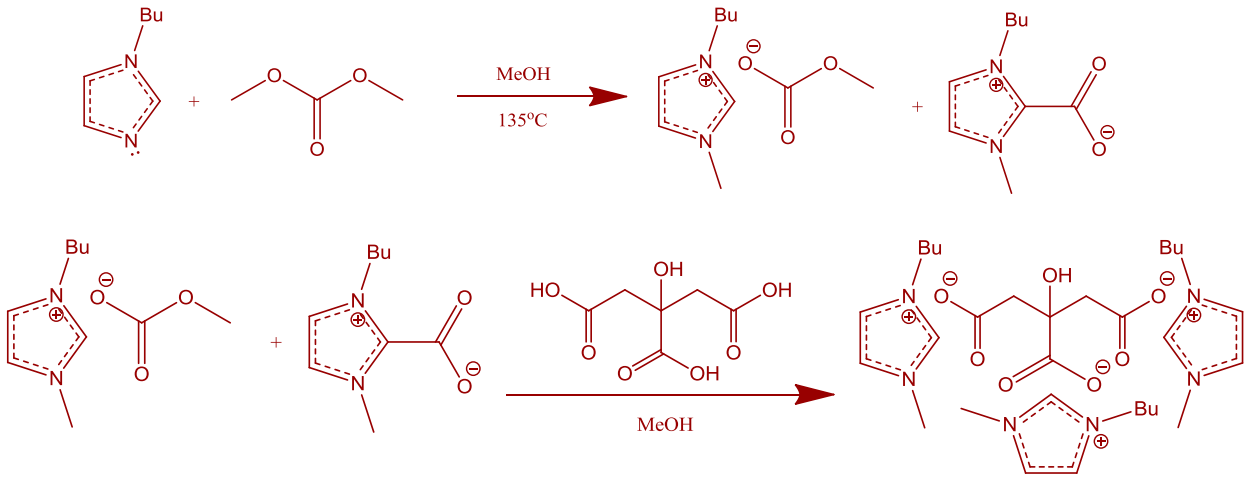

Figure 1. Initial synthesis of imidazolium methylcarbonate/zwitterion and anion exchange with citrate.

Chondroprotective Supplementation Promotes the Mechanical Properties of Injectable Scaffold for Human Nucleus Pulposus Tissue Engineering

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Abstract:

Statement of Purpose: As a result of intervertebral disc (IVD) degeneration, the nucleus pulposus (NP) is no longer able to withstand physiological load leading to low back pain and disability. The NP mechanical properties are due to the production of a functional extracellular matrix. As degeneration occurs, matrix catabolism exceeds matrix synthesis. Current treatments aim to alleviate the symptoms of IVD degeneration, but do not maintain the natural biomechanical function of the NP. The objective of this study is to fabricate a tissue-engineered injectable scaffold with chondroprotective supplementation *in vitro* to improve the mechanical properties of a degenerative NP

Materials and Methods: This research employs a tissue engineering strategy to fabricate a NP scaffold as a minimally invasive means to restore biomechanical function to a degenerated disc. Firstly, a tissue-engineered injectable scaffold was prepared using different concentrations of alginate and calcium chloride (CaCl2) and mechanically evaluated using confined compression. Fabrication conditions were chosen based on structural and mechanical resemblance to the native NP. Chondroprotective supplementation, glucosamine (GCSN) and chondroitin sulfate (CS), were added to scaffolds at concentrations of 0:0 μg/mL (0:0-S), 125:100 μg/mL (125:100-S), 250:200 μg/mL (250:200-S), and 500:400 μg/mL (500:400-S), GCSN and CS, respectively (Table 1). Scaffolds were used to fabricate tissue-engineered NP constructs (TE-NP-constructs) through encapsulation of human nucleus pulposus cells (HNPCs). The TE-NP-constructs were collected at days 1, 14, and 28 for biochemical and biomechanical evaluations.

Summary of Results: Confocal microscopy showed HNPC viability and rounded morphology over the 28 day period. MTT analysis resulted in significant increases in cell proliferation for each group. Collagen type II ELISA quantification and compressive moduli showed increasing trends for both 250:200-S and the 500:400-S groups on Day 28 with significantly greater compressive moduli compared to 0:0-S group. Results indicate the increased mechanical properties of the 250:200-S and the 500:400-S was due to production of a functional matrix.

Conclusions: The results show that the supplemented scaffolds promoted both mechanical and biochemical properties of the TE-NP-construct through the production of a functional extracellular matrix. This study demonstrated potential for a chondroprotective supplemented injectable scaffold to restore biomechanical function of a degenerative disc through the production of a mechanically functional matrix.

Design of Nanoscale Compositions for Remineralization of Human Dentin

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Statement of purpose: There are currently two materials on the market for tooth remineralization by supplying hydroxyapatite to the tooth surface with a phosphate-functionalized compound. The first material is Casein PhosphoProtein Amorphous Calcium Phosphate (Recaldent©) patented in 2006.[1] The second material NovaMin© was patented in 2010[2] as the remineralizing and desensitizing component of the Restore© toothpaste. This material is described as sodium calcium phosphosilicate of unspecified chemical structure.[2] Our research is aimed at the development of multifunctional materials for protection, remineralization, and desensitization of human dentin.

Methods: Remineralizing ability of dentifrices was measured by the FTIR reflectance spectroscopy of human dentin disks monitoring the relative ratio of the collagen and phosphate absorption bands. Affinity of silica nanoparticles toward hydroxyapatite was measured by fluorescent spectroscopy, and their affinity toward tooth enamel was estimated by SEM-imaging. New dentifrice components were characterized by TEM-imaging, powder XRD, and DLS experiments.

Results: 1. The pH value in the oral cavity raises to the level of stimulated saliva in a matter of minutes after the meal. 2. Remineralizing activity of Recaldent©, NovaMin©, and chondroitin-calcium citrate composite is affected by lecithin, modulating affinity to the tooth surface. 3. The phosphonato- surface groups maximize affinity of silica nanoparticles toward hydroxyapatite. 4. Exposure of dentin disks to diluted aqueous HCl solution brings pH to 6.4, and demineralization stops.

Conclusions: 1. The pH value in the oral cavity after a meal is little affected by the acidity of food, but rather determined by the acidity and flow of stimulated saliva.

2. Remineralization of dentine by known and new dentifrice compositions depends on functionalization of their components. 3. Functional groups on the surface of nanoparticles affect their affinity toward tooth enamel and synthetic hydroxyapatite.

4. Dentin brings pH of 0.001 M HCl to 6.4 due to its natural buffering capacity.

References: 1. Reynolds, E. Stabilized Calcium Phosphate Complexes. *International Patent*, **2006**, WO 2006/056013 A1 2. Zaidel, L.; Prencipe, M.; Chopra, S.K. Oral Compositions for Treating Tooth Sensitivity and Methods of Use and Manufacture Thereof *International Patent*, **2010**, WO 2010/115041 A2

Acknowledgments

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Contribution of Vaccine-Induced Antibodies Toward Protection Against Influenza Virus:*Streptococcus pyogenes* Super-infection

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Statement of Purpose: While primary influenza virus infections remain a serious healthcare concern, most fatalities associated with influenza are a result of secondary infections with bacterial pathogens such as *Streptococcus pneumoniae*, *Staphylococcus aureus*, and *Streptococcus pyogenes*. We developed the hypothesis that vaccinating against *Streptococcus pyogenes* would protect against influenza virus:*Streptococcus pyogenes* super-infection.

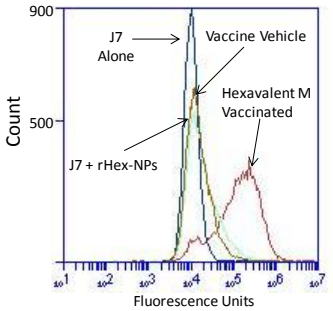
Methods: To test this hypothesis, we used a recombinant peptide *Streptococcus pyogenes* vaccine that has demonstrated safety in previous human clinical trials.[1] This vaccine incorporates the variable N-terminal domains (NTD) of the protective M protein from 6 different *Streptococcus pyogenes* variants that are currently circulating in humans.

Results: Complete protection was observed in vaccinated mice. Pooled sera from protected mice demonstrates the ability to induce macrophage uptake of nanoparticles conjugated with recombinant hexavalent M peptide. Furthermore, in nanoparticle-coupled opsonophagocytosis studies done on individual mouse sera, each mouse vaccinated with the hexavalent M vaccine showed a substantial increase in uptake compared with the individual mouse sera from mice vaccinated with a vaccine vehicle.

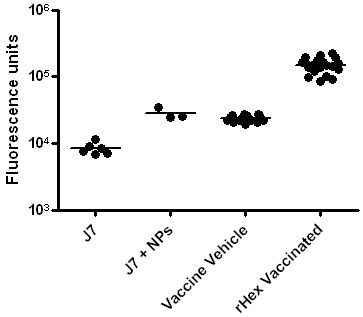
Conclusions: Our work demonstrates that vaccine-induced immunity can prevent the mortality associated with influenza virus:*Streptococcus pyogenes* super-infection, and provides evidence that antibody-mediated interactions with Fc receptor-expressing cells contributes to the protection observed.

References: James B Dale and others, 'Group A Streptococcal Vaccines: Paving a Path for Accelerated Development.', *Vaccine*, 31 Suppl 2 (2013), B216-22 <doi:10.1016/j.vaccine.2012.09.045>.

Figure 1. Nanoparticle uptake by J774A.1 macrophage with pooled mouse sera or individual mouse serum



Sample/Mouse	Mean Fluorescence
J7 Alone	11,334.03
J7 + rHex-NPs	34,050.81
Vaccine Vehicle	22,107.77
Hexavalent M Vaccinated	221,987.53



Novel PEG-Zein Nanomicelles for Topical Delivery of Retinol

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Statement of purpose: Retinol is used for various cosmetic and therapeutic applications on the skin including anti-aging agent, for treatment of keratinization disorders, psoriasis and cutaneous malignancies. However retinol has multiple delivery challenges including poor water solubility (Log P 6.3), poor skin permeability, chemical instability and skin irritation. The objective of this study is to explore the feasibility of addressing the delivery challenges of retinol using novel polyethylene glycol conjugated zein micelles.
Materials and Methods: Retinol was encapsulated in the PEG-zein micelles using dialysis method. Particle size, polydispersity index and zeta potential were determined by dynamic light scattering method (NICOMP™ 380 ZLS, USA). The surface morphology of PEG-Zein micelles were investigated by atomic force microscopy. The entrapment efficiency of retinol in PEG-Zein micelles was determined using UV spectrophotometry. In-vitro skin penetration studies were carried out using excised porcine skin in a vertical Franz diffusion cell. The amount of retinol in the skin and in the receptor medium was analyzed by radiochemical method of analysis.
Summary of results: The average particle size and polydispersity index of retinol micelles was 197nm, 0.234 respectively with an encapsulation efficiency of 79%. Photodegradation of retinol was significantly reduced (by 3-20 fold) after encapsulation in PEG-zein micelles. The micelles showed burst release followed by sustained release for 48 hours. PEG-zein micelles showed significantly higher skin retention (8 fold) of retinol PEG-Zein micelles compared to free retinol. At the same time, the amount of retinol in the receptor compartment was significantly reduced when encapsulated in micelles. Tape stripping studies showed that the higher amount of retinol (6 folds) was found in the stratum corneum and epidermis/dermis when treated with the retinol PEG-Zein micelle compared to the plain retinol dispersion.
Conclusions: The results show that the encapsulation of retinol in PEG-zein micelles improved the stability of retinol and retained retinol in the skin. Overall the study demonstrates that PEG-zein micelles are a potential skin delivery vehicle for retinol.

Effect of Vitamin-C on the Growth of Endothelial Cells for Stent and Vascular Graft Applications

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Statement of Purpose: Endothelialization is vital for preventing thrombosis on cardiovascular medical devices such as coronary stents and vascular grafts. The anti-proliferative drugs such as sirolimus (SIR) and paclitaxel (PAT) are currently released from stents and vascular grafts to inhibit the growth of smooth muscle cells (SMCs) and thereby preventing neointimal hyperplasia [1]. However, these drugs delay or impair the growth of endothelial cells (ECs) on implant surfaces causing late thrombosis [2] – a serious condition which results in heart attack or death. Hence, a drug which inhibits the growth of SMCs and concurrently encourages the EC growth is currently needed for these implants. Vitamin-C has been shown to inhibit SMC growth and promotes EC growth when orally administered in patients [3]. Hence, the research goal of this study is to investigate the effect of L-AA on the growth of ECs when the drug is directly added to the cells for potential applications in locally drug delivering stents and vascular grafts. For comparison purposes, the effect of SIR and PAT on the growth of ECs was also investigated in this study.

Methods: A density of 15×10^3 human aortic endothelial cells (HAECs) was seeded in a 24-well plate. A dose of 100µg/mL of L-AA in phosphate buffered saline (PBS) was added to the cells and incubated for up to 24 or 48 hours. Similarly, a dose of 100µg/mL of SIR and PAT in 0.5% ethanol was also added to the cells and incubated for the time period mentioned above. Three different control samples were also used in the study: (a) control # 1 – cells with no drugs; (b) cells with only PBS; (c) cells with only 0.5% ethanol. The viability and proliferation of ECs were investigated at 1, 3, 5, and 7 days using a Resazurin fluorometric assay. The live cells were stained with fluorescein diacetate and imaged using fluorescence microscopy. The morphology of cells was investigated using phase contrast microscopy. The expression of surface adhesion molecule, platelet endothelial cell adhesion molecules (PECAM-1), was investigated using immunofluorescent microscopy. One-way ANOVA was used to determine the statistical significance at $p < 0.05$.

Results: L-AA and the three controls exhibited a significant increase in cell viability and proliferation from day-1 to day-7 while SIR and PAT showed very limited viability and proliferation of ECs (Fig 1). L-AA showed the presence of maximum EC number even greater than that of controls at different time points (Fig 1). The viability and proliferation of ECs increased in the following order SIR < PAT < Control #1 < Control #2 = Control #3 < L-AA. These results showed that L-AA strongly favored the growth of ECs while SIR and PAT inhibited the EC growth. A spreading of ECs with typical polygonal shape was observed for L-AA and controls while an elongated oval shape was observed for SIR and PAT (Figs 2 and 3). The expression of PECAM-1 was stronger on L-AA and controls while a weaker expression was observed for SIR and PAT (Fig 4).

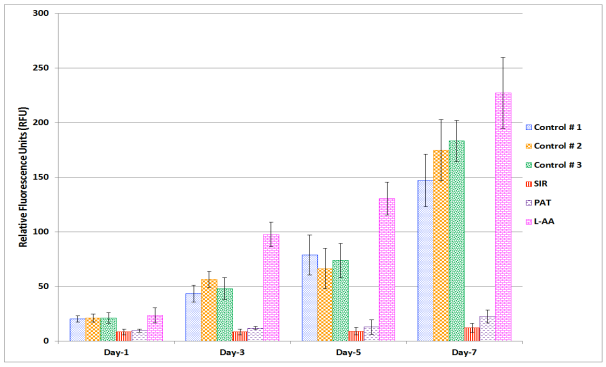

Fig 1. Quantitative EC viability and proliferation

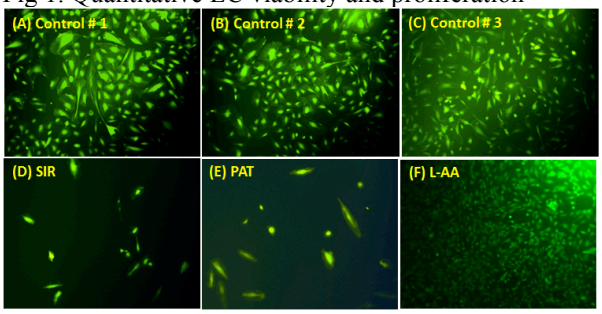

Fig 2. Fluorescence microscopy images of ECs

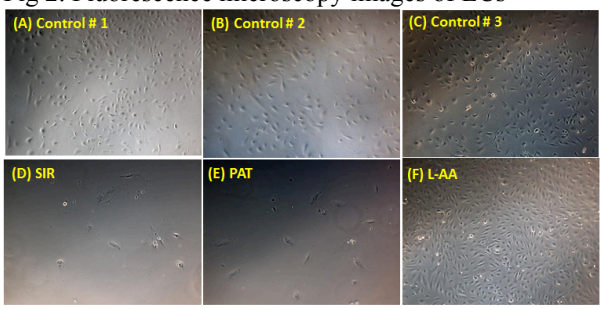

Fig 3. Phase contrast microscopy images of ECs

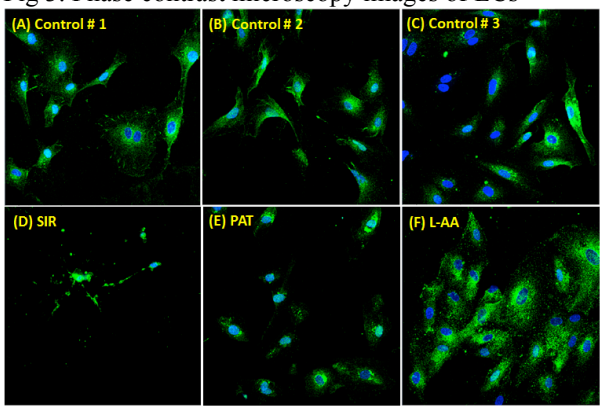

Fig 4. Immunofluorescent microscopy images of ECs

Conclusions: L-ascorbic acid strongly encouraged the growth of endothelial cells in *in vitro* conditions. Hence, L-AA is a promising drug for local delivery from stents and vascular grafts to promote endothelialization.

References: (1) Mani G. Biomaterials 2007; 28: 1689-1710; (2) Finn AV. Circulation 2007; 115: 2435-2441; (3) Aguirre R. Pharmacol Ther 2008; 119: 96-103.

Interaction of Endothelial and Smooth Muscle Cells with Paclitaxel-Immobilized Self Assembled Monolayers

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Introduction: Polymer-based drug delivery carriers used in drug eluting stents cause adverse reactions in patients [1]. The use of self-assembled monolayers (SAMs) as a polymer-free drug delivery platform to deliver an anti-proliferative drug, paclitaxel (PAT) has been previously demonstrated [2]. In this study, the interaction of endothelial cells (ECs) and smooth muscle cells (SMCs) with PAT immobilized SAMs on Co-Cr alloy was studied.

Methods: Co-Cr alloy plates used in this study were mechanically polished using SiC papers and chemically cleaned, followed by SAM and PAT deposition [2]. Surfaces were characterized using FTIR, SEM, Optical Profilometry (OP), and contact angle goniometry (CAG). The viability and proliferation of cells coated on these surfaces were investigated at 1, 3, and 5 days using a Resazurin assay, fluorescence microscopy (FM) and immunofluorescent assay.

Results: The observed IR peak confirmed the successful coating of SAMs and PAT on Co-Cr alloy surfaces (Fig 1). SEM (Fig 2A) and OSP (Fig 2B) images showed the spherical and oval shaped morphology of PAT crystals on alloy surfaces. The EC adhesion increased in the following order: PAT < control = SAMs (Fig 3A, day-1). The spreading of ECs on all the surfaces with typical polygonal shape indicated that these surfaces are conducive to endothelialization (Fig 3). The number of SMCs on PAT coated surfaces was significantly lesser when compared to that of other surfaces (Fig 4).

Conclusions: This study demonstrated the use of SAMs to obtain the sustained delivery of paclitaxel from stent material to inhibit the growth of smooth muscle cells.

References: (1) Virmani R. Circulation 2004; 109: 701-705; (2) Mani G. Biointerphases 2011, 6, 33-42.

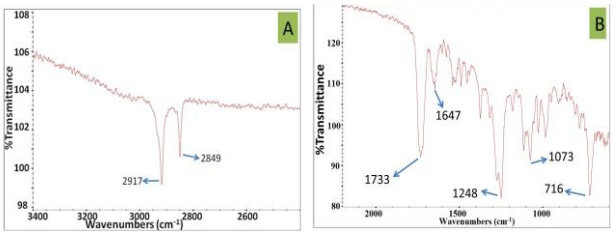

Fig 1: FTIR spectra of SAMs (A) and PAT (B).

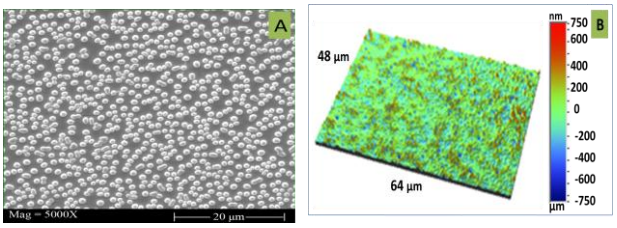

Fig 2: SEM (A) and OSP (B) images of PAT-Co-Cr.

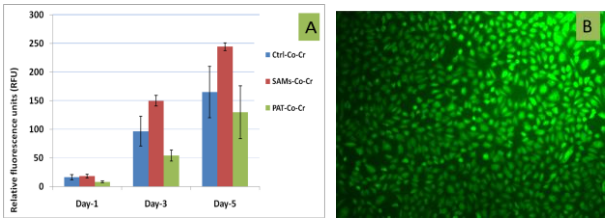

Fig 3: Resazurin data (A) and Fluorescent microscopy image (SAMs-CoCr, after day-5) (B) of HAECs.

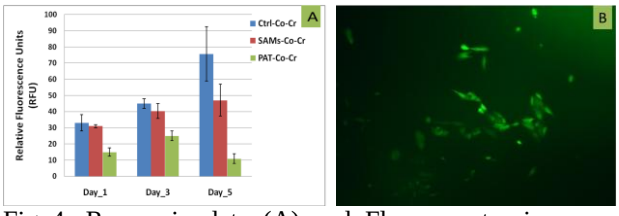

Fig 4: Resazurin data (A) and Fluorescent microscopy image (PAT-Co-Cr, after day-5) (B) of HASMCs.

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 concurrently 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 ℃ 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). The AFM images of control, PA coated and L-AA coated specimen were obtained using AFM.

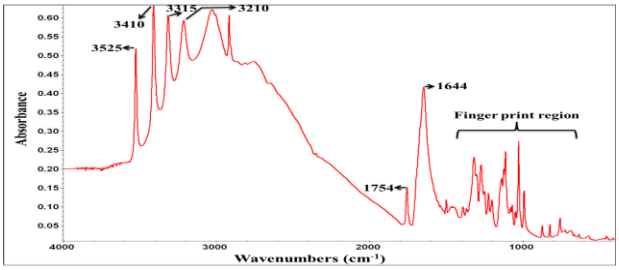

Fig 1. FTIR spectrum of L-AA coated Co-Cr alloy

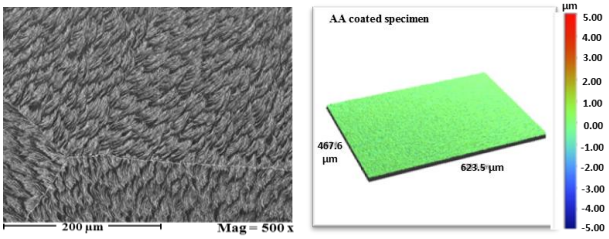

Fig 2: SEM (A) and OSP (B) images of L-AA on Co-Cr

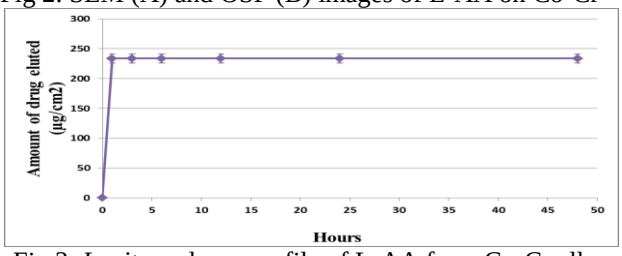

Fig 3. *In vitro* release profile of L-AA from Co-Cr alloy

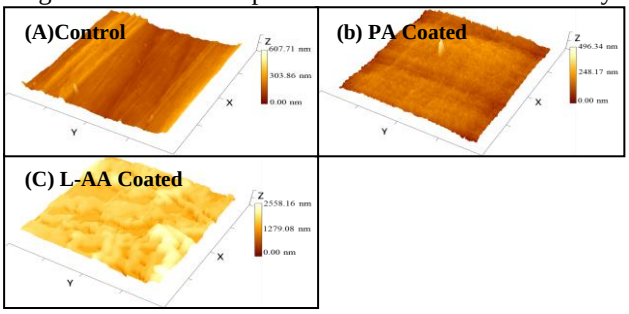

Fig 4. AFM images (Scan size = 10 × 10 µm) of (A) Control, (B) PA coated, and (C) L-AA coated Co-Cr specimens.

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.

Surface Functionalization of Cobalt-Chromium Alloy Using Phosphoric and Phosphonoacetic Acids

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Statement of Purpose: Cobalt-Chromium (Co-Cr) alloys have been extensively used for cardiovascular, orthopedic, and dental implants [1]. The functionalization of Co-Cr alloy surfaces with reactive functional groups such as hydroxyl (–OH) and carboxylic acid (–COOH) has abundant biomedical applications. A variety of biomolecules including proteins, peptides, antibodies, DNA, and therapeutic drugs can be immobilized and delivered from Co-Cr alloy surfaces using –OH and –COOH functional groups. In this study, the Co-Cr alloy was surface modified using phosphoric acid (PA) and phosphonoacetic acid (PAA) coatings to provide –OH and –COOH surface groups. The coatings were characterized using contact angle goniometry, Fourier transform infrared spectroscopy (FTIR), and atomic force microscopy (AFM).

Methods: Co-Cr alloy specimens (1cm x 1cm) were mechanically polished using 600, 800, and 1200 grit SiC papers and chemically cleaned by sonication in ethanol, acetone, and methanol for 10 minutes each followed by N_2 gas drying. The cleaned specimens were immersed in three different concentrations (1, 25, and 100 mM) of PA and PAA in deionized water for 24 hours. The specimens were then transferred to an oven without rinsing and heated in air at 120 ℃ for 19 hours. The specimens were cleaned by sonication in H_2O for 1 min to remove physically adsorbed molecules followed by N_2 gas drying. The control, PA, and PAA coated Co-Cr alloy were characterized using CAG, FTIR, and AFM.

Results: The contact angles of control, PA, and PAA coated specimens are provided in Fig 1. The contact angle measured for the control Co-Cr alloy was 53 ± 5°. For 1 and 25 mM PA, no significant difference in the contact angle was observed when compared to that of control. However, the 100 mM showed a significant decrease in the contact angle (8 ± 2°). For PAA coating, the 1 and 25 mM PAA showed a significant decrease in the contact angle when compared to that of control. The 100 mM PAA showed a contact angle of 6 ± 3°. These results suggested the presence of dense –OH and –COOH groups on Co-Cr alloy surfaces after coating with 100 mM of PA and PAA, respectively. The FTIR spectra of PA and PAA coating were provided in Fig 2 and Fig 3, respectively. For PA, the IR peaks at 807, 1107, and 1265 cm^{-1} were assigned to P–OH, P–O–Metal, and P=O, respectively. The peak at 3743 cm^{-1} belongs to the –OH groups of PA. For PAA, the IR peaks at 945, 1120, and 1295 cm^{-1} were assigned to P–OH, P–O–Metal, and P=O, respectively. A peak at 1695 cm^{-1} belongs to the C=O of –COOH groups. Also, the peaks observed at 3745 and 3860 cm^{-1} belong to the –OH of –COOH groups. Fig 4 shows the AFM topography images of control, PA, and PAA coated specimens. No significant difference in the surface topography was observed between the specimens. The RMS roughness values measured by AFM for the control, PA, and PAA coated specimens were 22 ± 7, 25 ± 9, 31 ± 6 nm, respectively. No significant difference in the roughness values were observed between control and PA or PAA. This suggests that PA and PAA formed a uniform coating which followed the contour of polished Co-Cr alloy surface.

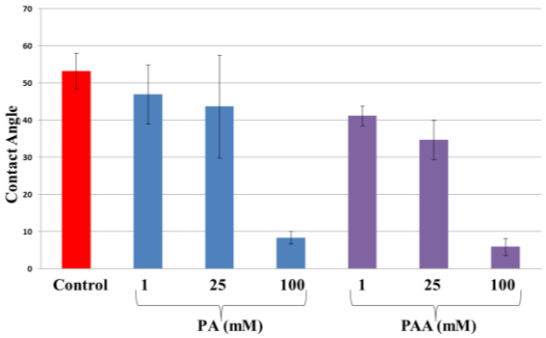

Fig 1. Contact Angle of PA and PAA coated Co-Cr alloy

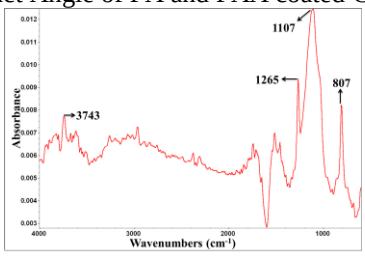

Fig 2. FTIR spectrum of PA (100 mM) coated Co-Cr

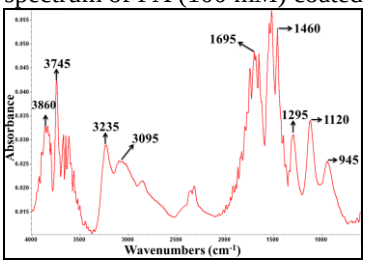

Fig 3. FTIR spectrum of PAA (100 mM) coated Co-Cr

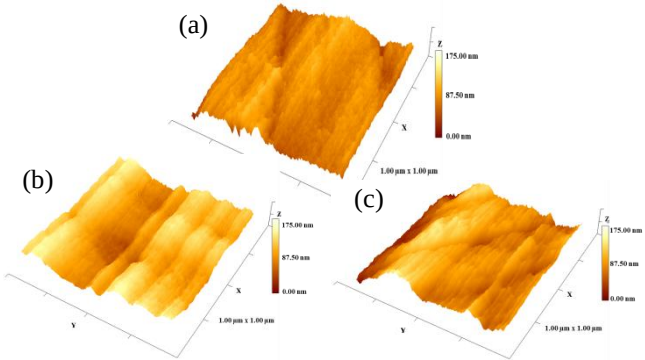

Fig 4. AFM images of control (a), PA (b) and PAA (c)

Conclusions: PA and PAA were successfully coated on Co-Cr alloy to provide –OH and –COOH surface groups. Thus, the surface modification technique employed in this study has potential applications for immobilizing and delivering therapeutic drugs and biomolecules from Co-Cr alloy surfaces.

Reference: (1) Mani G. Applied Surface Science 2009; 255: 5961-5970.