**A Dual-Functional Polymeric Coating Combining Rapamycin and Nitric Oxide Release**

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**Statement of Purpose:** Thrombus formation and smooth muscle cell (SMC) proliferation at the inteface of implanted medical devices are two major adverse outcomes of the placement of blood contacting biomedical devices such as vascular grafts and coronary artery stents. (Young KA. Catherter. Cardio. Inte. 1999; 48:324-330) Herein, we desciibe a dual-functional polymeric coating that releases both rapamycin (Sirolimus, an antiproliferative drug) and nitric oxide (NO, an antiplatelet agent). The new coating can potentially suppress SMC proliferation and blood clot formation, simultaneously.

**Methods:** Diazeniumdiolated *N,N'*-dibutyl-1,6-hexanediamine (DBHD/$N_2O_2$) was synthesized by treating *N,N'*-dibutyl-1,6-hexanediamine in $CH_3CN$ with NO (80 psi) (Batchelor, M. M. J. Med. Chem. 2003; 46:5153-5161) A cocktail containing well-suspended DBHD/$N_2O_2$ in the DMAc/THF solution of PurSil™ (a commercial silicone-poly(ether)urethane copolymer) was made. Silicone rubber (SR) tubings were then dip-coated with this cocktail to form an NO release under-layer. A 1:1 molar ratio of potassium tetrakis(4-chlorophenylborate) (KTpClPB, a lipophilic borate salt) was added to help reduce pH change within polymeric coatings during the decomposition process of DBHD/$N_2O_2$. The tubings were further dip-coated in the DMAc/THF solution of rapamycin and PurSil™ to form a rapamycin releasing top-layer. Both NO and rapamycin release kinetics were measured at 37 °C in PBS (10 mM, pH = 7.4). Scanning electron microscopy (Philips XL30 FEG SEM) was used to characterize the morphology of the coatings on the SR tubings. NO flux was measured by NO analyzer (NOA™ 280, Sievers Instruments, Inc.) in a continuous monitoring mode while rapamycin release was measured by HPLC (Hewlett Packard 1050 HPLC system) via an intermittent sampling method with 1-h intervals.

**Results/Discussion:**

*1) SEM Characterization*

SEM results (Fig. 1) illustrate that the DBHD/$N_2O_2$ containing under-layer has a thickness of approx. 30 μm while that of the rapamycin containing top-layer is approx. 15 μm.

*2) NO Release Measurement*

The NO flux released from the coatings stabilizes at approx. $3.5\times10^{-10}$ mol·cm$^{-2}$·min$^{-1}$ after an initial burst (Fig. 2). This level is comparable to the NO released from a healthy endothelium (~$1\times10^{-10}$ mol·cm$^{-2}$·min$^{-1}$) (Feelisch, M. Methods in Nitric Oxide Research; John Willey and Sons Ltd.: West Sussex, 1996). The duration of NO release can be as long as one week (data not shown).

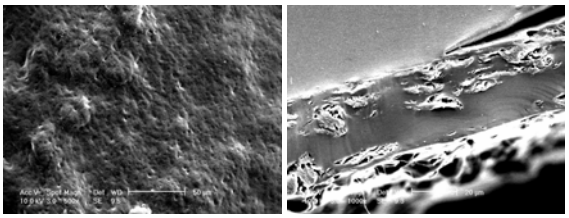

Figure 1. SEM pictures of the bilayer polymeric coating. Left : surface morphology of the coating ; Right : cross section of the double layer polymeric coating on the SR substrate.

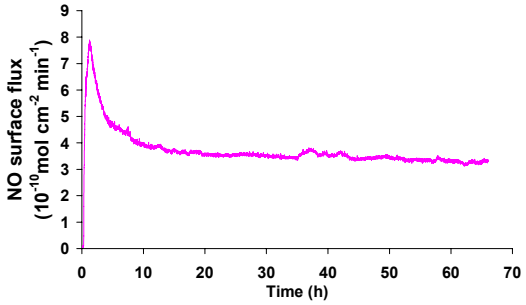


Figure 2. NO level of DBHD/$N_2O_2$-loaded SR tubings with rapamycin-releasing top-coatings. Tubing was incubated in PBS (10 mM, pH = 7.4) under 37 °C.

*3) Rapamycin Release Kinetics*

The rapamycin release kinetics was studied by HPLC measurements. A stable flux (approx. 1.5 μg·cm$^{-2}$·h$^{-1}$) was obtained and around 30% of total rapamycin was released during the first day (Fig. 3)

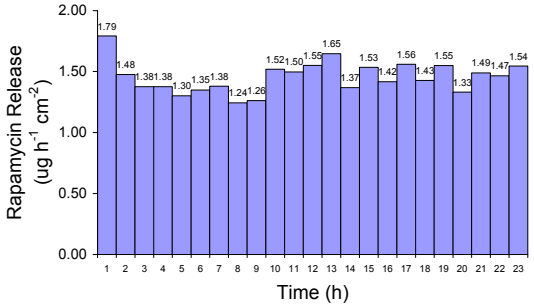


Figure 3. HPLC measurement of rapamycin release from the polymeric coating during the first day. The tubing was incubated in PBS (10 mM, pH = 7.4) under 37 °C.

**Conclusions:** A dual-function polymeric coating was made that can simultaneously release both NO (as an antithrombotic agent) and rapamycin (as an antiproliferative agent). Further studies will focus on the *in vitro* and *in vivo* biocompatibility studies of this novel bilayer polymeric coating.