

Controlled Photoinitiated Nitric Oxide Release from S-nitroso-N-acetyl-DL-penicillamine Derivatized Silicone Rubber (SNAP-SR)

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Statement of Purpose: Polymeric materials are used for a wide variety of blood-contacting and tissue contacting biomedical devices. A significant problem for all tissue contacting devices is biological response toward the material, which often time will lead to device failure and adverse physiological reactions in patients treated with these devices. Nitric oxide (NO) is an endogenously produced, free radical gas that has been shown to have many biological functions *in vivo*, including as a neurotransmitter, to fight bacterial infection, to control blood pressure, and is known to act as an anti-tumor agent. NO is also a potent inhibitor of platelet adhesion and activation. NO has been implicated in mediating the inflammatory response in subcutaneous tissue and has been shown to be important in the wound healing process. Because NO has an extremely short half-life (seconds) in oxygenated solutions such as the physiological environment, the affects of NO are localized specifically at the implant site, thus eliminating systemic effects caused by releasing NO from implanted devices. These properties of NO make it an ideal candidate for mediating the biological response toward implanted materials if materials can be developed that allow for controlled release of NO.

Herein, we describe the synthesis and controlled release of NO from a silicone rubber material modified to contain S-nitroso-N-acetyl-DL-penicillamine covalently linked to aminopropyl trimethoxysilane used to crosslink polydimethylsiloxane (PDMS). The light mediated NO generation from this polymer provides a means of precisely controlling spatial and temporal delivery of NO in biomedical applications.

Methods: Three different molecule weights (700-800 cSt, 2,000 cSt and 18,000 cSt) of hydroxyl-terminated PDMS were obtained from Gelest, Inc., (Morrisville, PA). Aminopropyl trimethoxysilane, dibutyltin dilaurate, N-acetyl-DL-penicillamine, cyclam and toluene were obtained from Sigma-Aldrich Co. (St. Louis, MO). Tert-butyl nitrite (90% technical grade) (t-BuONO) was purchased from Acros Organics (Geel, Belgium). All reagents were used as received except t-BuONO. The t-BuONO was first extracting over 15 mM aqueous cyclam in order to remove copper added as a stabilizing agent during manufacturing. A self-protected thiolactone was synthesized from N-acetyl-DL-penicillamine.

1.6 g of hydroxy-terminated PDMS was mixed with 0.3 g of aminopropyl trimethoxysilane, 24 mg of dibutyltin dilaurate and 8 mL of toluene. The solution was stirred for 24 hours to cross link the PDMS. 200 mg of thiolactone was then added and the solution was stirred for an additional 24 hours to allow the thiol-containing compound to react with the primary amine groups present on the cross-linking agent. 1 mL of cleaned t-BuONO was

then added at which point the polymer was placed on shaker table for 30 minutes which resulted in an emerald green polymer. This color change is indicative of the formation of SNAP linked to the PDMS backbone (final product is SNAP-SR). The SNAP-SR polymer was then dip-coated onto 500 μ m de-clad polymethylmethacrylate (PMMA) optical fiber. We have developed a wireless device that will transmit an sequence of illumination to a light emitting diode (LED) coupled to a fiber optic cable was. NO generated from the illumination sequence was monitored using a Seivers Nitric Oxide Analyzer 280i (GE Instruments, Boulder, CO, USA) (NOA) to establish the relationship of light output to NO generation.

Results: We have developed a novel cross-linked silicone rubber containing the RSNO S-nitroso-N-acetyl-DL-penicillamine covalently linked to the polymer backbone (SNAP-SR) whose NO release can be controlled by using light as an external on/off trigger. Figure 1 shows patterned NO generation from a PMMA optical fiber coated with 2,000 cSt SNAP-SR as current was turned on and off and increased to an LED. Increased current to the LED increases light output from the LED which precisely generated more NO. Surface fluxes were obtained of 1.0, 1.58, 2.50, 3.34, and 4.11 $\times 10^{-10}$ mol \cdot cm $^{-2}\cdot$ min $^{-1}$ with 10, 25, 50, 75 and 100% current through the LED used.

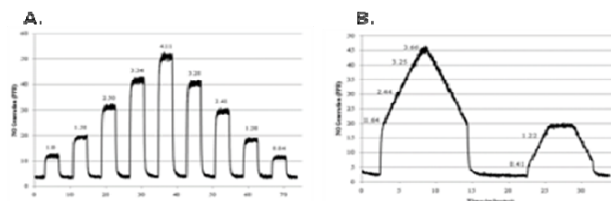


Figure 1: Nitric oxide (NO) generation from polymethylmethacrylate fibers coated with SNAP-containing silicone rubber using a custom built LED light source to photolytically generate NO with precise control of surface flux generated. Panel A shows NO generation when pulsing the light source on for 4 minutes and off for 4 minutes at increasing current to the LED. Panel B shows NO generation steadily increasing as current is ramped higher and decreased or held steady then decreased.

Conclusions: We have developed light-sensitive NO releasing silicone rubbers that can be coated on optical fibers which are able to generate NO with an external on/off trigger and precise temporal and spatial control. We have developed a system that will allow precise surface fluxes and duration of NO to be generated. This system will be used to quantitatively assess what level of NO causes specific cellular response. Ultimately, this will increase our fundamental understanding of the cellular response to NO and develop potentially useful polymeric systems that generate NO locally to be used to actively mediate the biological response toward materials that are placed in contact with blood and tissue.