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Controlled Photoinitiated Release of Nitric Oxide from Polymer Films Containing *S*-Nitroso-*N*-acetyl-DL-penicillamine Derivatized Fumed Silica Filler

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There has been considerable interest in creating nitric oxide (NO)-releasing materials that can be used in a wide variety of biomedical applications. Indeed, NO has been shown to be a potent inhibitor of platelet activation and adhesion,[1,2] to inhibit smooth muscle cell proliferation associated with restenosis[3] and neointimal hyperplasia,[4] inhibit bacterial cell adhesion,[5] and to be involved in macrophage function via cytotoxic effects.[6] The use of exogenous NO to mimic such functions is likely to require a different level and duration of NO generation and release. To date, a variety of materials have been synthesized in this laboratory and elsewhere that incorporate NO donors (e.g., diazeniumdiolates) into polymer matrixes by blending discrete donors into the polymer[7−12] or covalently linking the donor to the backbone of polymers or polymer fillers.[12−15] The kinetics of NO release from these materials have been modulated by changing the polymer matrix or structure of the NO donor. All of these materials continuously release NO upon initiation of the reaction (e.g., exposure to an aqueous solution or blood or continuous NO release due to thermal dissociation). Herein, we report the first hydrophobic NO-releasing material that utilizes light as an external on/off trigger that enables precise control of the rate of NO released by the polymer films depending on the duration and intensity of the light used to initiate NO release.

Several reports have previously utilized light to generate NO by decomposing *S*-nitrosothiols that are either endogenously present in biological systems[16] or have been incorporated into monolayers or hydrogels.[11,17,18] Etchenique et al.[17] used photolytic release of NO from monolayers of nitrosated dithiothreitol attached to gold surfaces. Although this work suggests the possibility of photoinitiated temporal and spatial control of NO release, it is limited in application because there is a very small reservoir of NO actually available in the monolayer. Shishido et al.[18] blended *S*-nitrosoglutathione or *S*-nitroso-*N*-acetylcysteine into hydrogels that release NO at body temperature and demonstrated enhanced NO release when the materials were simultaneously irradiated with light. This material again continuously released NO upon initiation via the thermal decomposition pathway. The new material described herein has the potential to be used to answer fundamental questions about the levels of NO required to achieve desired therapeutic effects in different applications because of the ability to fine-tune and fully reverse the rate of NO release with external control.

The strategy employed here to impart controllable NO-release character to silicone rubber films involves blending *S*-nitroso-*N*-acetyl-DL-penicillamine derivatized fumed silica (FS) (7−10 nm diam) filler particles into the center layer of a trilayer film, as illustrated in Figure 1. Covalent attachment of the nitrosothiol to the surface of the particles prevents leaching of any reaction byproducts as well as loss of the NO donor due to diffusion out of the polymer matrix, thereby ensuring NO release occurs at the polymer/biological sample interface. The first step in the derivatization process is to react the free silanol groups on the surface of the fumed silica with (3-aminopropyl)trimethoxysilane. The resultant pendent amine groups are then reacted with self-protected *N*-acetyl-DL-penicillamine, covalently linking the thiol molecules to the surface of the fumed silica particles. The tethered thiol groups are then converted to the corresponding *S*-nitroso-*N*-acetyl-DL-penicillamine (SNAP-FS) by reaction with *tert*-butylnitrite (see Supporting Information for details of the synthesis, Scheme 1S). The derivatized fumed silica contains 1.38 (±0.29) × 10^{-7} moles of NO per milligram of fumed silica. Films are cast by dissolving RTV-3140 silicone rubber (SR) (Dow Corning, Midland, MI) in toluene and casting the polymer cocktail into 2.5-cm diam Teflon rings on Teflon plates at 4-h intervals. The central layer of the trilayer film contains 20 wt % of the derivatized fumed silica. Cured films (∼100 μm total thickness) are cut into 0.7-cm diam disks. Nitric oxide generation from the polymer films is monitored via chemiluminescence detection (Siever's 280 nitric oxide analyzer, Boulder, CO) at 24 °C.

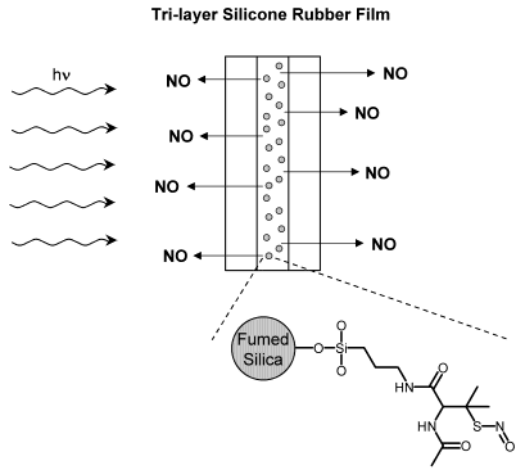

Figure 1. Schematic of photoinitiated NO release from silicone rubber trilayer films containing *S*-nitroso-*N*-acetyl-DL-penicillamine derivatized fumed silica blended into the middle layer.

S-Nitrosothiols are reported to decompose by three pathways: (a) copper ion mediated decomposition, (b) ascorbate mediated decomposition, and (c) photoinitiated decomposition.[19] The presence of copper ions and/or ascorbate in solutions that bathe these trilayer films do not initiate NO release, probably because of the hydrophobic nature of silicone rubber (see Supporting Information, Figure 1S). Soaking the films in fresh rabbit plasma also does not initiate NO release from these films (see Supporting Information, Figure 1S). This indicates that endogenous reducing species present in blood are not be able to initiate NO generation from the silicone rubber matrix containing SNAP-FS. However, Figure 2 shows NO generation from these same trilayer films when exposed to different intensities of visible light. Figure 2A illustrates NO release from a dry trilayer film exposed to dark, 40, 60, 75, and 100 W light bulbs (Philips Director bulbs). Figure 2B shows NO release from a second

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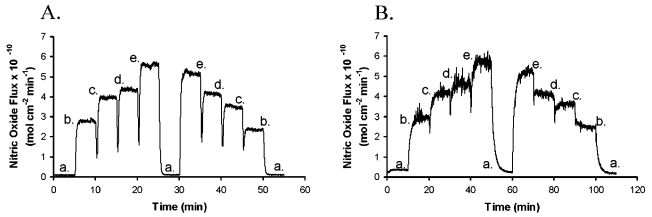

Figure 2. Photoinitiated NO release from trilayer silicone rubber films containing *S*-nitroso-*N*-acetyl-DL-penicillamine derivatized fumed silica (SNAP-FS) in the central layer when the film is (A) dry and (B) immersed in PBS buffer. The intensity of the light used increased from darkness, 40, 60, 75, and 100 W (a through e, respectively).

piece of the same trilayer film immersed in phosphate-buffered saline (PBS) (pH 7.4) exposed to the same increasing intensities of light. Both the dry film and the film soaked in PBS exhibit the same steady state NO flux for each power of light used to irradiate the sample. The total amount of NO released from the film during the first series of increasing light intensity exposures is 6.0% for the dry film and 12.9% for the film soaked in PBS. The slightly decreased NO flux observed for the second series of exposures to light results from the lower concentration of SNAP-FS remaining in the film after NO release during the initial cycle.

Evidence suggests that 590-nm light is primarily responsible for the release of NO from the SNAP-FS particles embedded in SR films. Indeed, free *S*-nitroso-*N*-acetyl-DL-penicillamine has absorbance maxima at 340 nm (with significant absorption through 400 nm) and 590 nm (see Supporting Information, Figure 2S), with the molar absorptivity at 590 nm, ca. 100-fold less than that at 340 nm. To determine definitively which wavelengths are responsible for releasing NO, a quartz cuvette sample holder was used with cutoff filters that allow light either longer than 500 nm or shorter than 500 nm to irradiate a SNAP solution. The longer wavelengths of light are responsible for approximately 67% of the photoinitiated NO generation, while the shorter wavelengths of light account for approximately 33% of the NO generated (see Supporting Information, Figure 3S). Since the incident intensities of light at 300−400 nm and 550−600 nm are within the same order of magnitude in such experiments, and given the much lower molar absorptivity of SNAP at the longer wavelengths, it is evident that absorption of photons at the longer wavelengths more efficiently releases the NO from the SNAP species. The glass sample vessels used for the polymer film experiments described above do not allow appreciable levels of shorter wavelength light to pass through the reaction cell, indicating that light >500 nm is initiating the vast majority of NO generation from the films containing the SNAP-FS (see Supporting Information, Figure 2S).

The total amount of NO available to be released and the specific fluxes achieved can be adjusted by changing the amount of derivatized particles blended into the middle polymer layer, varying the thickness of the polymer film, and/or adjusting the intensity of light used to initiate NO release from the cured polymer. These different control points offer the ability to create a wide variety of NO releasing materials with variable and fully reversible control of the NO fluxes obtained. This type of control will allow fundamental studies to be carried out that can define useful NO fluxes required to achieve specific therapeutic effects (e.g., inhibition of platelet activation, inhibition of bacterial growth, etc.). Additionally, the use of light as an on/off trigger to initiate release allows a means of temporal control of the NO flux. Such control would be important in efforts to potentially utilize local NO release within tumor masses to kill cancerous cells. This could be accomplished by coating the SNAP-FS doped SR material described here on the end of a fiber optic probe, inserting the probe precisely within the tumor mass, and delivering a defined NO dose by controlling the duration, intensity, and wavelength of light used to illuminate the fiber.

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Supporting Information Available: Detailed scheme for derivatizing fumed silica, experimental data showing NO generation from trilayer silicone rubber films exposed to Cu(II), ascorbate, and rabbit plasma, and experimental data showing NO generation using optical filters and the relative irradiance of incident light used to generate NO and the UV−vis absorbance spectrum of SNAP (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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