S-nitrosation of poly(propylene sulfide) nanoparticles for nitric oxide delivery

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Statement of Purpose: Nitric oxide (NO) is one of the most important small molecules in physiology. Its large diffusivity and high reactivity allow it to contribute to a wide spectrum of physiological functions including vasodilation, immune-cytotoxic defenses, blood clotting, and lymphatic contraction. NO therefore represents an exciting putative drug candidate for the treatment of a myriad of immune-regulated pathologies. However, as the result of scavengers such as haemoglobin and its short half-life, NO produced *in vivo* largely mediates its effects proximal to its synthesis source. Delivery of exogenous NO to deep tissue and/or cellular targets therefore remains a challenge and requires a delivery vehicle capable of stabilizing and transporting NO amidst these physiological pressures. While several NO donors and NO-loaded synthetic polymer platforms have been previously developed, few biocompatible formulations have been explored for NO delivery that can exploit the improved tissue and cell targeting activity of colloidal formulations. To address this we have explored a putative NO donor platform based on a previously reported Pluronic-stabilized, poly(propylene sulfide) (PPS)-core nanoparticle (NP) technology [1]. These Pluronic-PPS NP demonstrate improved penetration into lymphoid tissues as a result of their small size characteristic [2] and are extensively taken up intracellularly [3] allowing loaded drugs to exert increased and targeted bioactivity [4]. Here, we modify this technology and exploit the chemical reactivity of NO to form stable adducts capable of efficient donation under physiological conditions.

Methods: Pluronic-PPS NP are synthesized using block co-polymer Pluronic F-127 to create a hydrophobic micelle in which propylene sulfide is polymerized via anionic ring-opening emulsion polymerization [1]. When exposed to oxygen some of the linear PPS chains form disulfide bonds stabilizing the hydrophobic core, while the rest remain free thiols as measured by Ellman's assay. These free thiols can be S-nitrosated by addition to an equal volume of acidified sodium nitrite in 1N hydrochloric acid. After the reaction period, excess ammonium sulfamate is added to quench the S-nitrosation reaction. S-nitrosothiols are measured by the method of Saville and free nitrite is measured using the Griess assay. We thus assessed the activity of S-nitrosated NP (SNO-NP) in the loading and release of NO in comparison to another commercially available NO donor, S-nitroso-N-acetyl-D,L-penicillamine (SNAP).

Results: Increasing the concentration of initiator added to the NP synthesis reaction increased NP thiol content (data not shown). Incubation with acidified nitrite led to NP S-nitrosation and the extent of S-nitrosation increased concomitantly with NP thiol concentration (Figure 1A). This NP S-nitrosation was found to be dependent on acidic pH and the presence of oxygen (data not shown), while decomposition results in the formation of NP disulfides (data not shown), results which are consistent with the existing literature on nitrosothiol reactivity and degradation mechanisms. When compared to the widely used NO donor SNAP, SNO-NP display dramatically longer NO retention times when stored at 4°C (days vs hours, respectively) yet roughly equivalent cupric chloride-catalyzed NO release profiles (Figure 1B). SNO-NP can also donate NO to physiological low molecular weight thiols (LMWSH), a process termed transnitrosation, at increasing extents with increasing ratio of CYS:SNO-NP (Figure 1C).

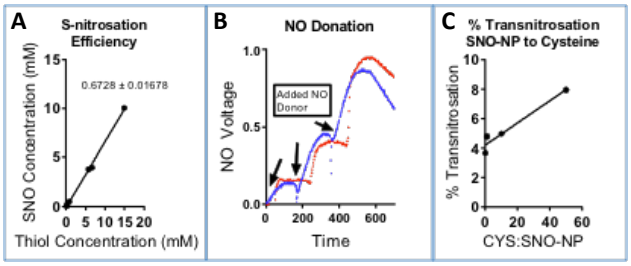

Figure 1: A) S-nitrosylation increases with increasing NP thiol concentration. The slope indicates an average efficiency of S-nitrosation of ~67%. B) SNO-NP donate NO as efficiently as commercially available NO donor SNAP as measured by NO concentration sensor indicated by voltage. The arrows represent the addition of NO donor. Red, SNAP; Blue, SNO-NP. C) Increasing cysteine (CYS) to SNO-NP ratio increases the extent of transnitrosation.

Conclusion: These data suggest that Pluronic-PPS NP can be used as a NO delivery platform as the result of their tunable free thiol content and by exploiting the physiological S-nitrosothiol bond. SNO-NP can donate NO on par with commercially available small molecule donor SNAP, facilitate transnitrosation to physiologically relevant LMWSH, and retain SNO functionality under prolonged storage conditions. In ongoing work, the capacity of SNO-NP to induce NO-mediated signaling effects in physiological environments *in vitro* and *in vivo* will be explored.

References:

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