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Nitric oxide-releasing silica nanoparticles with varied surface hydrophobicity

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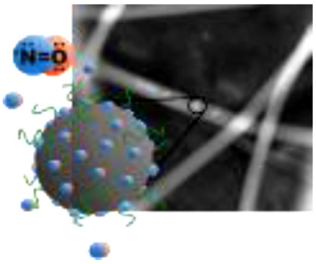
HIGHLIGHTS

- Surface modification of silica nanoparticles to tune nitric oxide-release kinetics.
- Hydrophobic modifications result in extended nitric oxide-release duration.
- Method allows for reduced particle leaching from polymers.

GRAPHICAL ABSTRACT



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ABSTRACT

Hydrocarbon- and fluorocarbon-based silanes were grafted onto the surface of amine-containing silica nanoparticles to achieve materials of varied surface hydrophobicity. X-ray photoelectron spectroscopy and direct polarization solid-state nuclear magnetic resonance spectroscopy were used to confirm the presence of surface-grafted functionalities. Changes in hydrophobicity were illustrated by assessing the stability of aqueous particle suspensions using dynamic light scattering. Following surface modification, the amines were converted to *N*-diazeniumdiolate nitric oxide (NO) donors to yield nanoparticles with tunable nitric oxide (NO) release kinetics dependent on the rate of water uptake into the silica structures. Moreover, modification of the particle surface hydrophobicity allowed for the preparation of NO-releasing electrospun polymer fibers with improved particle incorporation and decreased particle leaching compared to fibers doped with unmodified particles. Methods for tuning particle surface chemistry (e.g., hydrophobicity) while maintaining control over size and structure will enable the optimization of such NO-release vehicles for specific therapeutic applications.

1. Introduction

Nitric oxide (NO) is produced endogenously as a regulator of numerous physiological processes including angiogenesis, wound healing, vasodilation, and the immune response [1]. As such, much research has been devoted to designing methods for delivering exogenous NO in a manner that mimics its biological generation in order to treat medical conditions such as hypertension, ischemia/reperfusion injury, thrombosis, restenosis, and cancer [2]. The major factor inhibiting the clinical success of NO-based therapeutics stems from the gaseous and reactive nature of NO that make it difficult to control its delivery both in terms of release kinetics and location.

Improved control over NO delivery can be achieved by modifying macromolecular scaffolds with NO donors including *S*-nitrosothiol and *N*-diazeniumdiolate varieties to achieve biologically relevant NO release activity [3]. For example, Zhou et al. designed polymers doped with *S*-nitrosothiol NO donor-modified silica particles to achieve NO fluxes that mimicked NO generation by endothelial cells (i.e., 1–4 × 10^{-10} mol cm^{-2} s^{-1}) [4]. The

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advantage of *N*-diazeniumdiolate (NONOate) chemistry over *S*-nitrosothiols is the ability to store two molecules of NO per amine site with subsequent spontaneous release under physiological conditions (i.e., no need for an external trigger). Unfortunately, the labile nature of the NONOate functionality in aqueous media often results in materials with short NO release durations (i.e., less than 24 h). While fast release of large NO payloads (i.e., micromolar) are sufficient for some applications, such as eradicating tumor or bacteria cells, the release of lower levels of NO (i.e., picomolar) for extended periods are required for other therapies (e.g., wound healing and treatment of cardiovascular dysfunctions) [1]. Methods for extending the NO release duration of *N*-diazeniumdiolate NO donor-based macromolecules are thus needed to broaden their therapeutic impact.

Due to the proton-initiated decomposition of *N*-diazeniumdiolate NO donors, the chemical environment surrounding the NONOate governs NO release kinetics [5]. The zwitterionic NONOate can be stabilized directly through hydrogen bonding. For example, silica nanoparticles composed of the diamine *N*-(6-aminohexyl)aminopropyl-trimethoxysilane (AHAP) exhibit slower release kinetics compared to particles composed of the monoamine *N*-methylaminopropyltrimethoxysilane (MAP) as the primary amine of AHAP can stabilize through hydrogen bonding [6]. Alternatively, protecting groups can be added to the NONOate functionality such that a rate-limiting hydrolysis step must first occur prior to NO release. Silica particles containing O^2-protected NONOates exhibited the longest NO release kinetics to date ($t_{1/2}$ = 2 weeks) [7]; however, the NO flux from these particles was well below that which is necessary for many applications. While NO release can be tuned by switching from one type of aminosilane to another, the altered NO-release kinetics are often accompanied by a drastic change in particle formation and size [8]. Indeed, exchanging one aminosilane for another may even completely inhibit particle formation. Thus, tuning NO release requires proper selection of the silane precursors and systematic study to determine the appropriate synthetic parameters. Given the strong influence of particle size on the effectiveness of NO delivery [6,9,10], the ability to tune hydrophobicity without affecting particle size would be useful synthetically.

Another strategy for prolonging NONOate lifetimes is to increase the hydrophobicity of the matrix surrounding the NO donor, thus slowing water diffusion and protonation of the *N*-diazeniumdiolate-bearing amine. For example, Koh et al. doped *N*-diazeniumdiolate-based silica particles into bulk polyurethanes of increasing hydrophobicity to achieve materials with prolonged NO release [11]. Similarly, NO-releasing silica particles were doped into electrospun polyurethane fibers as these porous scaffolds are more attractive for applications such as wound healing and implant coatings [12,13]. Unfortunately, the NONOate-based silica particles leached from the fibers upon submersion in physiological conditions (i.e., pH 7.4, 37 °C). Moreover, entrapment of the NO donor within a polymer matrix to delay NO release is often not a viable option for applications requiring direct interaction of the particles and cellular targets.

Based on the proton-initiated decomposition of *N*-diazeniumdiolates to release NO, we hypothesized that NO-release durations from silica nanoparticles could be prolonged via the surface grafting of hydrophobic functional groups. The goal of this work was thus to tune particle hydrophobicity without affecting particle size, which would be impossible using traditional core–shell type particle designs. While reports of "shell"-modified silica particles are vast, little has been reported on the surface grafting of silanes onto hybrid particles pre-formed by the co-condensation of two silane precursors or the use of such chemistry to modify NO-release kinetics. We also postulated that increasing the surface hydrophobicity of the NO-releasing particles would improve their stability in hydrophobic polymers.

2. Materials and methods

N-(6-aminohexyl)aminopropyltrimethoxysilane (AHAP), ethyltrimethoxysilane, isobutyl-trimethoxysilane, octadecyltrimethoxysilane, (heptadecafluoro-1,1,2,2-tetrahydrodecyl)-trimethoxysilane, and tetramethoxysilane (TMOS) were purchased from Gelest Inc. (Morrisville, PA). Ethanol, aqueous ammonium hydroxide solution (28 wt%), anhydrous toluene, anhydrous *N*,*N*-dimethylformamide, anhydrous tetrahydrofuran, anhydrous methanol, and sodium methoxide (5.4 M solution in methanol) were purchased from Fisher Scientific (Fair Lawn, NJ). Sodium trimethylsilanolate was purchased from Sigma–Aldrich (St. Louis, MO). Nitrogen (N_2), argon (Ar), and nitric oxide calibration (26.81 ppm, balance N_2) gases were purchased from National Welders (Raleigh, NC). Pure nitric oxide gas (99.5%) used for *N*-diazeniumdiolate formation was purchased from Praxair (Sanford, NC). Distilled water was purified using a Millipore Milli-Q UV Gradient A-10 system (Bedford, MA), resulting in a total organic content of $\leq$6 ppb and a final resistivity of 18.2 mΩ cm.

2.1. Stöber synthesis of amine-containing silica nanoparticles

Hybrid inorganic/organic silica nanoparticles were synthesized via a modified Stöber method. Briefly, AHAP (1.173 mL) and TMOS (0.708 mL) were premixed and added to a mixture of ethanol (59.16 mL), water (27.84 mL), and 28 wt% ammonium hydroxide (9.8 mL). The reaction was stirred at room temperature for 2 h. The resulting white particle precipitates were collected by centrifugation (3645 × *g*, 10 min, 4 °C) and washed thrice with ethanol to remove unreacted reagents. The particles were then dried in vacuo and stored in a sealed container until further use.

2.2. Grafting of organosilanes onto the surface of amine-containing silica nanoparticles

A suspension of AHAP silica particles (50 mg) in 10 mL toluene was prepared via sonication. Pyridine (100 μL) and an organosilane (200 μL) were added, and the reaction was refluxed overnight. An excess of organosilanes was used as the silanes were found to condense with available silanols on the glassware. The surface-modified silica particles were collected by centrifugation (3645 × *g*, 10 min, 4 °C), washed twice with toluene, then twice with ethanol. The particles were then dried in vacuo and stored in a sealed container until further use.

2.3. N-diazeniumdiolate-modification of AHAP and surface-modified AHAP silica nanoparticles

Each particle composition was suspended in a 1:9 methanol:*N*,*N*-dimethylformamide mixture via sonication at a concentration of 5 mg/mL. Sodium methoxide was then added in a 1:3 mol ratio of secondary amine to base. The mixtures were vortexed, aliquoted into 4 mL glass vials, placed in a 160 mL Parr general purpose pressure vessel, and connected to an in-house NO reactor. The solutions were stirred magnetically with removal of oxygen by purging with Ar. The vessel was then filled to a pressure of 10 bar with NO that had previously been scrubbed with KOH. After 72 h, the NO was released from the vessel, and the solutions were again purged with Ar to remove unreacted NO. The resulting *N*-diazeniumdiolate-modified silica nanoparticles were collected by centrifugation (3645 × *g*, 10 min, 4 °C), washed thrice with