

# Nitric oxide-releasing sol–gels as antibacterial coatings for orthopedic implants

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## Abstract

To assess the benefits of nitric oxide (NO)-releasing sol–gels as potential antibacterial coatings for orthopedic devices, medical-grade stainless steel is coated with a sol–gel film of 40% *N*-aminohexyl-*N*-aminopropyltrimethoxysilane and 60% isobutyltrimethoxysilane. Upon converting the diamine groups in these films to diazeniumdiolate NO donors, the NO release from the sol–gel-coated stainless steel is evaluated at both ambient and physiological temperature. Sol–gel films incubated at 25°C have a lower NO flux over the first 24 h compared to those at 37°C, but release more than five times longer. The bacterial adhesion resistance of NO-releasing coatings is evaluated in vitro by exposing bare steel, sol–gel, and NO-releasing sol–gel-coated steel to cell suspensions of *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Staphylococcus epidermidis* at 25°C and 37°C. Cell adhesion to bare and sol–gel-coated steel is similar, while NO-releasing surfaces have significantly less bacterial adhesion for all species and temperatures investigated.

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**Keywords:** Nitric oxide; Sol–gel; Stainless steel; *Pseudomonas aeruginosa*; *Staphylococcus aureus*; *Staphylococcus epidermidis*

## 1. Introduction

Bacterial infection due to an implanted medical device is a potentially serious complication, typically leading to premature implant removal [1]. Although the removal of a vascular catheter is not difficult, orthopedic implant removal procedures are costly, traumatic to the patient, and potentially lethal. With the increased use of orthopedic devices to nearly one million per year for hip replacements alone, [1] the incidence of infected implants will likely increase.

Sterilization, meticulous surgical procedure, and proper infection control guidelines greatly diminish the likelihood of infection. Despite these preventative measures, invasive bacteria can be found at ~90% of implantation sites immediately after surgery [2,3]. Complicating matters, the procedure damages neighboring tissue, leaving the implant environment susceptible to the colonization of infectious organisms [1]. Trans-

dermal devices, such as catheters and bone screws for external fixators, pose an additional infection risk from skin flora creeping into the skin lesion around the implant [1].

Virulent bacteria typically secrete polymeric materials after association to form protective coatings known as biofilms. The biofilm further impedes the activity of the host defenses and antibiotic therapy, making implant removal the only effective option [1]. The staphylococci species including *Staphylococcus aureus* and *Staphylococcus epidermidis* are responsible for the majority of biofilms found on explanted orthopedic devices [4,5]. *Pseudomonas aeruginosa* and other gram-negative species have also been identified in implant infections, but are less frequent [6].

Numerous localized methods have emerged to address the concerns associated with orthopedic implant infections. While antibiotic-doped bone cements (e.g., polymethylmethacrylate, acrylic cement) [7,8] and wound irrigation [9] with antibiotic solution reduce the risk of infection, the occurrence of infected implants is still of concern. Furthermore, the use of antibiotics as a preventative measure is questionable because of the

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high occurrence of antibiotic resistance among bacteria and the risk of developing more resistant bacteria. The addition of immunoglobulins to surgical lavage has demonstrated potential as a supplementary treatment, but in vivo testing has demonstrated its limited benefit [10]. Alternative methods to further diminish the risk of implant infections are clearly necessary.

A localized, persistent concentration of nitric oxide (NO) in the vicinity of an invasive medical device may prove to be a novel approach for reducing the likelihood of implant-associated infection. An early microbiological study demonstrated that NO gas destroys plated colonies of bacteria [11]. NO has also been discovered as an important mediator in the immune response. The arginine-dependant synthesis of NO by activated macrophages create both cytotoxic and cytostatic effects on pathogenic organisms including tumor cells, [12–15] protozoa, [16,17] fungi, [18] and bacteria [19–21]. These deleterious effects are typically the results of oxidative and nitrosative stress generated by reactive intermediates of NO (e.g., peroxynitrite and dinitrogen trioxide) that may result in damage to DNA, proteins, and/or cells [22]. Contrary to more conventional pharmaceutical therapies generally used to reduce bacterial adhesion and related infection, NO's half-life in vivo is on the order of seconds, [23] thereby confining the effects of NO to the immediate surrounding of the implant and avoiding systemic toxicity concerns.

Sol–gel coatings capable of NO release have recently been shown to decrease bacterial adhesion [24,25]. The NO-releasing surface is prepared by incorporating diamine-containing organosilanes into a sol–gel matrix. Upon exposure to high pressures of NO (g), the diamine coordinates two molecules of NO to form a NO donor molecule known as a diazeniumdiolate [26–28]. When the sol–gel is introduced into an aqueous environment, the diazeniumdiolate decomposes to NO and the diamine precursor. The local surface flux of NO generated from these sol–gel materials significantly reduces the adhesion of *P. aeruginosa* up to 95%, demonstrating that NO release may represent a new class of antibacterial biomaterials [25].

Sol–gels are easily manipulated and applied to a broad range of substrates because of their chemical versatility. Herein, NO-releasing sol–gels are examined as antibacterial coatings for orthopedic applications using medical-grade stainless steel. The antibacterial effectiveness of these coatings is evaluated with *P. aeruginosa*, *S. aureus*, and *S. epidermidis*.

## 2. Methods and materials

Distilled water was purified with a Millipore Milli-Q Academic System (Billerica, MA). Final resistivity of the ultrapure water was 18.2 M $\Omega$ /cm with a total organic

content of <5 ppb. Butyltrimethoxysilane (iso form; BTMOS) was purchased from Aldrich (St. Louis, MO). *N*-(6-aminohexyl)-aminopropyltrimethoxysilane (AHAP3) was purchased from Gelest (Morrisville, PA). Silanes were stored under nitrogen and used as received. Polished steel (316L) slides (25 × 8 mm) were a gift from Orthofix (Winston-Salem, NC). NO and argon (Ar) were purchased from National Welders (Raleigh, NC). *P. aeruginosa* (ATCC #19143), *S. aureus* (ATCC #29213), and *S. epidermidis* (ATCC #22322) were acquired from American Type Culture Collection (Manassas, VA). A BacLight Live/Dead cell viability fluorescence kit was obtained from Molecular Probes (Eugene, OR). All other chemicals were purchased through Fisher Scientific (St. Louis, MO) and used as received.

### 2.1. AHAP3 sol–gel synthesis

Sol solutions were prepared by mixing 240  $\mu$ l BTMOS, 160  $\mu$ l AHAP3, 500  $\mu$ l ethanol (EtOH), 120  $\mu$ l H<sub>2</sub>O and 20  $\mu$ l 0.5 M hydrochloric acid (HCl). A two-step synthesis was used by initially mixing the EtOH, H<sub>2</sub>O, HCl, and BTMOS for 1 h. AHAP3 was subsequently added to the solution and stirred for 1 h. Stainless-steel slides were cleaned with a BioForce UV-cleaner (Ames, IA) for 30 min prior to use. Sol–gel films were prepared by casting 60  $\mu$ l of the solution onto the slides. The films were allowed to solidify in a hood for ~15 min, and then dried at 70°C for 3 days. Resulting sol–gels were stored in a desiccator at 25°C until used.

### 2.2. NO-donor formation

Diazeniumdiolates were synthesized by placing the sol–gel-coated stainless-steel slides in a 500 ml in-house NO reactor [26]. Prior to introducing NO, the reaction chamber was flushed with 5 atm Ar to remove atmospheric oxygen and water. NO stored in a vessel containing potassium hydroxide (to remove NO degradation products) was then introduced into the reaction chamber at 5 atm for 2.5 days. Residual NO was removed by flushing the reaction chamber by repeating the Ar rinsing procedure as described above. NO donor-modified sol–gel films were stored in individual vials purged with nitrogen, and stored at –20°C until used. These storage procedures were necessary since atmospheric water and ambient temperature initiate diazeniumdiolate decomposition [29].

### 2.3. Sol–gel stability

Sol–gel-coated stainless-steel slides were placed in 5 ml of PBS and incubated at either 25°C or 37°C. At fixed intervals, the samples were removed and transferred to fresh solutions of PBS. Silicon (Si) concentration in the soak solutions was determined using a direct

current plasma optical emission spectrometer (DCP; ARL-Fisons Spectraspan 7; Beverly, MA). The instrument was calibrated with 0–50 ppm standard Si solutions in PBS. The percent fragmentation was calculated based on the total mass of Si in the soak solutions and the mass of the cast sol–gel.

#### 2.4. Characterization of NO release

NO release from diazeniumdiolate-modified sol–gel films was characterized using a chemiluminescent NO analyzer (NOA; Sievers Model 280; Boulder, CO). Diazeniumdiolate-modified sol–gels were immersed in 10 ml of 25°C and 37°C deoxygenated PBS solutions (pH 7.4). Solutions were purged continuously with 300 ml/min nitrogen (wet) gas. NO release was measured at an instrument collection flow rate of 200 ml/min. Overflow was monitored with a flow meter. Measurements were performed at discrete intervals until the NO release was undetectable. Sol–gel films were stored in 5 ml of PBS between measurements.

#### 2.5. Bacterial adhesion

*P. aeruginosa*, *S. aureus*, and *S. epidermidis* were cultured at 37°C in tryptic soy broth (TSB), pelleted, rinsed with ultrapure water, resuspended in 15% glycerol, and stored at –80°C. Cultures were grown from a –80°C stock in TSB for 12 h at 37°C. A 1 ml aliquot of the cell culture was inoculated into 200 ml of TSB and incubated for ca. 6 h at 37°C until a  $\sim 10^8$  cfu/ml culture was obtained. Mature cultures were centrifuged and resuspended in PBS. BacLight fluorescent probes were added to this bacterial suspension for 20 min. The labeled cells were pelleted, rinsed, and resuspended in PBS. Viable cell counts through serial dilutions of the final cell suspension and cell plating on tryptic soy agar were performed to ensure the cell concentrations were consistent between experimental sets.

Bare steel, sol–gel-coated steel, and diazeniumdiolate-modified sol–gel-coated steel were first incubated in PBS at 25°C for 30 min to initiate steady NO release. The preconditioned samples were immersed into 5 ml solutions of the labeled cell suspension at 25°C and 37°C for 30 min with gentle shaking, then removed and rinsed gently with DI water. Fluorescent optical micrographs were obtained with a Zeiss Axiovert 200 inverted microscope (Chester, VA) equipped with a Syto 9 filter set from Chroma (Brattleboro, VT). Images from at least five different regions ( $870 \times 430 \mu\text{m}^2$ ) on three different sol–gel films were acquired with a Zeiss AxioCam (Chester, VA). Images were digitally processed by thresholding the cells from the background to obtain bacterial coverage values as percentage of cellular fluorescence in the micrograph. Statistical differences

between groups were evaluated using single-tailed analysis of variance testing. Experimental sets were considered significantly different at  $p < 0.01$ .

### 3. Results and discussion

#### 3.1. Sol–gel characteristics and stability

The development of sol–gel materials capable of diverse NO release has previously been reported [25,30]. The AHAP3/BTMOS sol–gel system was chosen as a candidate for orthopedic coatings because of its consistent film quality, and the amount and longevity of NO release. NO fluxes  $> 1 \text{ pmol cm}^{-2} \text{ s}^{-1}$  can be achieved for periods greater than 14 days. As the percentage of NO donor in the sol–gel is increased above 40%, improved release properties are obtained but film stability is compromised. On glass substrates, the sol–gel often delaminates after only a few days of solution incubation regardless of the NO donor amount. For stainless-steel substrates, however, delaminating of the film is not observed even at PBS incubation periods greater than 2 months.

Although AHAP3/BTMOS sol–gels bind more strongly to stainless-steel substrates, material fragmentation in aqueous media is still a concern for biomedical applications. The extent of sol–gel fragmentation in PBS was thus determined from silicon DCP analysis of the sol–gel soak solutions. As shown in Table 1, sol–gels incubated at higher temperatures (37°C vs. 25°C) are slightly less stable and leach greater amounts of Si during the initial 3 days. Over 14 days, the AHAP3/BTMOS sol–gels continue to degrade slightly (3.5 and 5.2  $\text{nmol cm}^{-2}$  at 25°C and 37°C, respectively). Notably, this Si leakage accounts for only ca. 0.01% of the total Si content in the sol–gel films at both ambient and physiological temperatures.

Sol–gel surface chemistry changes after diazeniumdiolate formation may influence bacterial affinity for the coating, and obscure the importance of NO release. The effect of NO exposure has previously been characterized using water contact angle measurements [25,30]. Similar to previous studies, the wettability of the sol–gel surface did not change after exposure to high pressures of NO. The similar water contact angles between control and

Table 1  
Silicon fragmentation of sol–gel films

Temp (°C)	Fragmented Si content ( $\text{nmol/cm}^2$ ) <sup>a</sup>			Total 14 days
	0–3 days	3–7 days	7–14 days	
25	$0.4 \pm 0.1$	$1.2 \pm 0.1$	$1.9 \pm 0.1$	$3.5 \pm 0.1$
37	$2.8 \pm 0.2$	$1.2 \pm 0.3$	$1.2 \pm 0.3$	$5.2 \pm 0.9$

<sup>a</sup> Calculated from Si DCP results from sol–gel soak solutions.

NO-releasing films ( $\sim 90^\circ$ ) is attributed to the organic component of the supporting BTMOS sol–gel matrix and justifies that any variation in bacterial adhesion is due to NO release.

### 3.2. NO release

The NO release properties of AHAP/BTMOS sol–gels are investigated at both  $25^\circ\text{C}$  and  $37^\circ\text{C}$  to model a transdermal orthopedic implant that resides outside and inside of the body. As shown in Fig. 1, significant differences in the NO flux from sol–gels are observed at these temperatures. The initial NO flux at  $25^\circ\text{C}$  is relatively low at  $42.0\text{ pmol cm}^{-2}\text{ s}^{-1}$ . The flux decreases exponentially over the initial 24 h to  $13.1\text{ pmol cm}^{-2}\text{ s}^{-1}$ . Beyond 24 h, the NO flux continues to diminish to 1.6 and  $0.2\text{ pmol cm}^{-2}\text{ s}^{-1}$  at 13 and 48 days, respectively. Of note, the NO flux values at  $25^\circ\text{C}$  for 40% AHAP3/BTMOS films are greater than those reported earlier because twice the amount of sol–gel has been cast on the steel substrate. The relationship between the amount of sol–gel cast and NO release is described by Marxer et al. [30].

The NO release for these sol–gel films at  $37^\circ\text{C}$  is more exponential. The initial NO flux is greater than at  $25^\circ\text{C}$  ( $190.8\text{ pmol cm}^{-2}\text{ s}^{-1}$ ). At 24 h, the NO flux at the different temperatures is analogous, with sol–gels incubated at  $37^\circ\text{C}$  releasing  $13.5\text{ pmol cm}^{-2}\text{ s}^{-1}$ . After the initial 24 h, sol–gels incubated at  $37^\circ\text{C}$  release less NO than those films at  $25^\circ\text{C}$ . Negligible NO flux is detected beyond 7 days at  $37^\circ\text{C}$ .

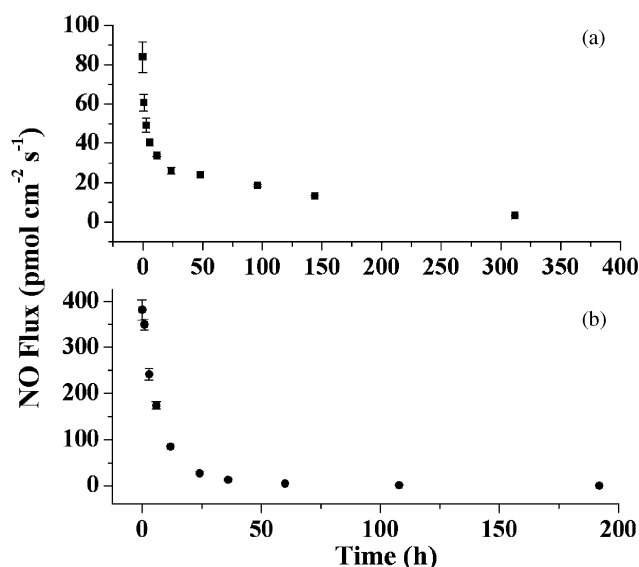


Fig. 1. NO release from 40% AHAP3 sol–gel coatings on stainless steel. NO flux was measured at: (a)  $25^\circ\text{C}$ , and (b)  $37^\circ\text{C}$ . Time and flux scales are expanded to depict the NO release profiles at each temperature.

The diazeniumdiolate dissociation rates change considerably with temperature. The total moles of NO released is also influenced by temperature. Total NO release was determined by integrating the NO release curves. The 40% AHAP3/BTMOS sol–gels yield 22.7 and  $13.1\text{ }\mu\text{mol}$  at  $25^\circ\text{C}$  and  $37^\circ\text{C}$ , respectively. By calculating the number of diamine moieties from sol–gel casting and assuming a NO:diamine ratio of 2, the theoretical amount of total NO release is estimated to be  $73.2\text{ }\mu\text{mol}$ . The NO release efficiency for 40% AHAP3 at  $25^\circ\text{C}$  is thus 31%, suggesting that only a third of the available diamines in the film are converted to NO donors. Two plausible explanations for the lack of NO release include (1) not all diamines are converted to diazeniumdiolates during NO exposure due to diamine inactivity or NO inaccessibility; and, (2) the diazeniumdiolates are oxidized to other byproducts (e.g., nitrosoamines, nitrite) during NO release measurement. The NO release efficiency decreases to 18% when the temperature is increased to  $37^\circ\text{C}$ . A reasonable rationalization for the further loss of NO release is enhanced diazeniumdiolate oxidation at higher temperatures, thus increasing the rate of byproduct formation. Investigations to examine and improve the NO release efficiency are currently underway.

The data presented above are for sol–gels stored at  $-20^\circ\text{C}$  for  $<6\text{ h}$ . The effect of long-term storage on NO-releasing films was investigated to examine the practical implementation of these coatings. When stored at  $-20^\circ\text{C}$ , no significant deviations in the extended NO release are observed through 28 days of storage, the longest period studied (data not shown). Therefore, NO-release coatings could be preapplied to the medical device, without the necessity of on-site NO.

### 3.3. Preventing *P. aeruginosa* adhesion

In a previous report, the resistance to *P. aeruginosa* adhesion of NO-releasing sol–gels was characterized using a number of sol–gel formulations [24,25]. Herein, AHAP3/BTMOS sol–gel is applied to a stainless-steel substrate to investigate the potential of NO-releasing coatings to reduce the likelihood of bacterial adhesion to steel-based orthopedic implants. The bacterial adhesion experiments are performed at both  $25^\circ\text{C}$  and  $37^\circ\text{C}$  to mimic conditions of a transdermal orthopedic device (e.g., external fixator bone screws).

The bacterial adhesion resistance of bare and sol–gel-coated steel substrates is investigated by exposing samples to high concentrations of *P. aeruginosa* suspensions (ca.  $10^8\text{ cfu/ml}$ ). These bacterial concentrations are far greater than those an implant would encounter in the surgical theatre. Adhesion of *P. aeruginosa* to bare stainless steel is characterized by an integrated network of cells that cover roughly half of the surface, regardless of temperature (Fig. 2a and c). The NO-releasing sol–gel

coating significantly decreases the bacterial adhesion observed (Fig. 2b and d). The bacterial adhesion of NO-releasing surfaces at ambient temperature is lower, and characterized by little to no cell aggregation, while a greater amount of cell aggregation is observed at 37°C.

To semi-quantitatively relate the differences in *P. aeruginosa* adhesion, surface coverage values were computed from the fluorescent images of bare, sol-gel-coated, and NO-releasing sol-gel-coated stainless steel (Table 2). Variation in bacterial adhesion between the bare and sol-gel-coated steel was expected because of the difference in water contact angle (ca. 70° and 90°, respectively). Despite the change in surface wettability, bare and sol-gel-coated stainless-steel samples are characterized by similar amounts of bacterial adhesion

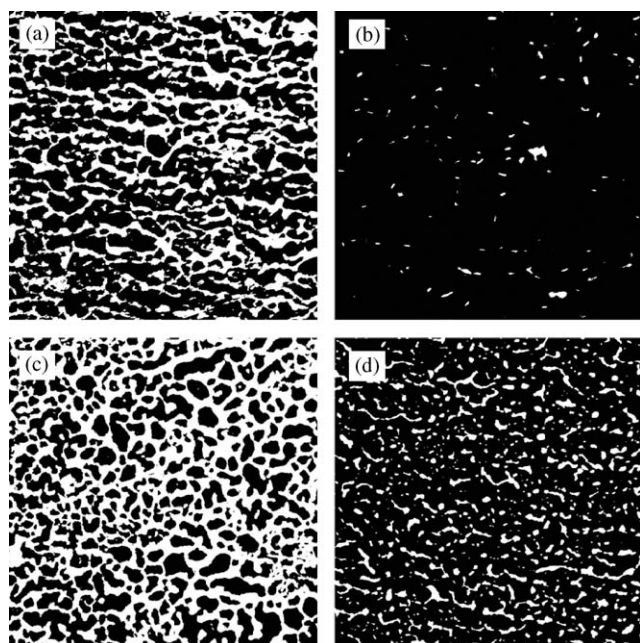


Fig. 2. Fluorescent images of *P. aeruginosa* adhesion to: (a,c) bare steel, and (b,d) NO sol-gel coatings. Adhesion performed at: (a,b) 25°C, and (c,d) 37°C. Cells are white. Images are 300 × 300 μm<sup>2</sup>.

at different temperatures (37 ± 18% at 25°C and 45 ± 12% at 37°C). Although the average bacterial surface coverage for bare and sol-gel-coated substrates at 37°C appear to be slightly greater than that observed at 25°C, large variations in the measurements render no significant difference between stainless steel and control coatings. For sol-gels capable of NO release, the bacterial adhesion coverage decreases to 5 ± 5% at 25°C. At 37°C, the *P. aeruginosa* coverage on the NO-releasing films is slightly greater (12 ± 8%) but still noticeably less than controls. The reduction in bacterial adhesion is significant for both temperatures ( $\rho \ll 0.01$ ), indicating that the NO-release coating is more resistant to bacterial biofouling than bare stainless steel. Despite the increased NO release, however, a significant increase in bacterial adhesion to NO-releasing coatings at 37°C ( $\rho \ll 0.01$ ) is observed. This is surprising since previous studies have shown that bacterial surface coverage decreases with increasing NO release [24]. Conceivable hypotheses for this include (1) an increase in the reaction rate of NO with oxygen in aerobic media may convert more of the NO to NO<sub>x</sub><sup>-</sup> byproducts (i.e., nitrites and nitrates), thus lowering the effective surface concentration of NO; (2) a threshold NO flux exists for the resistance of *P. aeruginosa* adhesion and additional NO release is extraneous; and/or, (3) *P. aeruginosa* is more effective at catabolizing NO into inactive byproducts at 37°C. Experiments aimed at better understanding both the mechanism of NO release from sol-gel-bound diazeniumdiolates and NO's effect on bacteria are currently underway.

### 3.4. Resisting staphylococcal adhesion

Although *P. aeruginosa* is a medically relevant species, the *Staphylococcus* genus is responsible for the majority of orthopedic implant infections [4,5]. Thus, the impact of NO-releasing coatings on *S. aureus* and *S. epidermidis* adhesion was evaluated.

Table 2  
Bacterial adhesion to stainless steel substrates

Bacterial species	Temp (°C)	Bacterial coverage (%) <sup>a</sup>			
		Bare steel	Sol-gel	NO release	$\rho$ -value <sup>b</sup>
<i>Pseudomonas aeruginosa</i>	25	36.5 ± 18.9	37.3 ± 18.7	4.7 ± 4.6	2 × 10 <sup>-13</sup>
	37	44.9 ± 13.8	45.1 ± 11.8	11.6 ± 7.6	6 × 10 <sup>-15</sup>
<i>Staphylococcus aureus</i>	25	7.1 ± 2.9	4.8 ± 1.3	1.7 ± 1.7	2 × 10 <sup>-12</sup>
	37	9.0 ± 5.0	4.2 ± 2.5	0.6 ± 0.4	9 × 10 <sup>-11</sup>
<i>Staphylococcus epidermidis</i>	25	1.8 ± 0.9	3.1 ± 1.7	0.3 ± 0.3	6 × 10 <sup>-11</sup>
	37	2.1 ± 0.9	2.6 ± 1.2	0.3 ± 0.2	1 × 10 <sup>-14</sup>

<sup>a</sup> Computed from threshold optical micrograph to quantify cell coverage areas ( $n = 15$ ).

<sup>b</sup> ANOVA results comparing bare steel to NO-releasing coatings.  $\rho < 0.01$  is considered significantly different.

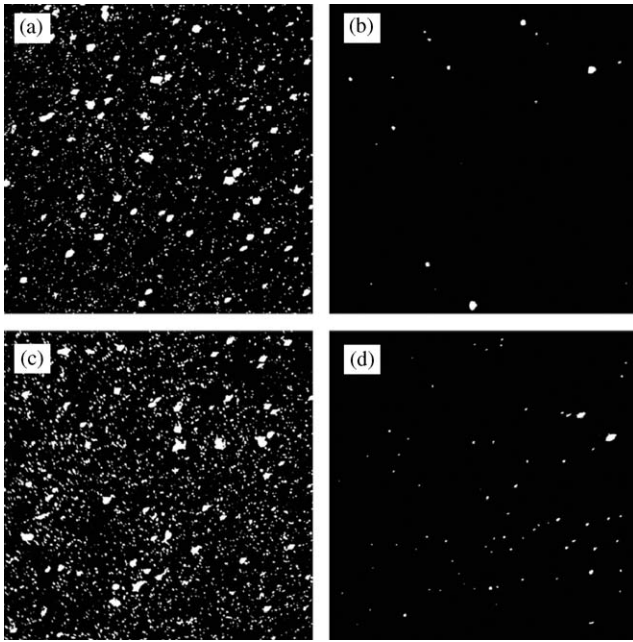


Fig. 3. Fluorescent images of *S. aureus* adhesion to: (a,c) bare steel, and (b,d) NO sol-gel coatings. Adhesion performed at: (a,b) 25°C, and (c,d) 37°C. Cells are white. Images are  $300 \times 300 \mu\text{m}^2$ .

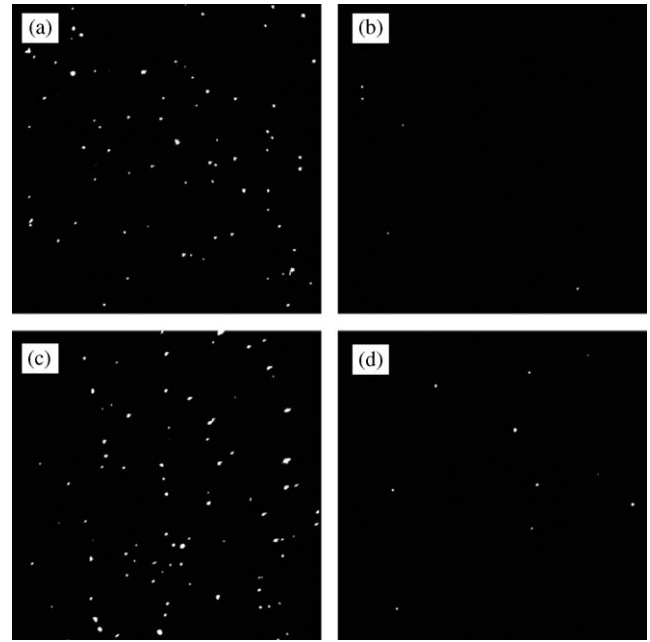


Fig. 4. Fluorescent images of *S. epidermidis* adhesion to: (a,c) bare steel, and (b,d) NO sol-gel coatings. Adhesion performed at: (a,b) 25°C, and (c,d) 37°C. Cells are white. Images are  $300 \times 300 \mu\text{m}^2$ .

The trends observed with *S. aureus* adhesion to bare and NO-release coated stainless steel are similar to those of *P. aeruginosa*. Bare steel samples are characterized by a large degree of cellular adhesion at both 25°C and 37°C (Fig. 3a and c). The bacteria are distributed evenly across the surface with some sites of cellular aggregation. A clear decrease in *S. aureus* adhesion is observed for NO-releasing surfaces at both temperatures (Fig 3b and d). NO release effectively increases the substrate resistance of *S. aureus* adhesion (Table 2). Steel surface coverage for *S. aureus* adhesion is five times less than *P. aeruginosa* with ca. 7–9% coverage at 25°C and 37°C. Notably, the blank 40% AHAP3 coating appears to resist bacterial adhesion significantly more than bare stainless steel. The cell coverage is reduced from 7–9% to less than 5% at both temperatures ( $\rho < 0.01$ ). This decrease in bacterial adhesion is further improved with NO release, lowering the average *S. aureus* surface coverage to 1.7% and 0.6% for 25°C and 37°C, respectively ( $\rho \ll 0.01$ ). The NO release coating imparts a greater resistance against *S. aureus* adhesion to stainless steel with the addition of both the sol-gel and NO release.

*S. epidermidis* has a poor affinity for all of the surfaces studied here (Fig. 4). The bacteria are dispersed evenly across the bare steel samples with some sites of cellular aggregation (Fig. 4a and c). *S. epidermidis* coverage values for bare steel and the sol-gel coating are equally low (ca. 2–3%). Despite the low overall coverage on bare steel, a substantial reduction in *S. epidermidis* adhesion is observed for NO-releasing sol-gels (Fig. 4b

and d). The bacterial surface coverage decreases to 0.3% for 25°C and 37°C ( $\rho \ll 0.01$ ) in the presence of NO release. Although polished stainless steel performs satisfactorily as a biomaterial against *S. epidermidis* adhesion, NO release improves the resistance against *S. epidermidis* even more. Such enhancement may further diminish the likelihood of implant-associated infections.

#### 4. Conclusion

An effective method for improving the bacterial adhesion resistance of stainless-steel materials through the utilization of a sol-gel-derived NO-releasing coating is reported. The adhesion capabilities of *P. aeruginosa*, *S. aureus*, and *S. epidermidis* are diminished in the presence of a low-level, surface-localized NO flux at both ambient and physiological temperature. The degree of bacterial adhesion is not temperature dependent for the *Staphylococcus* species. However, the NO release effectiveness is hindered at 37°C for *P. aeruginosa*. The amount of adhesion to the bare steel and the control sol-gel-coated steel is nearly identical for *P. aeruginosa* and *S. epidermidis*, while the blank sol-gel coating appears to present a favorable passive barrier to *S. aureus* adhesion. Therefore, the addition of the sol-gel coating does not adversely affect the bacterial adhesion resistance properties of stainless steel. When the majority of NO has been released from the coating, bacterial adhesion to the NO-depleted sol-gel is similar to control sol-gel films, [30] exemplifying the utility of NO as an antibacterial agent.

With excellent material stability and increased bacterial biofouling resistance, NO-releasing sol–gel coatings may prove to be a unique method for reducing the risk of orthopedic implant infections. Since the resistance to bacterial adhesion extends from ambient to body temperature, NO-releasing coatings may prove to be a protective method for transdermal medical implants, where infection due to environmental wound exposure is of high risk.

Although these results encourage the use of NO as an antimicrobial for medical devices, the implications of NO release on the neighboring tissue remains a concern. Since the late 1980s, NO has been found to be an important mediator in numerous mammalian biological processes, including neural transmission, [31,32] vasodilatation, [33] inflammation, [34,35] septic shock, [36,37] and apoptosis [38,39]. Although NO has a short half-life in vivo, [23] localized influences of NO are expected. In an implant coating model, the impact of NO release on the inflammation and wound healing process is of primary interest. During these processes, the vascular endothelium and immunological cells release NO in response to the injury. The consequence of increased NO exposure from actively releasing NO coatings may either benefit the site by enhancing the efficiency of wound healing (e.g., increase migrations of immunological cells, [35] promote angiogenesis, [40,41] sustain an antimicrobial environment [19–21]) or be detrimental (i.e., cytotoxic) to neighboring tissue [42–44]. We are currently investigating both the in vitro and in vivo impact of NO-releasing sol–gel films to elucidate the host response to such coatings.

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