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Supporting Information

Facile solvent-free fabrication of nitric oxide releasing coating for prevention of biofilm formation

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Experimental details:

Materials:

Allylamine (98%) and diallylamine (99%) were obtained from Sigma-Aldrich and used without further purification. Griess' reagent kit G7921 (Molecular Probes, Inc, Eugene, OR) was purchased from ThermoFisher Scientific. Silicon wafers (1 × 1 cm^2) were obtained from M.M.R.C. Pty Ltd, Malvern Australia. The wafers were cleaned with RBS solution in a sonicator bath for 30 min followed by thorough rinsing with MilliQ water and dried prior to use. Nitric oxide gas (99.9%) was purchased from Linde Gas and used without further purification.

Methods:

Allylamine and diallylamine plasma treatment

Allylamine and diallylamine plasma coatings were obtained using a custom built reactor according to a previously established procedure.[1] Briefly, 1 × 1 cm^2 silicon wafers were placed on the lower rectangular electrode of the plasma reactor in a glass chamber (height = 350 mm, diameter = 170 mm). The distance between the lower and upper electrodes was 150 mm. The allylamine or diallylamine monomer was degassed 5 times prior to deposition. The deposition was carried out twice for 25 s with an initial pressure of 0.2 mbar (200 kHz, 20 W).

Nitric oxide (NO) treatment

The untreated and plasma-treated silicon wafers were placed in a Parr apparatus. The apparatus was sealed and then purged and evacuated with nitrogen gas three times, followed by charging with excess NO gas at 25 °C at 5 atm for 48 h. After 48 h, the excess NO was vented by purging with nitrogen and the samples were kept under vacuum for a minimum of 3 days before further use.

Determination of NO release

The release of NO from the treated samples was measured using a Griess reagent kit. Diazeniumdiolates readily release NO upon contact with water at physiological pH. The samples to be tested were placed individually in glass sample tube with 1 mL PBS (pH 7.4) and the sample tubes were sealed. The samples were incubated in room temperature (25 °C) or at 37 °C for 1, 24, 48, 72 or 168 h. After incubation, 150 µL of the solution was placed in 96-well plate in triplicate, followed by the addition of

130 μL MilliQ water and 20 μL of freshly made Griess reagent consisting equal volume of component A and B. The solution was then incubated in the dark at room temperature at 500 rpm for 30 min. Nitrite concentrations were then measured using an UV-vis spectrometer at 548 nm. A standard curve was prepared using dilutions of sodium nitrite between 0-100 μM in PBS (pH 7.4, ambient temperature). The amount of NO (or nitrite) release was calculated by extrapolation from the standard curve using the measured absorbance. Experiment was performed in triplicate and repeated in 3 independent experiments.

Antimicrobial activity

Staphylococcus aureus strain 38 and *Pseudomonas aeruginosa* PAO1 were used for this study. These strains were streaked onto Luria Bertani (LB) agar and incubated overnight at 37 °C. A single colony was cultured overnight in 10 mL of tryptone soya broth (TSB; Oxoid, UK) medium at 37 °C. The resulting bacteria were collected by centrifugation and re-suspended in the same volume of TSB twice. Optical density of the resulting culture was adjusted to OD660 = 0.1 (10^8 CFU/mL) in TSB. The samples to be tested were disinfected with 70% w/v ethanol/water and air-dried before being placed individually in 12-well plates, followed by addition of 3 mL of the bacterial suspension. The samples were placed in an upright position to avoid bacterial deposition due to gravity. The plates were incubated at 37 °C for 18 h. The surfaces were then gently rinsed twice with phosphate buffered saline (PBS) to remove non-adherent bacteria before examination by fluorescent microscopy.

The samples with adhered bacteria prepared as described above were stained with Live/Dead BacLight Bacterial Viability Kits L-7007 (Molecular Probes, Inc, Eugene, OR) according to the manufacturers' procedure. Briefly, 2 μL of the two components were mixed thoroughly in 1 mL of PBS. 10 μL of the solution was then trapped between the sample and a glass microscopy slide and allowed to incubate at room temperature in the dark for 10 min. The samples were observed and imaged with an Olympus FV1200 Confocal Inverted Microscope. For bacterial adhesion, images from 10 representative areas on each of triplicate samples for each surface were taken. Cells that were stained green were considered to be viable, those that stained red were considered to be dead as were those that stained both green and red.

All confocal images were analyzed using ImageJ software, which measured the area fraction covered by green (live) or red (dead) cells in the field of view. The image analysis results were reported as the average percentage coverage of live cells and dead cells in the fields of view. The results are mean values of three independent experiments.

Statistical analysis

Data were analyzed by the one-way analysis of variance (ANOVA) using IBM SPSS Statistics software (version 22). Differences between the groups were analyzed using post hoc Dunnett's T3 test, and results with $p < 0.05$ were considered significant.

Equipment:

X-Ray Photoelectron Spectroscopy (XPS)

Surfaces were analyzed using X-ray photoelectron spectroscopy (XPS; ESCALAB220-iXL, VG Scientific, West Sussex, England). The X-ray source was monochromated Al Kα and the photo-energy was 1486.6 eV with a source power of 120 W. Vacuum pressure was ≤ 10^{-8} mbar.

Profilometry

Film thickness was measured on allylamine or diallylamine plasma coated Si wafers via stylus profilometry. Prior to measurements, the edge of a fine spatula was used to produce a scratch across the Si wafer, whereby the coating was removed in a line without any damage to the wafer surface. A Veeco Dektak 6M stylus profilometer was used for the thickness measurements. Briefly, the force of the stylus (having a diameter of 12.5 µm) was set to 3 mg and traced across a distance of 500 µm over 15 seconds. Average values are reported from at least 3 repeats per sample.

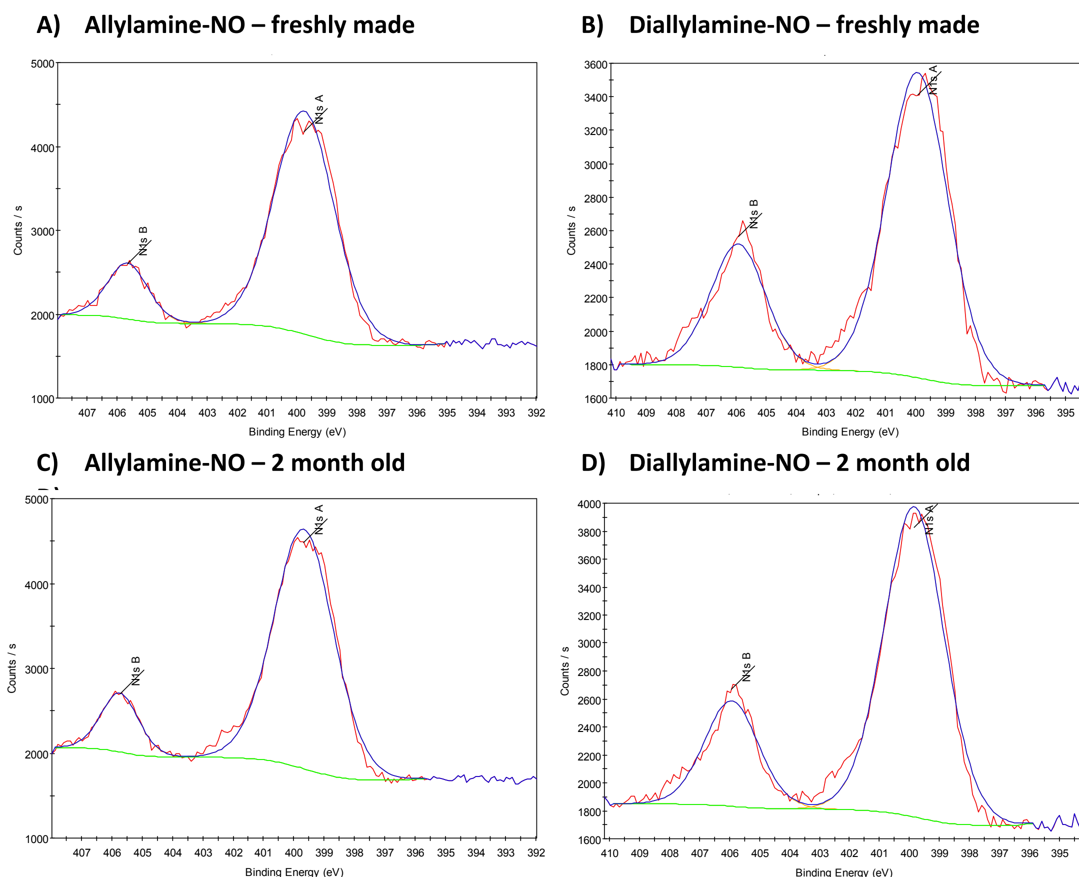

Fig. S1 XPS high resolution N 1s spectra of allylamine-NO and diallylamine-NO samples that were freshly made (A and B) and samples that were kept in ambient conditions for 2 months (C and D). Little to no difference was observed between the freshly made and 2 month old samples.

Reference:

1. Ozcelik, B., K.K.K. Ho, V. Glattauer, M. Willcox, N. Kumar, and H. Thissen, ACS Biomater. Sci. Eng., 2017. **3**(1): p. 78-87.