# Supporting Information

## Experimental

### Sample preparation of NDs

NDs with a mean particle size of 150 nm (PolyQolor, ND-150, HPHT) were surface oxidized in air at 500 °C for 1 h. The result NDs were dispersed in distilled water and sonicated for 1h to obtain colloidal dispersion. After sonication, the NDs were purified with distilled water three times by centrifugation of 10,000 rpm for 10 min, and the purified NDs were re-dispersed in 0.01 M phosphate buffer saline (PBS) and sonicated for 10 min to obtain (0.02 – 10 mg/mL) HPHT NDs buffer for further study. The NDs surface might need to be modified to get stable dispersion in buffers. However, we aim to investigate the NDs in their intrinsic status (mostly common form), i.e., air-oxidized surface, and thus don't introduce any other surface modification. One can expect that any of the surface modification might introduce additional effects to the planktonic cells and biofilms, although the stability of NDs could be improved.

### Characterizations of NDs

The crystallinity and morphology of NDs were analyzed using X-ray Powder Diffraction (XRD, D8 Advance, Bruker), transmission electron microscopy (TEM, FEI Tecnai G2 20 S-TWIN, USA) and scanning electron microscopy (SEM, Hitachi S-4800, Japan). The dynamic light scattering (DLS) size distribution and the zeta potential of NDs (0.02 mg/mL of NDs in 0.01 M PBS) were recorded with a Malvern Zetasizer (Malvern Instruments Ltd., UK). For the Fourier transform infrared (FTIR) spectroscopy measurements, the dried powder samples were placed on top of the diamond prism of the FTIR-ATR (Attenuated total reflectance) spectrometer (PerkinElmer, Spectrum Two, USA) and measured in the 400−4,000 $cm^{-1}$ range at 1 $cm^{-1}$ resolution. The wide-field fluorescence image of the NDs was measured on a home-built wide-field fluorescence microscope. To simplify, a continuous-wave 532 nm laser was used for the excitation of the NV centers in NDs, and an oil immersion objective (Olympus, UAPON100XOTIRF, 1.49 NA) was used for both the excitation and fluorescence collection. The NV fluorescence was filtered by a long pass 647 nm detection filter and detected by an EMCCD camera (Photometrics, Evolve 512 Delta). And the fluorescence spectrum of the NDs was also measued using the same wide-field set-up that equipped with a spectrometer (Thorlabs).

### Coating performance of the NDs on substrate

200 µL of NDs solutions with different concentrations (10, 7.5, 5, 2.5, 1.25, and 0.625 mg/mL) were coated on chamber slides and dried overnight to determine their coating performance on substrate.

### Microbial strains and culture conditions

### Culture maintenance

*Candida albicans* SC5314, *Candida glabrata* ATCC2001, *Porphyromonas gingivalis* ATCC33277 and *Streptococcus mutans* ATCC700610 were acquired from American Type Culture Collection (ATCC). *C. albicans* and *C. glabrata* were maintained in Sabouraud

Dextrose Agar (SDA) at 37 °C in aerobic incubator, while *S. mutans* was maintained in Columbia Horse Blood Agar (CHBA) and *P. gingivalis* was maintained in CHBA supplemented with 0.5 mg/mL of Hemin and 10 mg/mL of Vitamin K at 37 °C in an anaerobic chamber (85% $N_2$, 10% $H_2$, 5% $CO_2$).

**Inoculum preparation**

For all experiments, the *Candida* species were grown overnight in Yeast-Peptone Dextrose (YPD) broth at 37 °C in aerobic conditions. Prior to each experiment, the $OD_{520}$ was adjusted to 0.327-0.351 to obtain a final concentration of $1.5 \times 10^7$ CFU/mL cells. *P. gingivalis* was grown for 72 h in Tryptic Soy Broth (TSB) supplemented with 5 g/L of Yeast extract, 0.5 mg/mL of hemin and 10 mg/mL of Vitamin K, while *S. mutans* was grown overnight in Brain-Heart Infusion broth (BHI) + 0.2 % sucrose at 37 °C in an anaerobic chamber (85% $N_2$, 10% $H_2$, 5% $CO_2$). The bacterial inoculum was prepared by adjusting the $OD_{660}$ of 0.271-0.279 to obtain a final concentration of $2 \times 10^8$ CFU/mL cells.

**The effects of NDs on planktonic cells**

The bactericidal and fungicidal activity of NDs was determined by assessing cell viability using the well-established XTT assay (Fig. S2). Bacterial and fungal suspensions were inoculated with different concentrations of NDs in sterile 96-well polystyrene plates and incubated in aerobic conditions (for fungi) and anaerobic conditions (for the bacteria) at 37 °C for 60 min. Untreated bacterial and fungal cultures were considered as control. After incubation, XTT solution (1 mg/mL) was freshly prepared with menadione (0.007 g/mL) in PBS in the ratio of 79:20:1 (PBS: XTT: Menadione). The plates were then incubated in dark at 37°C for 3 h with 100 µL of XTT dye in each well, after which the plates were centrifuged at 3000 rpm for 5 min. The supernatant was carefully transferred to another sterile 96-well plate and the absorbance was measured at 492 nm using a SpectraMax 340 tunable microplate reader (Molecular Devices, San Jose, California, USA). All experiments were performed in triplicates on three independent occasions. Cell viability for each concentration was normalized to the control and presented as percentage of dead cells.

**The effects of NDs on biofilm formation**

Different sub-inhibitory concentrations of NDs were coated on chamber slides (idibi µ 8 well plate-chamber slides) to determine their efficiency to inhibit biofilm formation. 200 µL of NDs with different concentrations were added to each well of a chamber slide, and dried overnight (O/N) at 37 °C. Control wells were coated only with PBS. Then, the bacterial and fungal suspensions were prepared at the concentrations of $2 \times 10^8$ and $1.5 \times *10^7$ CFU/mL respectively, as mentioned above and inoculated into the NDs (treatment) and PBS (control)-coated chamber slides. The chamber slides were then incubated for 24 h at 37 °C in aerobic conditions for fungi and anaerobic conditions for the bacteria. Following incubation, planktonic cells were removed, and biofilms were carefully washed with PBS. The slides were then stained with 200 µL OF freshly prepared Live/Dead™ stain (Molecular Probes, Life Technologies, Eugene, Oregon, USA) and kept undisturbed for 30 min in dark conditions.

The stained biofilms were examined under a Confocal Laser Scanning Microscope (CLSM; FV1000 Spectroscopic Confocal System, Olympus, Tokyo, Japan) Z-stack images were obtained from 5 different fields. The obtained images were analyzed using the Cell-C software

to quantify the percentage of dead cells and the total number of attached cells by counting the adhered cells in every scaffold, to indicate the effects of treatment on biofilm development. And the fungal biofilms were also imaged under SEM (Fig. S4).

**The effects of NDs on gene expression in biofilms**

The effect of NDs on growth, metabolism, virulence and biofilm formation of the treated biofilm cells was assessed using gene expression analysis. *C. albicans* and *S. mutans* biofilms were developed in the presence of 0.625 and 0.15 mg/mL of ND respectively, as described previously. Following 24 h of incubation, the planktonic cells were removed, and biofilms were washed with PBS to remove loosely adhered cells. Biofilm cells were then scraped out, and centrifuged at 14,000 rpm for 10 min. The supernatant was discarded, and total RNA was extracted from the pellet as per the manufacture's guidelines of the Promega SV Total RNA isolation system (Promega Corporation, Madison, Wisconsin, USA). The concentration and purity of RNA extracted were verified using Nanodrop (Thermo Scientific, USA). Total RNA was then converted into cDNA using High-Capacity cDNA Reverse Transcription Kits (Applied Biosystems, Foster City, California, USA) using the manufacturer-recommended protocol. Gene expression analysis was performed using quantitative Real Time Polymerase Chain Reaction (qRT-PCR). Relative gene expression (fold change) was calculated using the $2^{-\Delta\Delta CT}$ method with 18srRNA and 16srRNA as the housekeeping genes for *C. albicans* and *S. mutans* respectively.

**The effects of NDs on preformed biofilms**

To demonstrate the effects of NDs on preformed biofilms, 48 h biofilms of the microbes were formed in sterile 96-well polystyrene plates. The planktonic cells were removed, and the biofilm was washed with PBS to remove loosely adhered cells. NDs treatment was performed at the concentrations of 5 and 7.5 mg/mL (2×, 3× MIC) for *C. albicans,* 10 mg/mL (3× MIC) for *C. glabrata*, 1.25 and 2.5 mg/mL (2×, 3× MIC) for *S. mutans* and 10 mg/mL (5× MIC) for *P. gingivalis*. Untreated biofilms with added fresh media were considered as control. The plates were incubated at 37 °C in their respective conditions as mentioned earlier.

The biomass and viability of NDs exposed mature biofilms were quantified using safranin and XTT assay, respectively. To assess the biomass, the NDs treated biofilms were washed with PBS twice and dried for 10-15 min. Safranin (0.1%) was added to each well and incubated for 20 min at room temperature. Then, the safranin was removed and washed twice with PBS, and the plates were dried for 30 min. The dye stained in the biofilm was retained using 33% acetic acid and absorbance was measured at 492 nm. To analyze the viability of ND-treated Biofilms, the planktonic cells were removed, washed with PBS, and XTT assay was performed as mentioned earlier. To visualize the effect of ND treatment on preformed biofilms, 48 h biofilms were developed in chamber slides as mentioned earlier and treated as mentioned above. Then the biofilms were stained with the Live/Dead stain, visualized under CLSM and z-stack images analysed using the Cell-C software as described earlier. Additionally, the total percentage of dead (red-stained) cells was calculated by (number of red-stained cells in a Z-stack/total number of cells) x 100.

## Additional figures

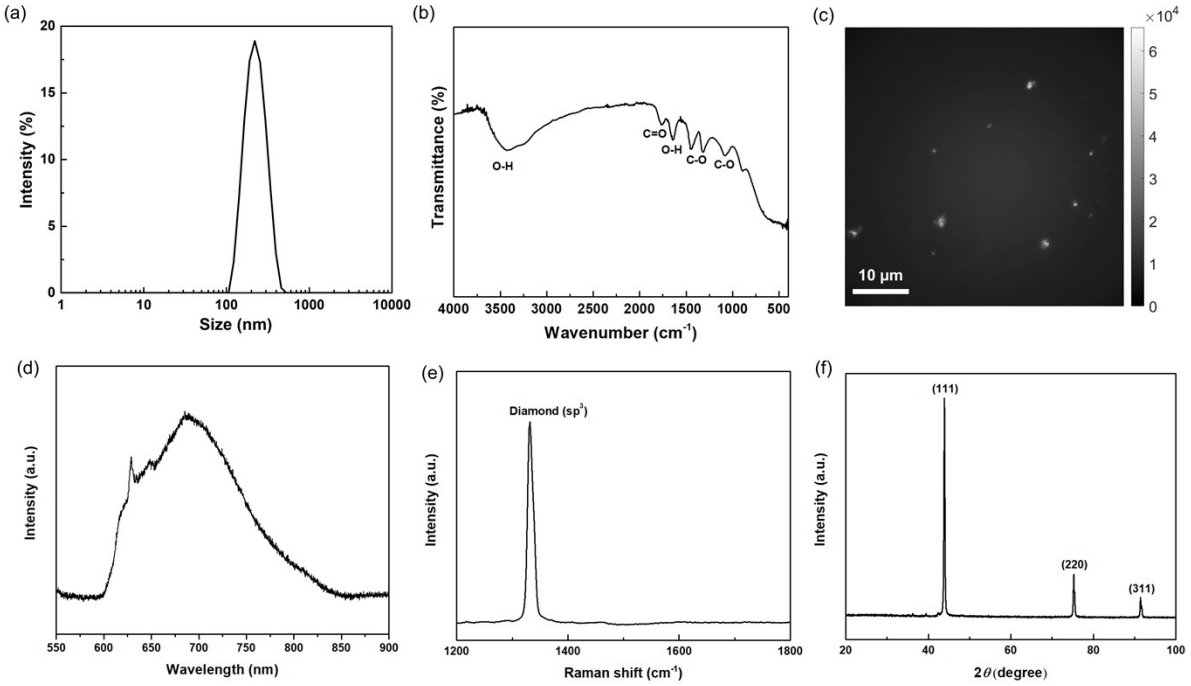


Fig. S1. (a) DLS size distribution, (b) FTIR spectrum, (c) wide-field fluorescence image, (d) fluorescence spectrum, (e) Raman spectrum and (f) XRD spectrum of the NDs.

The DLS result (measured in 0.01 M PBS buffer with NDs concentration of 0.02 mg/mL) indicates that the NDs are well dispersed in medium at the beginning of incubation (shown in Fig. S1a), no aggregate formed.

In Fig. S1b, the peak appearing at 1650 $cm^{-1}$ is owing to the −OH bending vibration, which comes from either the carboxyl group (−COOH) of the ND surface or water molecules adsorbed on the sample surface.[1] A broad peak around 3500 $cm^{-1}$ is similar because of the −OH stretching mode. The peaks at 1080, 1320 and 1450 $cm^{-1}$ are attributed to the C−O stretching and bending vibrations and asymmetric −CH bending vibration, respectively due to the air oxidation treatment.[2, 3] And the peak at ~1760 $cm^{-1}$ is attributed to the C=O stretching mode indicating the presence of carboxyl group on surface.[4] The new FT-IR spectrum clearly indicates there are carboxyl groups on the surface of air-oxidized NDs, which is matching with the zeta potential result (negatively charged surface). In fact, this is also in consistent with literature reports,[5] the carboxyl groups existing on the surface of air-oxidized NDs.

The sharp peak observed at 1333 $cm^{-1}$ in the Raman spectrum (Fig. S1e) is the symmetric stretching mode of the C−C bond of the diamond lattice structure (the so-called diamond line), and the peaks at 43.9°, 75.2° and 91.5° in the XRD spectrum (Fig. S1f) are corresponding to the (111), (220) and (311) planes of the cubic diamond lattice (ac=3.571 Å), respectively.

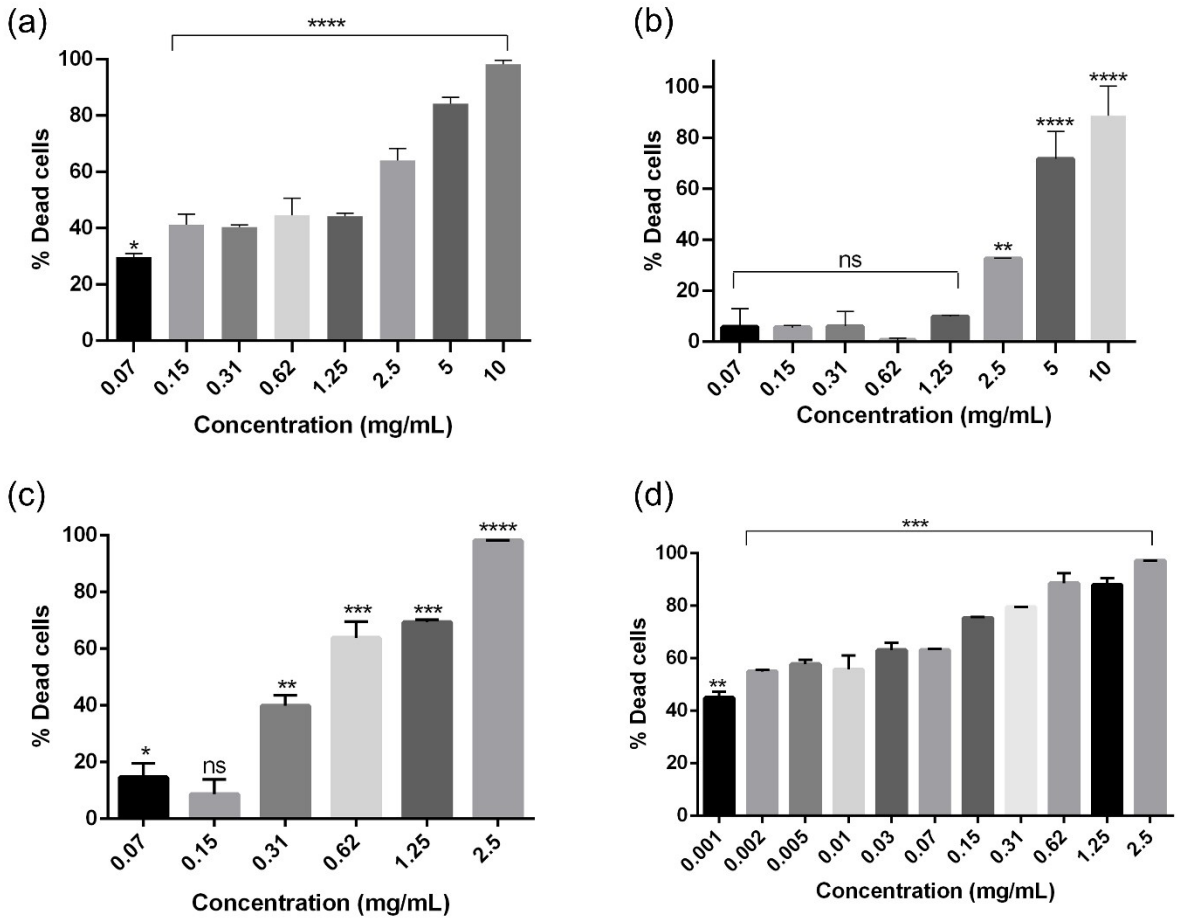


Fig. S2: Killing effect of varying concentrations of NDs on planktonic fungal and bacterial pathogens assessed using XTT assay: (a) *C. albicans*, (b) *C. glabrata*, (c) *S. mutans*, (d) *P. gingivalis*. Upon NDs treatment, the amount of dead cells percentage was calculated relative to the untreated controls. Statistical comparisons were made between each concentration and the drug-free control as well as between concentrations. Only statistically significant differences compared with the control have been indicated in the figure. * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$, and ns, not-significant.

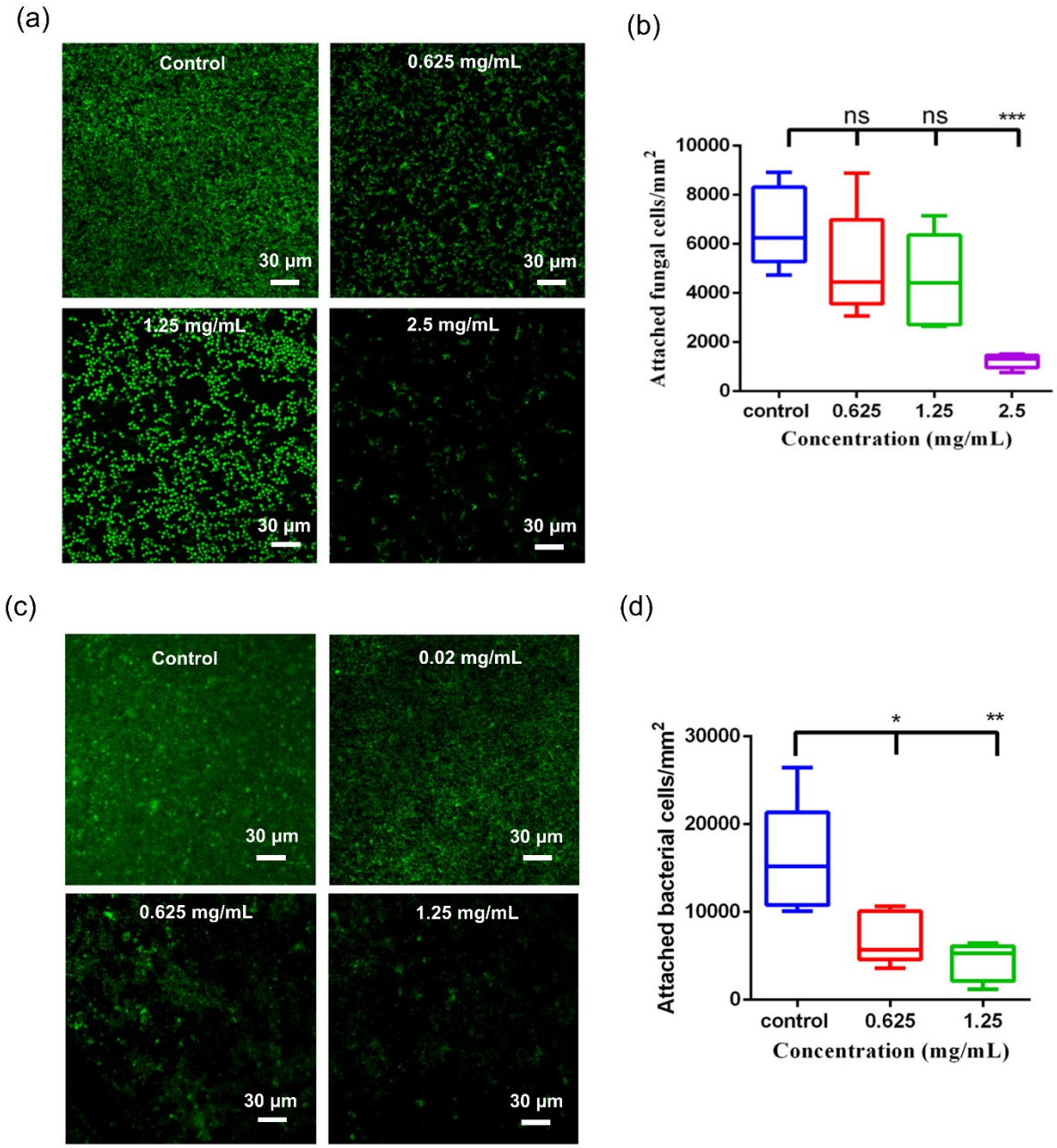


Fig. S3: The effects of NDs on biofilm formation (a, b) *C. glabrata* and (c, d) *P. gingivalis*. (a, c) CLSM images of the fungal and bacterial pathogens grown on chamber slides coated with sub-inhibitory concentrations of NDs, and visualized by Live/Dead staining (live cells were green labeled). (b, d) The quantitative analyses of attached cells/mm$^2$ calculated using Cell-C software. * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$, and ns, not-significant.

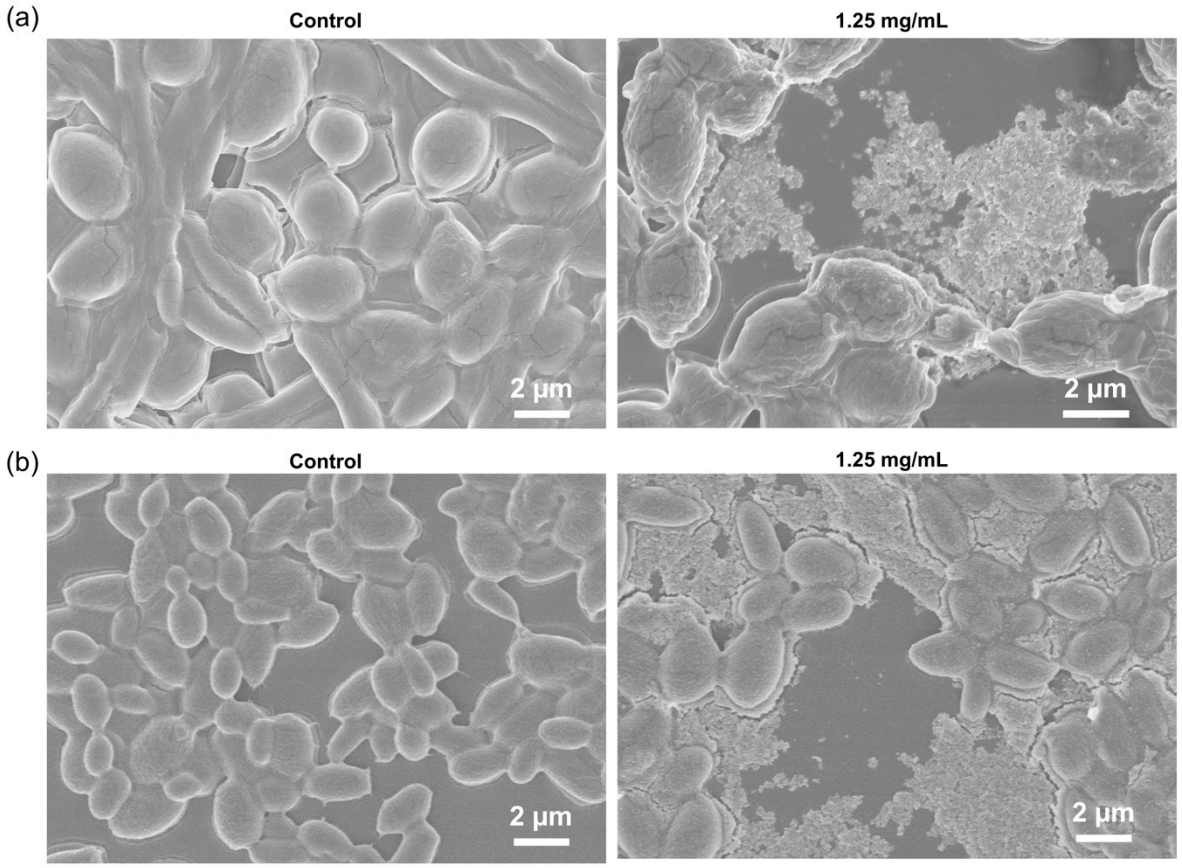


Fig. S4: SEM images of the (a) *C. albicans* and (b) *C. glabrata* biofilms grown on chamber slides coated with and without NDs.

One of the key points of our current work was to check whether biofilm development to a surface can be "inhibited" by NDs or not. The inhibition of biofilm, rather than killing, is more favored by clinic translation since oral biofilms contain both beneficial and pathogenic organisms. The SEM images clearly verify our hypothesis that sublethal concentrations of NDs can inhibit biofilms without lethal effects on cells, i.e., no morphology changes of the microbial cells by NDs. Therefore, it is not surprising that there were no obvious morphology changes of microbial cells (mainly intact cells) by NDs as indicated in the SEM images. The shown SEM images were mainly used to prove that the cell numbers have decreased compared to the control. In addition, these SEM data also suggested that there is no damage to cell membranes as expected. This is evidence that the adhesion and subsequent biofilm formation have been affected by the NDs.

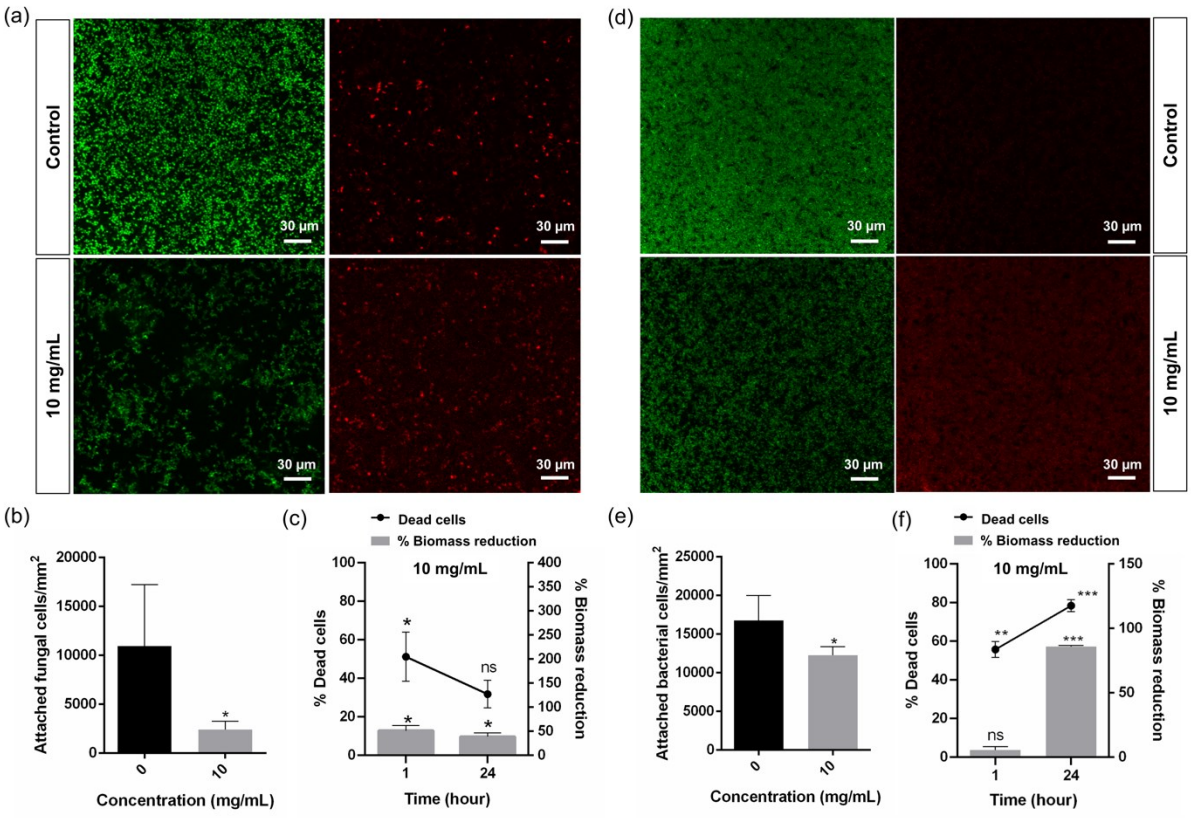


Fig. S5: The effects of NDs on preformed (a-c) *C. glabrata* (a-c) and (d-f) *P. gingivalis* biofilms. (a, d) The CLSM images of live (green labeled) and dead (red labeled) cells, clearly showing the reduction in biomass and increased number of dead cells compared to the control. (b, e) The quantitative analyses of attached live cells/mm$^2$ calculated using Cell-C software. (c, f) The reduction in biofilm biomass and the percentage of dead cells, quantitatively measured using safranin and XTT assays after treating the biofilms with NDs for varying time periods (1h, 24h). * p $\leq$ 0.05, ** p $\leq$ 0.01, *** p $\leq$ 0.001, **** p $\leq$ 0.0001, and ns, not-significant.

Confocal microscopic analysis of the dead cell percentage showed that 10 mg/mL NDs treatment of 24 h biofilms killed 94.94% *C. glabrata* and 73.3% *P. gingivalis*. As was described with *C. albicans*, the dead cell count in the XTT assay was significantly less (~20%) in the 24 h treated biofilms of *C. glabrata* compared to the 1 h treatment. While this difference of 20% was statistically significant, its biological relevance is questionable. The reasons for this effect are likely to be same as those described for the *C. albicans* biofilms. As was observed with *C. albicans* and *S. mutans*, the number of attached live cells was significantly reduced in the *C. glabrata* and *P. gingivalis* biofilms.

## References


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4. L. Gines, S. Mandal, I. A. Ashek, C. L. Cheng, M. Sow and O. A. Williams, *Nanoscale*, 2017, **9**, 12549−12555.

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