Bactericidal Activity of Partially Oxidized Nanodiamonds

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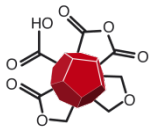
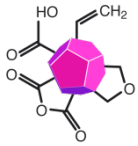
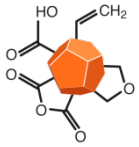
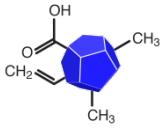
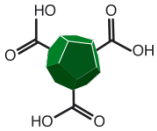
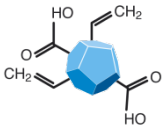
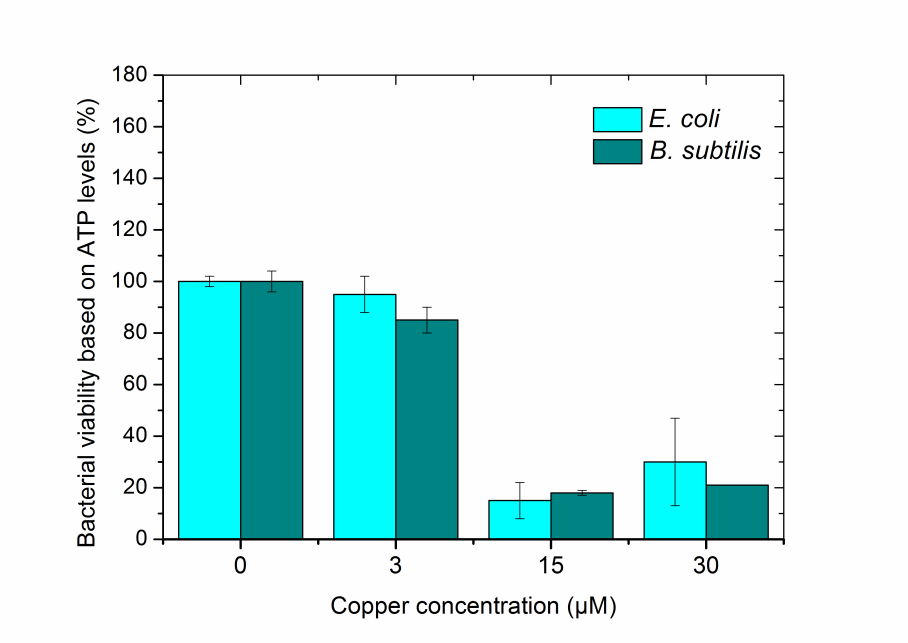
Copper(II) sulfate was incubated with bacterial suspensions to imitate possible side-effects caused by metal impurities in the ND dispersions. The highest concentration of around 3 µM copper that was present in ND- dispersions *(Supplementary Table 1)* did not lower bacterial ATP levels, indicating that copper impurities are not the reason for the ND toxicity *(Supplementary Figure 1).* Zinc impurities were measured in the different ND dispersions, but were determined to be comparable for all ND types, which excludes an effect of zinc on bacterial viability *(Supplementary Table 1)*.

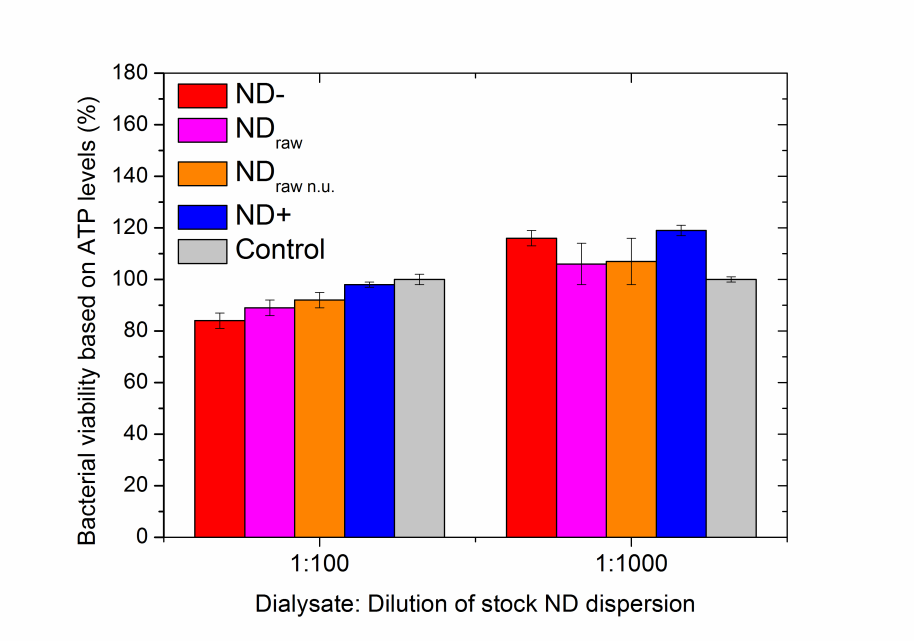
Supplementary Table 1| Copper and zinc impurities in the different types of ND dispersions (500 mg/L)

	Cu (µM)	Zn (µM)
ND- 	2.43 ± 0.56	0.32 ± 0.16
ND_{raw} 	1.67 ± 0.03	0.40 ± 0.16
$ND_{raw\ n.u.}$ 	0.94 ± 0.13	0.24 ± 0.08
ND+ 	1.54 ± 0.38	0.25 ± 0.10
ND_{pure}- 	0.19 ± 0.00	0.25 ± 0.10
ND_{pure}+ 	0.08 ± 0.04	0.25 ± 0.06



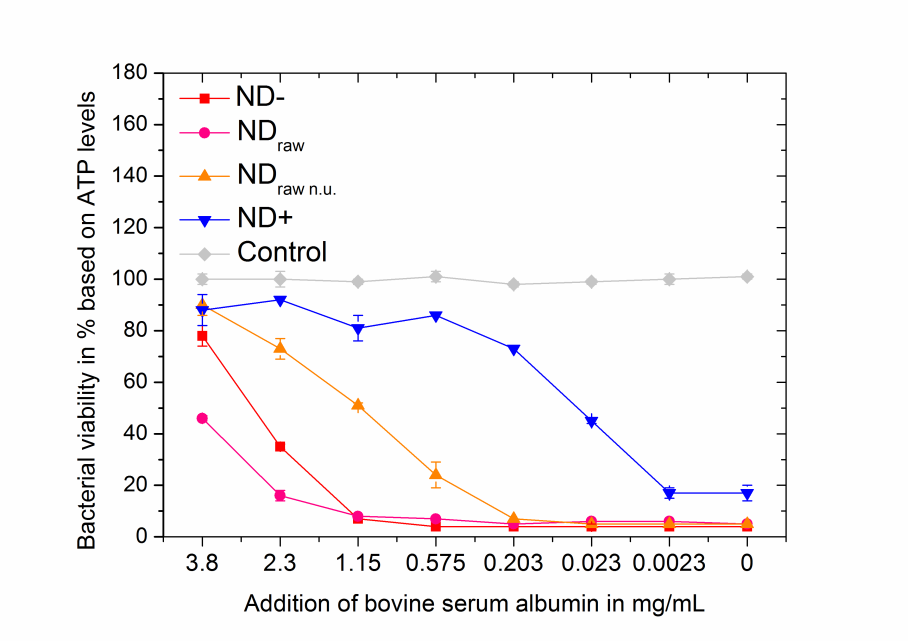
Supplementary Figure 1| Antibacterial effect of copper on *E. coli* and *B. subtilis*. Concentrations of 3 µM copper(II)sulfate that are present in toxic ND dispersions did not reduce bacterial viability. Therefore, copper impurities can be excluded as toxic agents in ND dispersions.

ND_{pure} were demonstrated to not act as a bactericides, although similar surface charges were present as on the toxic types ND- and ND_{raw}/$ND_{raw\ n.u.}$. Bacterial viability in the presence of particle-free ND dialysates of the toxic ND types was investigated to exclude low-molecular impurities causing the bactericidal effect. Dialysates correspond to 50 mg/L (1:100) and 5 mg/L (1:1000) of former ND dispersions due to higher volumes and were shown not to affect bacterial ATP levels in the extent that comparable ND dispersions did *(Supplementary Figure 2)*.



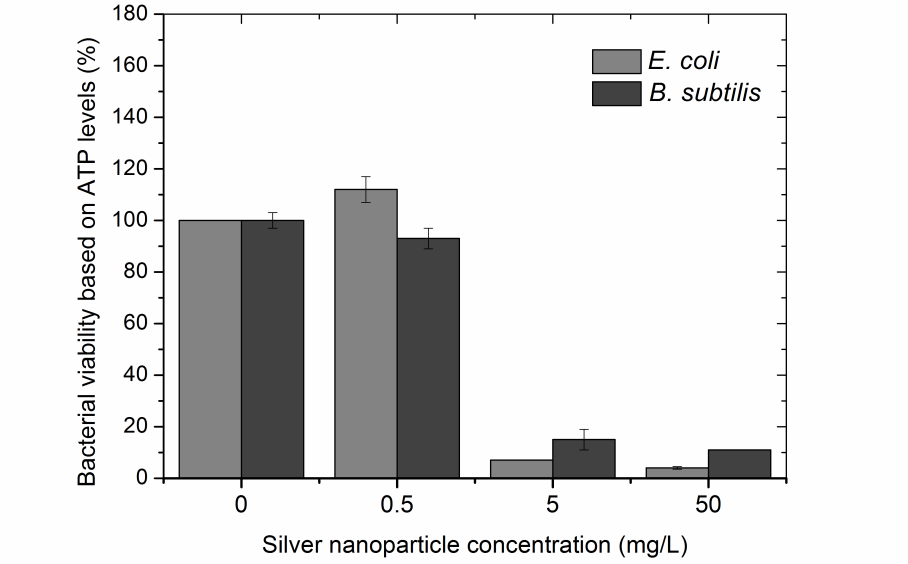
Supplementary Figure 2| Viability of *E. coli* after incubation with ND dialysates. Dialysates of the toxic ND types corresponding to 50 and 5 mg/L ND dispersions hardly compromised bacterial viability.

Additionally to FCS, the most prominent protein in FCS, bovine serum albumin (BSA), was added with NDs to bacteria to investigate the influence of the presence of proteins on ND toxicity. The presence of high concentrations of BSA (up to 3.8 mg/mL) reduced the antibacterial effect of ND. Compared to FCS, the reduction of toxicity was slightly less efficient. The presence of low concentrations of BSA (0.0023 – 0.575 mg/mL) did not prevent the toxicity of ND- and ND_{raw} (*Supplementary Figure 3*).



Supplementary Figure 3| Bovine serum albumine inhibits the antibacterial properties of NDs. The addition of increasing concentrations of BSA reduces the toxicity of NDs on *E. coli*.

Silver nanoparticles were used as well-known antibacterial material [36] for comparison with the antibacterial potential of NDs. Silver nanoparticles strongly reduced bacterial viability in concentrations of >5 mg/L, while the toxic types of ND were efficient in reducing bacterial viability at concentration of >50 mg/L*(Supplementary Figure 4)*.



Supplementary Figure 4| Antibacterial effects of silver nanoparticles on *E. coli* and *B. subtilis*.

The different types of ND slightly interact with the luminescence assay for the determination of bacterial ATP levels. To quantify the influence of the ND on the assay, 1 nM ATP was incubated with 5, 50 and 500 mg/L ND and luminescence counts were measured. The effects of the ND types on the luminescence counts were calculated and indicated as correction percentages. These correction percentages were subtracted from the results from bacterial tests. *Supplementary Figure 5* shows a comparison of the unmodified data for *E. coli and B. subtilis* and data where the ATP correction values were applied.

Supplementary Figure 5| Comparison of the raw data from bacterial ATP assays for *E. coli* (a) and *B. subtilis* (c) with the corrected data (b, d).