Sensitivity of bacteria to diamond nanoparticles of various size differs in gram-positive and gram-negative cells

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Abstract

In this study, the influence of the size and surface termination of diamond nanoparticles (DNPs) on their antibacterial activity against Escherichia coli and Bacillus subtilis was assessed. The average size and distribution of DNPs were determined by dynamic light scattering and X-ray diffraction techniques. The chemical composition of the DNPs studied by X-ray photoelectron spectroscopy showed that DNPs > 5 nm and oxidized particles have a higher oxygen content. The antibacterial potential of DNPs was assessed by the viable count method. In general, E. coli exhibited a higher sensitivity to DNPs than B. subtilis. However, in the presence of all the DNPs tested, the B. subtilis colonies exhibited altered size and morphology. Antibacterial activity was influenced not only by DNP concentration but also by DNP size and form. Whereas untreated 5-nm DNPs were the most effective against E. coli, the antibacterial activity of 18–50-nm DNPs was higher against B. subtilis. Transmission electron microscopy showed that DNPs interact with the bacterial surface, probably affecting vital cell functions. We propose that DNPs interfere with the permeability of the bacterial cell wall and/or membrane and hinder B. subtilis colony spreading.

Diamond nanoparticles (DNPs) are an emerging class of materials with several medically significant properties and promising potential in applications such as drug delivery (Huang et al., 2007; Chen et al., 2009), biomarkers (Yu et al., 2005; Faklaris et al., 2009) or vectors for gene delivery in gene therapy techniques (Zhang et al., 2009). Employing DNPs provides implicit benefits, as they generally exhibit good biocompatibility with eukaryotic cells (Schrand et al., 2007a, b, 2009), are chemically stable and resistant to wet etching (Mochalin et al., 2012).

Surprisingly, knowledge on the antibacterial properties of DNPs is scarce. In our previous study we showed that DNPs inhibit the growth of Escherichia coli on solid medium (Beranová et al., 2012). Sawosz et al. (2011) showed DNPs tend to attach to the outer cell structures of both gram-negative Salmonella enteritidis and gram-positive Listeria monocytogenes. They even suggested that DNPs enter the cells, although the evidence presented was not
very convincing. Polycrystalline nanodiamond thin films were also reported to confer high resistance to bacterial colonization (Jakubowski et al., 2004; Medina et al., 2012). However, more studies have been conducted using diamond-like carbon thin films that possess similar physical properties to diamond films but are amorphous and contain a higher proportion of nondiamond (sp²-hybridized) carbon. These have been shown to decrease bacterial attachment and subsequent growth on surfaces (Ishihara et al., 2006; Marciano et al., 2009, 2011).

In this study we focused on the antibacterial activity of DNPs against model bacteria. The influence of nanoparticle size (5, 18, 25 and 50 nm) and their surface terminations (nonoxidized and oxidized surfaces) on their activity against *E. coli* and *Bacillus subtilis* were assessed.

### Materials and methods

#### DNPs

DNPs of a nominal average size of 5 nm produced by a detonation process were provided by NanoAmando, New Metals and Chemicals Corp. Ltd. (Kyobashi, Japan). They were used either as delivered or as oxidized by annealing in air at 450 °C for 30 min (Kozak et al., 2013). Monocrystalline synthetic DNPs with a nominal average size of 18, 25 and 50 nm produced by the high-pressure, high-temperature method were purchased from Microdiamant AG (Lengwil, Switzerland). The lattice parameters and crystallite sizes of DNPs were determined by X-ray powder diffraction (XRD) using a Bruker D8 diffractometer (Cu, Kα radiation). The size of the DNPs and their distribution in deionized water were determined using a commercial dynamic light scattering (DLS) instrument (ALV, Malvern) and ZETASIZER Software. Before measurements, a water suspension of each type of DNP was ultrasonically treated for 5 h. The DLS measurements were performed both for unfiltered water suspensions of nanoparticles and samples filtered through a 220-nm filter (Supporting Information, Fig. S1). The filtration was used to remove large aggregates that otherwise interfere with the detection of the smaller ones due to high scattering.

The chemical composition of DNPs was analysed by X-ray photoelectron spectroscopy (XPS) with two spectrometers. XPS using an Al, Kα X-ray source (1486 eV; Specs) equipped with a hemispherical energy analyser (Phoibos 100, Specs) was used to analyse the chemical composition of the DNPs with diameter 18, 25 and 50 nm. The XPS photoelectron spectra of 5-nm DNPs were recorded using an ADES 400 angle-resolved photoelectron spectrometer (VG Scientific, East Grinstead, UK) equipped with a rotatable hemispherical electron energy analyser with an Mg, Kα X-ray source (1253.6 eV, Specs). For details on the measurement and data analysis, see Data S1.

Various concentrations of DNPs were tested for their effects on bacterial growth. DNPs were resuspended in deionized water (1–10 mg mL⁻¹ stock) and sterilized by autoclaving (121 °C, 20 min). The suspension was sonicated (35 kHz, 30 W) for 30 min prior to use to ensure a homogeneous suspension.

### Microorganisms and cultivation

*Escherichia coli* (strain K12) and *B. subtilis* (strain 168) were employed as model microorganisms. Bacteria were cultivated aerobically in Luria broth (LB) at 37 °C until the midexponential phase of growth. Cultures were appropriately diluted in sterile water to yield c. 80–100 colonies per plate, mixed with defined amounts of DNPs in water and plated immediately onto LB agar. Because of the differing antibacterial potential of DNPs against *E. coli* and *B. subtilis*, the following concentration ranges were tested: 20–2500 µg mL⁻¹ for *E. coli* and 200–5000 µg mL⁻¹ for *B. subtilis*. For the 50- and 5-nm DNPs used against *B. subtilis*, the lowest concentrations tested were 500 and 1000 µg mL⁻¹, respectively. Colonies were counted after overnight cultivation at 37 °C and expressed as colony-forming ability.

### Colony diameter measurement

The average size of at least 500 bacterial colonies was calculated from digital images of agar plates containing colonies grown in the presence of DNPs. For each colony, the area was measured using IMAGEl software (http://rsb.info.nih.gov/ij/) and normalized to the average colony area of the control without DNPs. Only the circular monocolonies were included in the analysis.

### Transmission electron microscopy

Transmission electron microscopy was used to identify the localization of DNPs and their interaction with bacteria. Negatively stained samples were prepared as follows. The DNP suspension was added to the bacterial culture (OD₄₅₀nm of 0.5) and gently mixed for 20 min on a rotary shaker at room temperature. Drops of DNP-treated culture were then applied onto activated Formvar/carbon-coated Cu grids (Benada & Pokorny, 1990). After 1 min of adsorption, the grids were negatively stained with 1% ammonium molybdate containing 0.1% trehalose. Final samples were observed in a Philips CM100 electron microscope at 80 kV. The images were recorded using a MegaViewII slow scan camera and processed with the ANALYSIS 3.2 software suite.
Results

Physical analysis of DNPs

The diffraction patterns of untreated DNPs (Fig. 1) indicate the presence of well-resolved peaks at 2θ = {43.7, 75.2, 92, 120 and 140}, which correspond to the (111), (220), (311), (400) and (331) reflections from the diamond. The average DNP crystallite sizes, i.e. the sizes of the primary particle, determined from the XRD measurements are summarized in Table 1. We did not observe any substantial change in the DNP crystallite size of 5-nm DNPs after their oxidation. This indicates that their cores have not been etched and all changes are mainly due to the modification of their surface and/or graphitic shell (Kozak et al., 2013). The average DNP diameters determined by the DLS of filtered samples (Table 1) were 5, 28, 37.8 and 58 nm for DNPs with nominal average sizes of 5, 18, 25 and 50 nm, respectively. For the 5-nm DNPs, the average diameter increased from 5 nm to 32 nm after their oxidation by annealing in air. When compared with the crystallite size deduced from XRD measurements, the DLS data show a considerably larger diameter for all DNPs with the exception of untreated 5-nm DNPs. This indicates that a certain proportion of nanoparticles form larger aggregates. These were shown to have a broad size distribution (Fig. S1). The DLS measurement of unfiltered DNP suspensions also indicated the presence of even larger aggregates (Fig. 1), which however due to their size could hardly have any effect on the viability of bacteria.

The concentrations of chemical elements and bonds on the surface of the DNPs, acquired from the peak-fitted C 1s spectra are expressed for each fit as a percentage of the total C 1s peak area and are summarized in Table 2 (details on peak fitting are provided in Data S1). We determined that DNPs contain 63–75% diamond (sp3) phase, 11–24% nondiamond (sp2) phase, 7–12% C–O bonds and 2–6% C=O bonds.

The data indicated minimal differences in the chemical compositions of the nanoparticles tested, except for the untreated 5-nm DNPs. We observed that for the untreated nanoparticles, the sp3/sp2 ratio increased with particle size, i.e. DNPs with a size of 5 and 18 nm had a ratio of about 2.8, while for nanoparticles with diameters of 25 and 50 nm, the ratio was 6.7 and 4.7, respectively. However, after annealing the 5-nm nanoparticles, their sp3/sp2 ratio increased to 5.5.

Moreover, the main size-dependent difference in untreated DNPs lies in their different oxygen content and amount of C–O and carboxyl C=O groups. A small amount of oxygen (5%) was detected for the untreated nanoparticles with a mean size of 5 nm compared with the others (more than 13%). As a result, these untreated 5-nm DNPs contain only 2% carboxyl groups and 7% C–O bonds. In contrast, the average concentration of C=O and C–O bonds for much larger nanoparticles is about 5 and 9%, respectively. This effect can be explained by the differences in the technologies used for nanoparticle fabrication. The surface chemical properties of the annealed 5-nm nanoparticles were comparable to the nanoparticles of larger sizes rather than to untreated 5-nm nanoparticles (Table 2). We consider that these distinct surface chemical properties of annealed 5-nm DNPs are responsible for the larger size of their aggregates compared with untreated particles of the same nominal size, as was also detected by DLS (Table 1).

Antibacterial activity of DNPs

DNPs of four different nominal sizes (5, 18, 25 and 50 nm) in concentrations ranging from 20 μg mL−1 to

### Table 1. Lattice constant and crystallite size of DNPs as determined by XRD and appropriate average diameter size of DNP aggregates calculated from DLS measurements

<table>
<thead>
<tr>
<th>Type of DNPs</th>
<th>XRD</th>
<th>DLS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Size (nm)</td>
<td>Lattice constant (A)</td>
<td>Crystallite size (nm)</td>
</tr>
<tr>
<td>5</td>
<td>3.5651 (8)</td>
<td>4.4</td>
</tr>
<tr>
<td>5 (annealed)</td>
<td>3.5634 (9)</td>
<td>5</td>
</tr>
<tr>
<td>18</td>
<td>3.5676 (2)</td>
<td>9.8</td>
</tr>
<tr>
<td>25</td>
<td>3.5675 (2)</td>
<td>12</td>
</tr>
<tr>
<td>50</td>
<td>3.5675 (1)</td>
<td>23.1</td>
</tr>
</tbody>
</table>

Fig. 1. XRD patterns of untreated DNPs with various average sizes: (a) 5 nm, (b) 18 nm, (c) 25 nm, (d) 50 nm.
5000 μg mL⁻¹ were tested for their effects on the growth of *E. coli* and *B. subtilis* on solid medium. With 5-nm nanoparticles, both untreated and DNP oxidized by annealing in air were used to study the effect of DNP surface modification on their antibacterial activity. Bacterial cultures from the exponential phase of growth, at densities of 1 × 10⁸ or 0.8 × 10⁸ cells mL⁻¹ for *E. coli* and *B. subtilis*, respectively, were appropriately diluted in water, mixed with a defined amount of nanoparticles of the indicated size and plated onto LB agar plates. After overnight cultivation, the average number of colonies was compared with an untreated control sample.

In the presence of DNP, the viability of *E. coli* decreased with increasing particle size and concentration (Fig. 2a). The antibacterial potential of DNP decreased with both their increasing size and surface oxidation. Untreated 5-nm DNP were the most effective against *E. coli*, and no other DNP of any size and concentration exhibited such a strong inhibitory effect. In our experimental setup, 5-nm untreated nanoparticles were almost 100% effective against *E. coli* at the final concentration of 100 μg mL⁻¹, whereas the oxidized form failed to substantially reduce the number of colonies, and even at a 25-fold higher concentration caused only an c. 80%

### Table 2. Concentration of chemical elements and bonds in DNP with various nominal sizes calculated from XPS

<table>
<thead>
<tr>
<th>Type of DNP</th>
<th>Chemical elements</th>
<th>Chemical bonds – C 1s</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>O (%)</td>
<td>C (%)</td>
</tr>
<tr>
<td>Size (nm)</td>
<td>Surface termination</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Untreated</td>
<td>5</td>
</tr>
<tr>
<td>5</td>
<td>Annealed</td>
<td>14</td>
</tr>
<tr>
<td>18</td>
<td>Untreated</td>
<td>13</td>
</tr>
<tr>
<td>25</td>
<td>Untreated</td>
<td>17</td>
</tr>
<tr>
<td>50</td>
<td>Untreated</td>
<td>14</td>
</tr>
</tbody>
</table>

**Fig. 2.** Effect of DNP on colony-forming ability of *Escherichia coli* (a) and *Bacillus subtilis* (b). The graphs represent the mean and standard deviation of at least three independent experiments, each performed in triplicate. 'oxi-' denotes oxidized (annealed) DNP. CFA 100% represents 1 × 10⁸ and 0.8 × 10⁸ CFU mL⁻¹ for *E. coli* and *B. subtilis*, respectively.
reduction in colony formation. The 18-nm DNPs were much less potent inhibitors of E. coli growth; a 70% reduction in colony-forming activity was only observed for the highest concentration used (2500 μg mL⁻¹). A weak antibacterial effect was observed in the presence of 25-nm DNPs, which at concentrations higher than 500 μg mL⁻¹ only decreased colony formation by 10–20%. The 50-nm nanoparticles were ineffective against E. coli at all the concentrations tested.

Interestingly, the response of the gram-positive bacterium B. subtilis to the presence of DNPs (Fig. 2b) was completely different from that of gram-negative E. coli. DNPs than 5 nm in size together with 5-nm DNPs with an oxidized surface inhibited the growth more effectively than the smaller, untreated DNPs. The presence of untreated 5-nm DNPs did not impair the growth of B. subtilis, even at the highest concentrations tested, i.e. 5000 μg mL⁻¹. In contrast, the 18-, 25- and 50-nm DNPs all decreased colony formation to c. 40% at a concentration of 5000 μg mL⁻¹. The presence of oxidized 5-nm DNPs resulted in a maximal reduction in colony counts to c. 50%, and thus their antibacterial effect was similar to that of nanoparticles of larger sizes.

Remarkably, an interesting phenomenon was observed with all types of nanoparticles, even when no antibacterial activity was detected: the presence of DNPs resulted in both smaller colonies and an apparent change in the morphology of B. subtilis colonies. The colonies on the control plates without DNPs were flat with slightly undulate margins, whereas DNP treatment resulted in more circular colonies (Fig. 3b and c). The extent of colony size reduction was influenced by both nanoparticle size and concentration. As seen in Fig. 3, untreated 5-nm nanoparticles were the most potent at reducing B. subtilis colony size, while 50-nm DNPs were the least effective. Despite their lack of antibacterial activity, the 5-nm DNPs decreased colony size by 50% at a concentration of only 1000 μg mL⁻¹ and at 5000 μg mL⁻¹ decreased colony diameter to 30% that of control colonies. DNPs had no effect on the size or shape of E. coli colonies (data not shown).

Transmission electron microscopy was used to directly observe the interactions of nanoparticles with bacterial cells (Fig. 4). Nanoparticles in large aggregates were often attached to bacterial cells. The size of DNP aggregates, as they appeared on transmission electron micrographs, was larger than the average diameter values detected by the DLS measurement of unfiltered samples (Fig. S1). We assign this mainly to the technique of sample preparation for microscopy. Unlike Sawosz et al. (2011), who used DNPs of comparable size (2–10 nm) and transmission electron microscopy, we failed to see DNPs preferentially attached to the flagella. With E. coli, we observed the interaction of DNP aggregates with the cell surface and flagella without any marked preference (Fig. 4c–e). Therefore, we assume that the main interaction responsible for the antibacterial effect of DNPs is the attachment of the DNP particles to the bacterial cell surface structures. We observed that 5-nm DNPs accumulate on E. coli cells in large aggregates and seem to affect cell morphology. We did not observe damage to the cell shape in general, although bacterial membranes seemed to exhibit some defects – their surface was rougher and more corrugated than the untreated control cells (Fig. 4c and d). With 50-nm DNPs, this effect was much less obvious (Fig. 4e). Bacillus subtilis cells appeared quite intact when exposed to both 5- and 50-nm DNPs (Fig. 4g and h); however, many more 50-nm DNPs nanoparticle aggregates were attached to the bacterial cells.

![Fig. 3. Average size of a colony of Bacillus subtilis on LB agar in the presence of various concentrations of DNPs of the indicated size. The graph (a) shows the mean and standard deviations of at least three independent experiments, each performed in triplicate. 100% represents a colony diameter of 4.9 ± 0.5 mm. ‘oxi’ denotes oxidized (annealed) DNPs. The images (b, c) show representative B. subtilis colonies: (b) control colony without DNP treatment; (c) colonies grown after treatment with DNPs (18-nm DNPs at a concentration of 5000 μg mL⁻¹). For both images, the scale bar = 2.5 mm.](https://academic.oup.com/femsle/article-abstract/351/2/179/429373)
From the data presented, it appears that it is the separate primary nanoparticles or small aggregates of nanoparticles that confer the actual antibacterial effect, while the large aggregates (above 200 nm) do not affect the cells. The antibacterial capacity of the tested nanoparticles correlates with individual particle size and the correlation with *E. coli* is the opposite to that with *B. subtilis*.

According to DLS data, the average diameter of DNP aggregates smaller than 220 nm for 18-, 25- and 50-nm DNPs is comparable, ranging from 28 nm to 58 nm (Table 1). This represents c. 2–3 times the value of the primary particle size detected by XRD. In contrast, the 5-nm DNPs were shown to occur more frequently unaggregated, with average particle diameter equaling the crystallite size (5 nm).

We observed pronounced differences in susceptibility to DNPs between *E. coli* and *B. subtilis*. These differences appear to be based on the basic morphological distinction between the structure of the gram-negative and gram-positive cell wall.

As the sensitivity of *E. coli* to DNPs was in general substantially higher than that of *B. subtilis*, we suppose that the target structure in *E. coli* is located on the outer membrane surface and the interaction with DNPs is therefore more specific. With the gram-negative cell wall, it is the lipopolysaccharide moiety protruding from the outer membrane that first interacts with nanoparticles. Together with porin channels of the outer membrane, this structure serves as a molecular sieve that only allows smaller molecules (up to 600 Da) to enter the periplasmic space. We suggest that porins are candidates for the target structure that is affected by DNPs in the gram-negative cell. As the diameter of an average *E. coli* porin channel is 1.1–1.2 nm (Benz & Bauer, 1988), we hypothesize that 5-nm DNPs might block the rather nonspecific transport of hydrophilic solutes through these channels and negatively affect the cell osmotic balance. In contrast, larger DNP aggregates may be prevented from moving closer to porins because they can be trapped in the lipopolysaccharide layer. On the other hand, with *B. subtilis*, the structure that comes into contact with the DNPs is the thick peptidoglycan layer. We suggest that the diffusion of nutrients through the peptidoglycan layer can be substantially blocked by DNPs, but that such a nonspecific effect requires higher concentrations of DNPs to be arrested in the peptidoglycan coat. It has also been reported that *E. coli* and *B. subtilis* differ substantially in the general hydrophobicity and charge of their surfaces; the surface of a *B. subtilis* cell was shown to be more hydrophobic and more negatively charged (Dickson & Koohmaraie, 1989). This feature can cause the different general affinity of DNPs to cells of these bacterial species. As we observed a higher susceptibility of *B. subtilis*.

Fig. 4. TEM images of *Escherichia coli* and *Bacillus subtilis* cells without DNPs and with untreated DNPs (5 and 50 nm). (a–e) *Escherichia coli*; (f–h) *B. subtilis*; (a, b, f) control samples without DNPs; (c, d, g) samples with 5-nm DNPs; (e, h) samples with 50-nm DNPs. The arrows indicate the areas of cell damage. In all images, the scale bar = 1 μm.
to larger DNPs, which were shown to form larger clusters, we hypothesize that the larger DNP aggregates are more effective in ‘plugging’ the otherwise permeable peptidoglycan mesh layer and consequently have a stronger antibacterial effect against gram-positive bacteria than smaller nanoparticles. However, further investigation would be needed to verify these hypotheses experimentally.

We examined the influence not only of DNP size but also their chemical modification on their antibacterial activity. The analysis of DNPs by XPS suggests that annealing makes the surface properties of 5-nm nanoparticles more similar in terms of oxygen content and sp³/sp² carbon ratio to untreated nanoparticles 25–50 nm in size. Also, DLS measurements showed that annealed 5-nm nanoparticles aggregate to a greater extent, forming aggregates with an average diameter of about 32 nm. The higher content of oxygen on their surface, compared with untreated 5-nm DNPs (Table 2), could underlie the different attachment capacity of these nanoparticles by making them more hydrophilic than untreated 5-nm DNPs. This effect of oxygen plasma treatment has been recently described by several authors. For example, Marciano et al. (2009) observed a substantial difference in the hydrophobicity between as-deposited (hydrophobic) and oxygen-plasma-treated (super-hydrophilic) diamond-like carbon thin films. The difference in sp³/sp² carbon ratio could also play a role, as it was reported that this influences bacterial adhesion to diamond-like carbon films (Zhao et al., 2007; Zhou et al., 2008; Marciano et al., 2009). The increased nanoparticle hydrophilicity may have induced larger cluster formation, which could explain the stronger antibacterial action of oxidized 5-nm nanoparticles against B. subtilis.

In our experimental setup, the interaction of DNPs with bacteria occurs in two distinct steps. The first step is the attachment of nanoparticles to the bacterial surface that takes place in the water suspension before the bacteria are plated on solid medium. We suggest that cells whose surface structures had been damaged or clogged by nanoparticles did not give rise to colonies on the agar plate and therefore we detected a lower colony-forming ability. The second interaction step may occur during colony growth. The cells that remained intact during the first step formed colonies. However, their colony formation might be influenced by the layer of nanoparticles that were spread on the agar surface together with the bacteria.

The obvious reduction in the diameter of B. subtilis colonies in the presence of DNPs of all sizes is a striking phenomenon. In this respect, the smallest diameter DNPs (5 nm) were the most effective, and this effect was independent of their surface modification. The reduction could be caused by a combination of several possible factors.

For example, the DNP layer could hinder the availability of nutrients from the medium, leading to a lower growth rate and subsequently to a smaller size of B. subtilis colonies. The specific surface of nanoparticles may play a role here, which would explain the fact that the smallest DNPs were most effective in affecting the colony size and shape of B. subtilis. Another possibility is that DNPs could physically modify or sterically block the agar surface otherwise available for colony spreading. Consequently, the cells do not prefer the usual colonization of the free agar surface and grow in a multilayered colony that is usually characteristic for a later stage of colony growth. In our future experiments, we would like to determine which of these effects are responsible for the differing colony morphology. The fact that E. coli colonies did not seem to be significantly influenced in terms of their size and/or shape may again be due to the distinct morphology and surface properties of the gram-positive and gram-negative cell, although the exact reason for this observation has yet to be determined.

Conclusions

The excellent chemical and physical properties of DNPs make them an attractive nanomaterial for fundamental antibacterial studies, especially as a standard for comparative studies with less stable nanoparticles. We have shown that DNPs exhibit remarkable antibacterial potential. They appear to attach to the bacterial cell surface and affect the cells in both a dose- and particle-size-dependent manner. Our study also shows that there are fundamental differences in the susceptibility of E. coli and B. subtilis to the growth-inhibiting effect of DNPs. Based on these observations, we proposed a hypothesis in which the cell-wall differences between gram-negative and gram-positive bacteria should play a key role.

DNPs belong to materials with significant potential for use in medicine. A wide range of bioactive molecules can be grafted onto their surface and several studies have been recently performed in which DNPs were successfully used as vectors for drug or gene delivery within eukaryotic organisms (Schrand et al., 2009). Therefore, based on our recent findings, we propose that these nanoparticles could represent a novel antibacterial agent themselves or – after appropriate modification – could also be used for the targeted delivery of conventional antibacterial substances to pathogenic bacteria.

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References


Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Fig. S1.** Dynamic light scattering measurement of DPNs.

**Data S1.** X-ray photoelectron spectroscopy measurement.