Physicochemical and Antibacterial Properties of PEGylated Zinc Oxide Nanoparticles Dispersed in Peritoneal Dialysis Fluid

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ABSTRACT: Owing to the peculiar broad-spectrum antimicrobial activities of zinc oxide nanoparticles (ZnO NPs), we envisaged their use to treat bacterial/mycobacterial/fungal infections during peritoneal dialysis (PD) of end-stage renal failure patients. However, a recent study from our lab showed that ZnO-NPs cannot be employed for the same in their naked form owing to their rapid agglomeration. Also, the naked ZnO-NPs showed strong interaction with organic acids present in the PD fluid (i.e., lactate and citrate present abundantly in almost all biological fluids) resulting in the formation of bioconjugates. Here, we propose that the surface coating of ZnO NPs may inhibit the binding interactions of NPs with the constituents of PD fluid. Therefore, in this study, we have carried out the surface coating of ZnO NPs with polyethylene glycol (PEG) of different molecular weights, followed by the investigations of physicochemical properties of PEGylated ZnO NPs dispersed in PD fluid using nuclear magnetic resonance (NMR) spectroscopy, dynamic light scattering (DLS), transmission electron microscopy (TEM), and Fourier transform infrared (FT-IR) spectroscopy. The interaction of PEGylated ZnO NPs has also been studied separately in glucose and lactic acid which are the main constituents of PD fluid and in citric acid. Although the X-ray diffraction and TEM results infer the colloidal stability of PEGylated ZnO NPs in PD fluid, FT-IR, UV−vis, and nuclear magnetic resonance results revealed the binding interactions of PEGylated ZnO NPs with the PD constituents. PEGylated ZnO NPs also interact strongly with the lactic acid and citric acid, leading to agglomeration, as observed previously for uncoated ZnO NPs. Further, the antibacterial activities of bare and PEG-coated ZnO NPs dispersion in PD fluid have been studied. A reduction in the bacterial inhibition effect against Staphylococcus aureus and Escherichia coli was observed for both the bare and PEG-coated ZnO NPs dispersed in PD fluid, indicating that the complex nature of PD fluid counteract on the efficiency of these nanobiotics.

INTRODUCTION

Peritoneal dialysis (PD) is a well-established therapy used for the treatment of patients with end-stage renal failure.1 Prolonged PD therapy is often associated with a high risk of infection of the peritoneum, subcutaneous tunnel, and catheter exit site.2,3 The recurrent and persistent infections many times lead to malfunctioning of peritoneal membrane, a condition known as infectious peritonitis, which is the main cause of mortality and morbidity in PD patients.4 Further, owing to weak and sabotaged immune system, the PD patients frequently require higher antibiotic dose to resolve the infection condition. However, the frequent intraperitoneal administration of higher antibiotic doses and their absorption through the peritoneum lead to serious side effects including peritoneal malfunctioning, hepatotoxicity, and multiple drug resistance (MDR). Therefore, there is an unmet need for alternative advances other than antibiotics to deal with the MDR bacterial strains in PD patients and hence to reduce the frequency of life-threatening episodes of infectious peritonitis. Owing to their broad spectrum antimicrobial activity, the metal oxide nanoparticles (NPs) have been proposed as alternative agents for the management of PD-related infections.5 Among the various metal oxide antimicrobial nanomaterials, zinc oxide NPs (ZnO NPs) exhibit excellent antibacterial and antifungal activities.7−10 But, before incorporation of any NPs in biological systems, the understanding of their stability and interaction with the biological fluids such as plasma and serum is very much essential.

Generally, the NPs surface could adsorb the components of biological fluids (e.g., electrolytes, proteins, lipids, and

Supporting Information

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metabolites) leading to the formation of a protein corona (PC) which may affect their antimicrobial activity.\textsuperscript{13–17} PD fluid is also a complex mixture of sodium, chloride, calcium, magnesium, lactic acid, and glucose which may get adsorbed onto the surfaces of NPs. Recently, we have investigated the detailed physicochemical properties of ZnO NPs dispersed in sterile PD fluid to explore if any such interactions and adsorption occur.\textsuperscript{18} It is found that ZnO NPs selectively interacts and binds with lactic acid, the main constituent of PD fluid rendering the NPs with a new biological identity. We presume that the surface modification of ZnO NPs may decrease the tendency of ZnO NPs to interact with the components of PD fluid. Among the various types of polymer coatings, the surface coating of NPs with polyethylene glycol (PEG) was found to be efficacious, making the NP system biologically compatible and partly reducing their cytotoxicity. In vivo compatibility is imparted owing to its ability to enhance the solution solubility and to limit the binding of NPs to blood proteins and macrophages, as well.\textsuperscript{19–21} Therefore, in the present study, we first carried out the surface coating of ZnO NPs with PEG, followed by the investigation of the effect of such surface modification on their colloidal stability in PD fluid and interaction with PD constituents. The interaction study of PEGylated ZnO NPs has also been carried out separately in glucose and lactic acid, which are the main constituents of PD fluid and in citric acid, the most abundant organic acid found in biological fluids. Further, the antimicrobial activity of ZnO NPs and PEG-capped ZnO NPs dispersed in PD fluid has been carried out and presented here.

## RESULTS AND DISCUSSION

PEGylated ZnO NPs have been obtained by adsorption of PEG molecules onto the surface of ZnO NPs (Figure S1, Supporting Information). The adsorption of the PEG was confirmed using thermogravimetric analysis (TGA). The TGA curves shown in Figure S2 for PEG-6000- and PEG-200-coated ZnO NPs reveals significant weight loss during the TGA test with two successive thermal transitions for both of the samples. The first transition up to 150 °C is attributed to water molecules on the surface of NPs, and the second consecutive transition observed in the range of 380–450 °C can be attributed to the decomposition of PEG molecules from the surface of ZnO NPs, as reported previously.\textsuperscript{22,23} The TGA curve depicts a net weight loss of 3 and 2.62% for PEG-6000- and PEG-200-coated ZnO NPs, respectively. The corresponding molar percentage of adsorbed PEG polymer on ZnO NPs was found to be $5 \times 10^{-4}$ and $1.3 \times 10^{-2}$ for PEG-6000 and PEG-200, respectively. These results revealed that the amount of adsorption of PEG molecules on the surface of NPs decreases with increase in the molecular weight of PEG which is in agreement with the previous studies.\textsuperscript{22} Figure 1A,B shows the X-ray diffraction (XRD) patterns of bare ZnO NPs and PEG-6000-capped ZnO NPs. The observed peaks in the XRD pattern of ZnO NPs are well-indexed to hexagonal wurzite structure.\textsuperscript{19} The absence of any other characteristic impurity peaks confirms the phase purity of the NPs. It can be seen that the PEG-6000-capped ZnO NPs have similar XRD pattern as that of ZnO NPs indicating no effect of surface coating on the crystal structure of ZnO NPs. Further, we do not find any significant change in XRD patterns of PEGylated ZnO NPs dispersed in PD fluid, lactic acid, glucose, and citric acid, as shown in Figure 1C–F, respectively, inferring no influence on the crystallinity of ZnO NPs. The retention of crystallinity of PEGylated ZnO NPs in the presence of PD fluid, lactic acid, and citric acid is an interesting observation owing to the fact that the uncapped/bare ZnO NPs have lost their crystallinity to a maximum extent in the presence of these biological fluids.\textsuperscript{18} Similar results were observed for PEG-200-capped ZnO NPs, as depicted in Figure S3A–F.

The size and morphological features of ZnO NPs, and PEG-6000- and PEG-200-capped ZnO NPs dispersed in deionized water, PD fluid, glucose, lactic acid, and citric acid were determined using transmission electron microscopy (TEM) (as shown in Figures 2A–F and S4A–F). It can be seen that bare ZnO NPs are well-dispersed having a spherical morphology with some elongated particles and particle-size distribution in the range of 20–50 nm, as shown in Figure 2G, with the average particle size of 31 ± 8 nm. Although a small degree of aggregation is seen for PEGylated ZnO NPs, we do not find any significant change in particle size both for PEG-6000 and PEG-200 capping. Although no morphological change was observed for PEGylated ZnO NPs dispersed in PD fluid and glucose, a complete agglomeration-forming clusters of NPs were seen for PEGylated ZnO NPs dispersed in lactic and citric acid similar to what was observed previously for bare ZnO NPs dispersed in lactic and citric acid.\textsuperscript{18} Strikingly, PEGylated ZnO NPs dispersed in PD fluid do not lead to any agglomeration which is contrary to the previous results observed for bare NPs inferring the enhanced colloidal stability of surface-coated ZnO NPs in the PD fluid.

The FT-IR absorption spectra of PEG, ZnO NPs, and PEGylated ZnO NPs, shown in Figure S5, confirm the surface coating of ZnO NPs with PEG. The FT-IR spectrum of bare ZnO NPs showed the presence of Zn–O bond characteristic...
absorption peak at ∼525 cm⁻¹ and the broad band at ∼3440 cm⁻¹, assigned to the characteristic absorption of the hydroxyl group (O–H). The surface adsorption of PEG on ZnO NPs is evident from the absorption peaks observed at 2930 and 2850 cm⁻¹ because of the asymmetric and symmetric C–H stretching mode of −CH₂− groups of PEG, C–O stretch coupled with C–C stretching peaks seen between 1050 and 1200 cm⁻¹, and C–O–H bending vibration at 1400 cm⁻¹, respectively.24 The peak observed at 830 cm⁻¹ can be attributed to C–CH aliphatic deformation vibration of the C–H bond. The interaction and binding of PEGylated ZnO NPs with PD fluid, glucose, lactic acid, and citric acid were also investigated using FT-IR spectroscopy (Figure 3). We do not find any significant change in the FT-IR spectra of PEGylated ZnO NPs dispersed in glucose, as shown in Figure 3A, respectively, inferring no interaction of PEGylated ZnO NPs with glucose. However, the FTIR spectra of PEGylated ZnO NPs dispersed in PD fluid, lactic acid, and citric acid show a redshift of ∼130, 140, and 170 cm⁻¹ in the C==O stretching band with respect to that observed at 1730 cm⁻¹ for free lactic acid and citric acid, confirming the interaction and binding of lactic acid and citric acid molecules with PEGylated ZnO NPs. Table S1 depicts the comparison of the observed C==O stretching frequency in the FT-IR spectra of bare ZnO NPs, PEG-6000-coated ZnO NPs, and PEG-200-coated ZnO NPs dispersed in various media. The observed redshift in the FT-IR spectra of PEG-6000-coated ZnO NPs and PEG-200-coated ZnO NPs dispersed in PD fluid, lactic acid, and citric acid is similar to the values, as reported previously, for bare ZnO NPs dispersed in PD fluid, lactic acid, and citric acid.18

The size and surface properties of PEG-6000- and PEG-200-capped ZnO NPs dispersed in PD fluid were further investigated using dynamic light scattering (DLS) technique. The corresponding zeta size and zeta potential are enlisted in Tables 1 and S2. The average particle size distribution and zeta potential for bare ZnO NPs in deionized water was 69 nm and
36.6 mV, respectively. After PEGylation, the average size distribution was found to be ~485 nm for PEG-6000-capped ZnO NPs because of the increase in the surface hydrophilicity leading to enhanced hydrodynamic diameter. The observed decrease in surface charge of ZnO NPs after PEGylation is in accordance with the previous reports. Further, we do not observe any significant change in size distribution for PEG-coated ZnO NPs dispersed in glucose. However, a significant increase in size distribution was observed for PEG-coated ZnO NPs dispersed in PD fluid, lactic acid, and citric acid, as shown in Table 1. The observed discrepancy in the DLS and TEM particle size distribution of NPs dispersed in PD fluid could be because of the effect of altered surface conjugation/interaction of NPs in the PD fluid along with the small extent of aggregation, as seen in TEM images. DLS and TEM have been reported to give similar particle size distribution for monodisperse samples; however, the DLS results contradict with TEM analysis for polydisperse samples. This is because of the fact that DLS is an intensity-based technique, and the measured analytical signal in DLS is directly proportional to the sixth power of particle diameter \( d^6 \). Therefore, for polydisperse systems, the scattered light from larger particles or aggregates will strongly mask the weaker light scattering from the smaller NPs. Further, the diffusion coefficient of NPs has also been reported to change upon the conjugation/interaction of biomolecules with the surface of NPs, which also affects the DLS measurements significantly. Zeta potential of PEG-6000-coated ZnO NPs has been found to decrease to +3.44, −4.37, and +2.16 mV when dispersed in PD fluid, lactic acid, and citric acid, respectively. Similar decrease in surface charge was observed previously for bare ZnO NPs. These results suggest that PEGylation of ZnO NPs is not effective in preventing the lactate and citrate ion interaction and/or adsorption onto the particle surface.

The UV–vis absorption spectra of PEG-6000- and PEG-200-coated ZnO NPs dispersed in deionized water exhibit a characteristic absorption peak at a wavelength of 363 and 368 nm, respectively, as shown in Figure 5A, B. This peak has been found to be red-shifted by 5 and 10 nm when compared to bare ZnO NPs \( (\lambda_{\text{max}} \approx 358 \text{ nm}) \) which might be because of the adsorption of PEG on ZnO NPs surface. The band gap energy of PEG-6000- and PEG-200-coated ZnO NPs, as calculated using the Tauc relation \( \epsilon h\nu = C(h\nu - E_g)^{1/2} \) comes out to be 3.26 and 3.19 eV, respectively, which is less than that observed for bare ZnO NPs (~3.29 eV), confirming the binding of PEG molecules onto the surface of ZnO NPs. Table S3 shows the comparison of the band gap energies for bare and PEGylated ZnO NPs dispersed in various media. The band gap energy was further found to be decreased for PEGylated ZnO NPs when dispersed in PD fluid, lactic acid, and citric acid similar to that observed previously for bare ZnO NPs inferring the interaction of PD constituents with PEG-coated ZnO NPs.

The time-course UV–vis absorption study of PEG-6000-capped ZnO NPs dispersed in deionized water and glucose does not reveal any change in the intensity of absorption maxima (data not shown for glucose), whereas the dispersion of these coated NPs in PD fluid (Figure 4A), lactic acid (Figure 4B), and citric acid (Figure 4C) showed a drop in the intensity of absorption maxima with increase in time, confirming the interaction of PEGylated NPs with lactate and citrate ions. Similar findings were observed for PEG-200-coated ZnO NPs, as shown in Figure S7. Further, the maxima has been observed to be red-shifted to 370 and 372 nm for PEG-6000- and PEG-200-coated ZnO NPs dispersed in the PD fluid, respectively, inferring the interaction of PD components with the NPs. Figure 4D–F shows the plots of change in absorption intensity as a function of time for PEG-6000-capped ZnO NPs dispersed in the PD fluid, lactic acid, and citric acid, respectively. Similar plots were shown for PEG-200-capped ZnO NPs dispersed in PD fluid, lactic acid, and citric acid in Figure S7D, E and F, respectively. The curves were fitted with the following exponential association equation.

### Table 1. Zeta Potential and Size Distribution of PEG-6000-Capped ZnO NPs Dispersed in Deionized Water, Glucose, PD Fluid, Lactic Acid, and Citric Acid

| PEGylated ZnO dispersed in | zeta potential (mV) | average size distribution (nm) |
|----------------------------|---------------------|-------------------------------|
| deionized water            | +30.3               | 485                           |
| glucose                    | +18.8               | 557                           |
| PD fluid                   | +3.4                | 2028                          |
| lactic acid                | −4.4                | 1133                          |
| citric acid                | +2.2                | 1149                          |

Figure 4. Time course of UV–vis absorption spectra of PEG-6000-capped ZnO NPs dispersed in (A) PD fluid, (B) lactic acid, and (C) citric acid. Change in absorption intensity plotted as a function of time for PEGylated ZnO NPs dispersed in (D) PD fluid, (E) lactic acid, and (F) citric acid.
based on Levenberg–Marquardt algorithm of Microcal Origin 7.5 (inset of Figure 4D–F) to determine the reaction kinetics

\[ I_t = I_0 + A_1 \left[ 1 - \exp \left( 1 - \frac{t}{t_1} \right) \right] \]

where \( I_0 \) and \( I_t \) are the absorption intensities at time zero and \( t \), respectively. The constant \( A_1 \) is the relative contribution and \( t_1 \) is the corresponding time constant of mechanism involved in interaction between PEGylated ZnO NPs and the solution constituents. The value for \( t_1 \) comes out to be 62 ± 7, 15 ± 2, and 14 ± 1 min for PEG-6000-capped ZnO NPs dispersed in PD fluid, lactic acid, and citric acid, respectively. The obtained \( t_1 \) values were compared with those reported for bare ZnO NPs, as demonstrated in Table S3. We found that the \( t_1 \) value for PEGylated ZnO NPs dispersed in PD fluid has been found to be two times greater than the values reported for bare ZnO NPs as dispersed in PD fluid. The increase in the time of interaction between PEGylated ZnO NPs and the components of PD fluid suggest that the PEG surface coating affects the interaction of ZnO NPs with PD constituents but do not completely suppress the interaction.

One-dimensional (1D) proton nuclear magnetic resonance (NMR) spectroscopic experiments were also carried out to study the interaction of PEGylated ZnO NPs dispersed in PD fluid, lactic acid, and citric acid. Figure 5A–C shows the stacked 1H NMR spectra of PD fluid, lactic acid, and citric acid containing different concentrations of PEG-6000-capped ZnO NPs (0.0, 0.5, 1, 2, 4, 6, and 8 mg/mL). A significant broadening of lactate resonances (at 1.3 and 4.1 ppm) can be seen with a very small change in the intensity of these peaks of NMR spectra of PD fluid up to 4 mg PEGylated ZnO NPs PD dispersion above which a sharp decrease in the intensity was marked. On the other hand, a significant decrease in the intensity of lactate and citrate resonances was observed even for 0.5 mg PEG-6000-capped ZnO NPs dispersed in lactic acid and citric acid, respectively. Similar results were observed for PEG-200-capped ZnO NPs dispersed in PD fluid, lactic acid, and citric acid, as shown in Figure S8A–C, respectively.

The reduced intensity of lactate and citrate resonances points toward the interaction of lactate and citrate ions with PEGylated ZnO NPs similar to that observed previously for bare ZnO NPs. The minimal intensity changes upon addition of the PEGylated ZnO NPs up to 4 mg and increased \( t_1 \) values along with their dispersed nature in PD fluid in TEM images, essentially establish that the surface modification/PEGylation enhanced the colloidal stability to a significant extent when these NPs are present at low concentrations in PD fluid.

The antibacterial activities of ZnO NPs and PEGylated ZnO NPs colloidal suspensions were investigated against the
*Staphylococcus aureus* and *Escherichia coli* strains, and the plots of inhibitory rate as a function of time are shown in Figures 6

Figure 6. Inhibition rate of (A) *S. aureus* and (B) *E. coli* in the presence of ZnO NPs, PEG-6000-coated ZnO NPs, PEG-200-coated ZnO NPs, ZnO NPs dispersed in PD fluid, PEG-6000-, and PEG-200-coated ZnO NPs dispersed in the PD fluid, each having the concentration of 0.5 mg/mL.

and S9 for 0.5 and 1 mg/mL concentration, respectively. Inhibitory rate represents the change in absorbance with respect to control (no treatment). We observed a small decrease of 10–20% in the inhibition rate of PEGylated ZnO NPs when compared to bare ZnO NPs. The observed decrease in the inhibition effect of PEGylated ZnO NPs compared to bare ZnO NPs is in line with the previous studies where cytotoxicity of PEG-coated ZnO NPs has been reported to reduce because of the decreased cellular uptake arising from a minimal PC. We have also measured the growth curves for PEG-6000 and PEG-200 polymers (with the concentration of 0.2 mg/mL equivalent to the percentage molar mass of PEG-200 on the surface of ZnO NPs, as obtained from the TGA results) for both *S. aureus* and *E. coli* strains, and the results are presented in Figure S10A,B, respectively. No change in growth was seen for both the PEG-6000 and PEG-200 polymers when compared to control. To elucidate the nature of activity of ZnO NPs, growth curves have been measured for ZnO-treated (0.5 mg/mL) *S. aureus* and *E. coli* bacterial strains till 48 h (Figure S11). No growth of bacterial cells up to 48 h revealed the bactericidal activity of ZnO NPs. The colloidal suspension of ZnO NPs and PEGylated ZnO NPs in PD fluid exhibited reduced inhibitory effect against both the *S. aureus* and *E. coli*, as shown in Figure 6A,B, respectively. Although for *S. aureus*, the inhibition of NP suspensions in the PD fluid was similar to that in water up to 2 h and decreases with further increase in time, a significant decrease in the inhibition mainly for PEGylated ZnO NPs was seen for *E. coli* from the starting time point.

The increase in the multidrug resistance of many well-known antibiotics against various bacterial strains poses a major challenge for the treatment of infectious diseases, resulting in severe morbidity and mortality of such patients. Antimicrobial NPs are a promising substitute to antibiotics having the potential to combat the infectious diseases. However, the prompt interaction of NPs with peptides, proteins, and lipids in biological media leading to the formation of PC on their surface strongly impact the biodistribution, clearance, activity, and toxicity of such NPs. Further, the adsorption and interaction of proteins onto the surface of such NPs have been reported to induce the structural and/or conformational changes of such proteins. As reported previously by our group, PD fluid being a complex mixture similar to biological fluids also exhibits the ability to interact with ZnO NPs, rendering the NPs with a completely new biological identity. Another important aspect required to be investigated is the effect of such interactions on the antibacterial activities of ZnO NPs, which will further enlighten the use of such NPs in PD-related infections. Therefore, the antimicrobial activity of the formed biological entity of ZnO NPs in PD fluid has been studied in the present work. Further, it was proposed that the surface coating of ZnO NPs may inhibit the interaction of PD components with NPs. Therefore, the surface coating of ZnO NPs with PEG polymers has been carried out in the present study, followed by the investigation of physicochemical properties of obtained PEGylated ZnO NPs when dispersed in the PD fluid.

Although the XRD and TEM data demonstrate the colloidal stability of PEG-capped ZnO NPs in the PD fluid, UV–vis, FT-IR, and NMR results reveal some sort of interaction of PEGylated ZnO NPs with PD constituents. In contrast to the PD fluid, we have seen a clear agglomeration of PEGylated ZnO NPs with PD constituents. As reported previously by our group, PD fluids also exhibit the ability to form PC on their surface strongly impact the biodistribution, clearance, activity, and toxicity of such NPs, having the potential to combat the infectious diseases. Antimicrobial NPs are a promising substitute to antibiotics resulting in severe morbidity and mortality of such patients. However, the interactions of NPs with bacterial membrane, dissolution of ZnO to Zn2+ ions, and the production of ROS, resulting in different cytotoxicity and genotoxicity, when compared to its bare counterpart. The antimicrobial activities of many NPs have been reported to diminish in blood serum because of the interaction of NPs with serum proteins. For instance, Divya et al. have shown that the silver (Ag) NPs lost their antibacterial properties against the bacteria grown in Luria-Bertani (LB) broth because of their interaction with serum proteins present in the medium. Therefore, the observed reduction of the antibacterial activities of bare and PEGylated ZnO NPs in PD fluid in the present study could be attributed.
to the surface modification of NPs with PD constituents, resulting in the suppression of direct interaction of NPs with the cell surfaces and thus the decrease in dissolution to Zn\(^{2+}\) ions and/or the release of ROS. It is seen that even PEG capping of ZnO NPs does not retain their antimicrobial activities in PD fluid. Few studies have shown that although the PEGylation of NPs typically reduce the nonspecific interactions of NPs with serum proteins when compared to their non-PEGylated counterparts; however, it does not completely rule out the possibility of the interaction of serum proteins with the underlying reactive surfaces.\(^{25}\) Similarly, the PEG coating of ZnO NPs may not completely prevent the interaction of PD constituents with ZnO NPs, and thus affect their antibacterial activities.

Although we have seen a reduction in the antibacterial activities of ZnO NPs when dispersed in peritoneal dialysate when compared to ZnO suspension in water, the antibacterial effect is not completely diminished despite the complex nature of the PD fluid. The potential of these NPs in combination with low-dose antibiotics can further be evaluated for PD-related infections which may help combat the growing resistance against currently used antibiotics. Also, the development of stable and robust noble NP-based formulations are required, which could be highly effective against the bacterial strains and hence can be used for the treatment of infectious peritonitis.

### CONCLUSIONS

In the present study, we have investigated the physicochemical and antibacterial properties of PEGylated ZnO NPs dispersed in PD fluid using various experimental techniques. XRD and TEM results revealed the colloidal stability of both the PEG-6000- and PEG-200-capped ZnO NPs in PD fluid in contrast to bare ZnO NPs, indicating a substantial role of PEG in acquiring the stability in the PD fluid. UV–vis, FT-IR, and NMR spectroscopy demonstrated the interaction of PEGylated ZnO NPs with PD constituents which infers that surface coating with PEG does not completely rule out the possibility of binding and/or interaction of PD components with ZnO NPs. Similar interaction of PEGylated ZnO NPs with lactic and citric acid was also revealed, leading to the agglomeration of NPs, as observed through TEM images. Further, the antibacterial properties of bare and PEG-coated ZnO NPs dispersion in the PD fluid were investigated. It has been demonstrated that although both the bare and PEGylated ZnO NPs exhibit excellent antibacterial properties against the S. aureus and E. coli bacterial strains, their efficacy gets reduced when dispersed in the PD fluid. All of these results suggest that PEG surface coating of ZnO NPs was unable to prevent the interaction of PD constituents with NPs, and their antibacterial activities get reduced when dispersed in PD fluid, although not completely diminished. The synergistic effect of antibiotics in combination with ZnO NPs can be investigated in future because it may help combat the growing resistance of PD infections against antibiotics.

### EXPERIMENTAL SECTION

#### Materials

Zinc oxide NPs dispersion purchased from Sigma-Aldrich (catalog no. 721077) with reported particle size less than 100 nm (measured by DLS) and an average particle size less than 40 nm (Aerodynamic Particle Seizer, APS) was used in this study. PEG-200 and PEG-6000 used for the surface modification of ZnO NPs were procured from Loba Chemie (CAS no. 25322-68-3) and S-D fine-Chem Limited (product code 3957P25; CASR no. 25322-68-3), respectively. PD solution IP (DIANEAL PD-2 with 2.5% w/v dextrose) was obtained from Baxter Healthcare. The sodium salt of trimethylsilylpropionic acid-d\(_4\) (TSP) and deuterium oxide (D\(_2\)O) used in NMR spectroscopy were procured from Sigma-Aldrich (DH5-Alpha) strains were used in antibacterial assessment of ZnO NPs. LB media purchased from Himedia (REF M575-500G) was used for the bacterial strain growth.

#### EXPERIMENTAL SECTION

**PEGylation of Zinc Oxide NPs.** The surface capping of ZnO NPs with PEG was carried out, as reported previously by Nabiyouni et al., with slight modifications.\(^{22}\) Briefly, 0.5 g of ZnO NPs was suspended in 50 cm\(^3\) of distilled water in a round bottom flask. PEG-200 (1.6 mL) was dissolved in 90 cm\(^3\) of distilled water separately and then added to the above suspension of ZnO NPs. The mixture was then kept for magnetic stirring (Glassco 1500.E.U.01) at room temperature for 48 h and left undisturbed for the next 48 h (illustrated in Figure S1). The particles were collected and washed with distilled water 3–4 times. The unreacted PEG molecules were removed from the suspension by membrane dialysis (3 kDa MWCO), followed by centrifugation (HERMLE Labortechnik Centrifuge Z326 K). The as-obtained powder was dried in an oven at 90 °C for 6 h and used for further studies. The aforementioned steps were repeated for coating with PEG-6000.

**CHARACTERIZATION**

**X-Ray Diffraction.** The structure and crystallinity of all samples were determined from the powder XRD patterns obtained at Bruker D8 X-ray diffractometer (equipped with a Cu K\(_{α1}\) source at 40 kV and 30 mA).\(^{3}\) The samples were placed in a glass holder and scanned from 10° to 90° with a scanning rate of 2.0°/min.

**Transmission Electron Microscopy.** The morphological characterization of PEGylated ZnO NPs and its dispersion in PD fluid, citric acid, lactic acid, and glucose were carried out by Tecnai G\(^2\) 20 S-TWIN Transmission Electron Microscope (FEI Netherlands) with an acceleration voltage of 200 kV. The samples for TEM analysis were prepared by depositing two to three droplets of dilute solution of the NPs onto a carbon-coated copper grid (Icon Analytical Equipment Pvt. Ltd).

**FT-IR Spectroscopy.** All samples were characterized by an Fourier transform infrared (FT-IR) spectrometer (PerkinElmer Frontier Instruments). FT-IR was performed on pressed pellets made of KBr and dried sample powder. The FT-IR spectrum was collected between 450 and 4000 cm\(^{-1}\).

**UV–Visible Spectroscopy.** UV–visible absorption spectra were acquired between the wavelength range of 200–800 nm at different time intervals using an Agilent Cary 300 (double-beam UV–vis spectrophotometer) with a spectral bandwidth of 2 nm. Quartz cuvette of 1 cm path length was used in this experiment.

**Particle Size Distribution and Zeta Potential Measurements.** Size distributions of the NPs and their zeta potential were determined with a Zetasizer Nano ZS90 (Malvern Instruments Inc, UK) by using DLS and electrophoretic light scattering (ELS).
Thermogravimetric Analysis. TGA was carried out using a TG analyzer (EXSTAR TG/DTA 6300) by heating the samples under nitrogen flow (200 mL/min). Approximately, 10 mg of the NPs was placed in an alumina crucible on the pan of the microbalance and heated from room temperature to 650 °C at a rate of 10 °C/min, using Al2O3 powder as a reference material.

NMR Spectroscopy. 1D 1H NMR spectra were acquired on a Bruker AVANCE III 400 MHz NMR spectrometer equipped with Smart BBFO probe with z-axis gradient at 298 K using CPMG (Carr–Purcell–Meiboom–Gill) pulse sequence (cpmgrp1d, standard Bruker pulse program) with the presaturation of water. NMR tubes (5 mm) (Wilmad Glass, USA) filled with 400 μL of the samples were used to carry out the NMR spectroscopy. A sealed capillary tube carrying 0.1 mM TSP (sodium salt of 3-trimethylsilyl-(2, 2, 3, 3-d4)-propionic acid) in D2O was inserted inside NMR tubes for the purpose of chemical-shift referencing. The following parameters were used for 1D CPMG pulse sequence: spectral sweep width: 20 ppm; data points: 64 K; flip angle: 90°; total relaxation delay: 5 s; number of scans: 32; window function: exponential; and line broadening: 0.3 Hz. Topperspin-2.1 (Bruker NMR data Processing Software) was used to process all of the spectra using standard FT procedure, followed by manual-phase and baseline correction. Chemical shifts were referenced to TSP methyl protons at 0.0 ppm (part per million).

Antimicrobial Study. Antimicrobial activity of ZnO NPs and PEGylated ZnO NPs was examined using growth inhibition studies against the Gram-positive S. aureus (MTCC-3160) and Gram-negative E. coli (DH5-Alpha) bacterial strains. All of the materials were freshly prepared and sterilized using an autoclave. To perform the antimicrobial experiments, the S. aureus and E. Coli strains were grown aerobically at 37 °C in 10 mL of LB medium in 18 mm × 150 mm borosilicate glass culture tubes (BOROSIL) with continuous shaking at 220 rpm under normal laboratory conditions. The optical density (OD) of culture at 600 nm wavelength was considered as cell growth measure, which was recorded using an Agilent Cary 300 UV–vis spectrophotometer. Briefly, the bacterial culture was grown in 10 mL of freshly prepared and autoclaved LB media up to ~0.6 OD at 600 nm wavelength. After that, this culture was used as a seed for 1 L of LB media. The culture was incubated under the same laboratory condition (as mentioned above) until OD reached again to 0.6. This bacterial culture was then aliquoted (5 mL each) in different culture tubes. The antimicrobial test was performed on these bacterial culture aliquots treated with 0.5 and 1 mg/mL of bare ZnO NPs and PEGylated ZnO NPs dispersed in water. The inhibition of bacterial growth was monitored every 2 h under UV–vis spectrophotometer by measuring the respective change in OD for three replicates. A similar study was carried out for both the ZnO and PEGylated ZnO NPs dispersed in the PD fluid.

ASSOCIATED CONTENT
Supporting Information
The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.9b02615.

Scheme illustrating the PEGylation of ZnO NPs; TGA of PEG-coated ZnO NPs; XRD pattern of PEG-200-coated ZnO NPs dispersed in various media; TEM images of PEG-200-coated ZnO NPs dispersed in various media; FT-IR spectra of bare and PEGylated ZnO NPs; comparison of the observed C=O stretching frequency in the FT-IR spectra; zeta potential and size distribution of PEG-200-capped ZnO NPs dispersed in various media; time course of UV–vis absorption spectra of PEGylated ZnO NPs; band gap energy of bare and PEGylated ZnO NPs dispersed in various media; time course of UV–vis absorption spectra of PEG-200-capped ZnO NPs inhibition rate of S. aureus and E. coli in the presence of ZnO NPs and PEGylated ZnO NPs each having the concentration of 1 mg/mL; growth curves of control, PEG-6000, and PEG-200 polymer-treated S. aureus and E. coli; growth curves of control and ZnO NPs-treated S. aureus and E. coli (PDF)

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Notes
The authors declare no competing financial interest.

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