

ZnO—Ag Hybrid Nanoparticles Used in the Antimicrobial Solvent-Based Coatings: Antibacterial Studies in the Darkness and Under Visible-Light Irradiation

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This study explores how hybrid nanoparticles can be used to improve the antibacteial properties of both single nanoparticles and their polymeric nanocomposite coatings. Hybridization of two different nanoparicles, such as nano-ZnO and silver nanoparticles (AgNPs) has been carried out to combine advantages of the individual particles. For these purposes, firstly ZnO-Ag hybrid nanoparticles were chemically fabricated by reducing Ag⁺ precursor on the as-received nano-ZnO using sodium borohydride in aqueous medium. Thereafter, these ZnO-Ag nanohybrids were introduced into the acrylic polyurethane matrix (at 2 wt.%) under sonication in xylene/toluene solvents. To reveal the effect of hybridization on the antibacterial activity against E. coli of both ZnO-Ag nanohybrids and their nanocomposite coatings, two antibacterial tests have been carrieried out in presence of visible light irradiation or without light (in dark). The agar-well diffusion method indicated that ZnO-Ag nanohybrids exhibited high antibacterial activity against E.coli at the low concentration (8 mg/mL). In addition, their larger inhibition zones under visible light exposure were observed,

Introduction

The organic coatings based on acrylic polyol resin and the curing agent polyisocyanate have been widely applied as topcoat in many fields, not only for indoor but also for outdoor applications, such as structural steelwork protection, paints for motorcycles and wood furniture.^[1,2] Incorporation of nanoparticles into the organic coatings can not only enhance their mechanical property,^[3] but also provide the antibacterial

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when compared to the dark condition. Similarity, antibacterial test (ISO 22196:2007 standard) indicated that nanocomposite coating under visible light exposure had a higher antibacterial activity than that in the dark condition. Data from this antibacterial test after 24 h indicated that the visible light exposure provided more bactericidal efficiency for APU/ ZnO–Ag coating (4.17 log), as compared to the dark condition (4.07 log). This increase in the bactericidal efficiency can be attributed to the hybridization of nano-ZnO and AgNPs in their hybrid nanostructure. From the experimental data, we propose the mechanism for antibacterial activity of ZnO-Ag hybrid nanoparticles. In addition, TEM photographs indicated that AgNPs (10-30 nm) were attached to the surface of nano-ZnO (<100 nm). Data from the diffused reflectance spectra indicated that the deposition of AgNPs on nano-ZnO reduced its band gap energy (E_{a}) from 3.2 eV to 2.75 eV. In case of nanocomposite coating, addition of 2 wt.% ZnO-Ag nanohybrids into the acrylic polyurethane matrix significantly increased their impact strength and abrasion resistance.

activity.^[4] In this direction, multifunctional coatings can be fabricated simply by using the inorganic nanoparticles. For example, as nanofillers, nano-TiO₂ (rutile) and nano-SiO₂ can be used to increase weathering resistance of organic coatings.^[2,5-7] The nano-TiO₂ (anatase) and nano-ZnO were used for antibacterial self-cleaning coatings.^[8-11] In case of nano silver particles (AgNPs), they have been proved to exhibit strong antibacterial property against a wide range of bacteria.

Traditionally, combination of ZnO and Ag in their composites, has been carried out by two main purposes: i) ZnO can act as supporting material for loading of Ag; and ii) Ag can act as doping material for defeating the large band gap of ZnO. The former purpose aims to control the solubility and toxicity of AgNPs, metal oxides have been used as supporting materials for loading of AgNPs.^[12] Whereas, in the latter purpose, the presence of Ag can enhance both antibacterial and photocatalytic behaviors of ZnO, especially under visible light irradiation (instead of using only UV light for pure ZnO). Thus, we expected that in the dark condition, AgNPs should provide a major contribution to the antibacterial activity of hybrid nanoparticles. Whereas, under visible light irradiation, nano-ZnO might contribute an extra contribution to the antibacterial activity of nanohybrids.

Recently, we have focused on the noble metal-metal oxide hybrid nanoparticles.^[13] Hybrid nanoparticles refers to the new



nanostructures, which are constructed from two different nanoparticles. Hybrid nanoparticles are fabricated due to several purposes, such as: i) to overcome the limits of individual nanoparticles, ii) to enhance their properties, iii) to achieve new property, and/or iv) to achieve the multi-functional nanoparticles. In this direction, hybridization of ZnO and Ag nanoparticles is new method to obtain higher antibacterial activity at lower content of single nanoparticles. This new approach has been reported recently in the literature for Fe_3O_4 –Ag,^[14,15] SiO₂–Ag,^[16] and TiO₂–Ag.^[17] There are several methods for synthesis of hybrid nanoparticles.^[18] Among them, chemical method has been considered as the simple and effective method for synthesis of ZnO–Ag nanohybrids.^[16,17]

In our previous works, ZnO–Ag nanohybrids have been used to provide antibacterial activity for polyethylene plastic,^[19] waterborne coating^[20] and photo-cured coating.^[21] In this study, we introduce these ZnO–Ag nanohybrids into the solventbased coating (acrylic polyol resin- based coating). This study is the first work that focuses on the comparison of antibacterial activity for organic coating in darkness or under visible light exposure.

Results and discussion

Characterization of ZnO-Ag nanohybrids

The microstructure of nanohybrid ZnO–Ag was evaluated by FE-SEM and TEM. The results were represented by Figure 1. Figure 1 showed that the as-received nano-ZnO has cylinder shape (width: 30-70 nm, length: 100-200 nm). The AgNPs in the spherical shape (size of 10-30 nm) was attached to the nano-ZnO. EDX diagram of ZnO–Ag hybrid nanoparticles is demonstrated in Figure 2. From this Figure, we can estimate the content of Ag in ZnO–Ag hybrid is about 1.2 wt.%. This content was lower than that in the ZnO–Ag hybrid fabricated previouly by seed-mediated growth method (8.55 wt.%).^[21] In the seed-mediated growth method, the same weight ratio of AgNO₃ : ZnO was used as the initial precusors.

Figure 3 presents the XRD patterns of nano-ZnO and ZnO–Ag hybrid nanoparticles. As can be seen in Figure 2, the as-received nano-ZnO exhibits the characteristic planes of (100), (002), (101), (102), (110), (103), (200) (112), (201), (004) and (202), which are indexed to the hexagonal wurtzite structure of ZnO (JCDPS file no. 36-1451). Whereas, the as-prepared ZnO–Ag nanohybrids contain not only the diffraction



Figure 1. FE-SEM (a) TEM images (b) of self-synthesized ZnO-Ag hybrid nanoparticles.



Figure 2. EDX diagram of ZnO–Ag hybrid nanoparticles nanoparticles.





Figure 3. XRD patterns of ZnO-Ag nanohybrids (ZnO-Ag) and ZnO nanoparticles (ZnO).

peaks of nano-ZnO (marked with "#" in black), but also the new diffraction peaks of AgNPs (marked with "*" in blue). For the contribution of AgNPs to the nanohybrids, their lattice planes of (111), (200) and (220) are attributed to the cubic structure of AgNPs (JCPDS file no. 04-0783).

To further verify the hybridization of Ag and ZnO nanoparticles, the E_g values of nano-ZnO before and after hybridization are calculated and compared (Figure 4). As shown in Figure 4, before hybridization, the E_g is 3.2 eV for as-received nano-ZnO (blue curve). Whereas, after hybridization, it reduces from 3.2 eV to 2.75 eV (red curve), due to the reduction of AgNO₃ precursor on the surface of nano-ZnO. This value of E_g was higher than that of ZnO–Ag hybrid fabricated previouly by



Figure 4. Eg values deducted from UV-Vis-DR spectra using the plots of (F(R)hv)2 vs photon energy (hv).^{[24,25]}

seed-mediated growth method (2.6 eV), due to the lower content of AgNPs in the nanohybrids (1.2 wt.% vs. 8.5 wt.%).^[21] Thus, this finding confirms the hybridization of ZnO and AgNPs. In comparison with other works, the E_g of nano-ZnO was only reduced from 3.25 eV to 3.18 eV, by Ag doping at 400 °C in the solid state milling and calcination of precursor powders.^[22] Similarity, for ZnO–Ag core-shell nanoparticles, the E_g value of nano-ZnO slightly reduced from 3.2 eV to 3.1 eV, once coated nano-ZnO by AgNPs.^[23] Thus, combination of TEM and UV-Vis-DR data confirmed the successful fabrication of ZnO–Ag nanohybrids.

Antibacterial activity of ZnO/Ag nanohybrids

Figure 5 presents the antibacterial results (agar-well diffusion method) of ZnO–Ag hybrid nanoparticles against *E. coli* bacteria, with or without visible light exposures. Table 1 shows their inhibition zones. As shown in Figure 5 and Table 1, control wells exhibited no inhibition zones with or without the presence of visible light. However, ZnO–Ag nanohybrids (at concentrations of 8–40 mg/mL) exhibited clearly the inhibition

Table 1. Antibacterial results for ZnO–Ag nanohybrids against E. coli bacteria.					
Concentration of nanohybrids	Inhibition zone (mm)				
(mg/mL)	In dark condition	under visible light exposure			
0	0	0			
8	2	8			
16	8	11			
40	10	13			





Figure 5. Photographs of the agar-well diffusion method for ZnO–Ag nanohybrids against *E. coli* bacteria. A) without visible light exposure; b) under visible light exposure; b) under visible light exposure). Concentration of nanohybrids: 0, 8, 16 and 40 mg/mL.

zones with or without light exposure. In other study, Ranjithkumar et al.^[26] reported the antimicrobial performance of Ag-doped ZnO nanoparticles (at concentrations of 0.1–50 mg/mL) against *E.coli* bacteria. As reported, 95% of toxicity to *E.coli* was obtained at the concentration of 50 mg/mL.

These inhibitory regions had extended when the concentration of nanoparticles increased. Interestingly, light irradiation provided the larger inhibition zones than that under dark condition (Table 1). This finding can be explained by the hybridization of AgNPs and nano-ZnO with reduction of bandgap energy (Figures 1 and 4).

Thus, the agar-well diffusion method indicated that ZnO–Ag nanohybrids provided a high antibacterial activity against *E.coli* at the low concentration (8 mg/mL). In addition,

their larger inhibition zones under light irradiation were observed, compared to that in the darkness.

In order to explain the reason why ZnO–Ag performed better under light irradiation, Figure 6 summerizes the antibacterial mechanisms of nano-ZnO (under UV irradiation), AgNPs and ZnO–Ag hybrid nanoparticles (in darkness or under light exposure). Regarding the lone nano-ZnO, as nanomaterials/nanoparticles, in general there are three possible pathways by which ZnO exhibit the antibacterial activity: ROS production, mechanical puncture/rupture of membranes, release of Zn²⁺ ions).^[27,28] Among them, ROS production has been considered as the major contribution to the antibacterial activity of nano-ZnO.^[4] However, ZnO has a wide band gap (3.2 eV), only UV light could be used for their activation in ROS production. In



Figure 6. Antibacterial mechanisms of nano-ZnO (under UV irradiation), AgNPs and ZnO-Ag hybrid nanoparticles (in darkness or under light exposure).

addition, this photocatalytic efficiency of lone nano-ZnO is also limited due to the high recombination rate of photo-generated electrons and holes.^[4] Under visible light irradiation, no antibacterial action was found for nano-ZnO (at concentrations of 8–40 mg/mL) against *E.coli* in the agar-well diffusion method.^[29]

For the lone AgNPs, the could attack bacteria simultaneously through various processes, such as (i) production of ROS, (ii) electrostatic interaction with the cell membrane, (iii) ion release, (iv) internalization.^[4,30,31] Thus, AgNPs can react against *E.coli* in both darkness and under light exposure.

In case of ZnO-Ag hybrid nanoparticle, it can kill E.coli in the synergic way by combination/hybridization of nano-ZnO and AgNPs. As result ZnO-Ag hybrid nanoparticle can kill E.coli in both the dark and under visible light iradiation. After hybridization with AgNPs, the band gap of ZnO is only 2.75 eV, which can be excited by photon from visible light iradiation (due to the visible-light localized surface plasmon resonance -LSPR of AgNPs in the nanohybrid).^[32] In addition, after hybridization with AgNPs, the Schottky barrier could minimize the recombination of photogenerated electron-hole pairs in the nanohybrid, thus enhance it photocatalytic activity.[32] Therefore, in the dark condition, AgNPs could provide a major contribution to the antibacterial activity of hybrid nanoparticles. Whereas, under visible light irradiation, nano-ZnO could contribute an extra contribution to the antibacterial activity of nanohybrids.

Effect of ZnO–Ag nanohybrids on the morphology and mechanical property of APU- based coatings

FE-SEM photographs of APU/ZnO–Ag nanocomposite coatings containing 2 wt.% and 4 wt.% of ZnO–Ag nanohybrids are presented in Figure 7. In Figure 7, we can see that at percentage of 2 wt.%, the nanohybrids dispersed fairly homogeneously into polymer base with an average size of 100 nm, the coatings have tight structure without any defects. Meanwhile, at high content (4 wt.%) when the agglomoration occurs, the average size is 500–600 nm. These results contribute to

explain the decline of mechanical properties of coatings at too high nanohybrid content (4 wt.%).

Gold, From the cross-cut test, the coating adhesion was determined. The obtained results yield a zero level (level 0/ classification 0) of detachment for APU/ZnO–Ag coating samples with the low contents of nanohybrids from 0 wt.% to 2 wt.%. However, coating adhesion is level 2 for the nano-composite coating with higher content of 4 wt.%, due to the aggregation of nanoparticles (as seen in Figure 7).

Figures 8 present the values of impact resistance and abrasion resistance for APU/ZnO-Ag nanocomposite coatings with various contents of nanohybrids, respectively. As shown in Figure 8, when the content of nanohybrid increase from 0 to 2 wt.%, the impact resistance and abrasion resistance rise proportionally with the nano hybrid content, improving from 180 kg.cm and 132 L/mil to 195 kg.cm and 186 L/mil, correspondingly, whilst the adhesion keeps unchanges. However, the further increase in ZnO-Ag nano hybrids content up to 4 wt.% led to the decrease of adhesion, impact resistance and abrasion resistance, from level 1, 195 Kg cm and 186 L/mil to level 2, 190 kg.cm and 153 L/mil, respectively. These enhancements can be explained by the fact that rigid nanohybrids (at suitable content \leq 2 wt.%) could fill the pores in coating. Thus, nanohybrids can play a role as reinforcement to improve the mechanical properties of the coating. Unfortunately, higher percentage of nanohybrids (e.g. 4 wt.%) may create the large agglomorations, leading to the weak (phase) interaction between nanohybrids and polymer base. This could be the reason why the mechanical properties of the coatings decreased when the nanohybrid content reached a high value of 4 wt.%.

Effect of ZnO–Ag nanohybrids in APU coating on its antibacterial activity

Growth rate of bacterial cultures in the darkness

Figure 9 shows the effect of ZnO–Ag nanohybrids in APU coating (2 wt%) on the growth rate of *E. coli* during the cell



Figure 7. FE-SEM photographs of APU/ZnO–Ag nanocomposite coatings: 2 wt.% (left) and 4 wt.% (right).



Figure 8. Values of impact resistance (a) and (b) Values of abrasion resistance for APU/ZnO-Ag nanocomposite coatings with various contents of nanohybrids.



Figure 9. Growth rate of E. coli bacteria in liquid culture without (control) or with the presence of the coating (APU)/nanocomposite coating (APU/ZnO-Ag).

cultures. As shown in Figure 8a, the growth rates of control culture and blank (mixed culture with APU neat coating) were nearly similar, after 2 hours of cultivation (77%). Their difference of 1% was found only after 150 and 180 minutes of cultivation. Thus, APU neat coating has no antibacterial activity against *E. Coli*.

However, in case of APU/ZnO–Ag coating (with 2 wt.% nanohybrids), it's growth rate of *E. Coli* was lower then that of both control culture and blank (mixed culture with APU coating). As can be observed in Figure 9, the log phase of the control culture and blank (mixed culture with APU) was 90% after 180 minutes of cultivation, whereas it was 84% when mixed the culture with APU/ZnO–Ag coating. Thus, the presence of ZnO–Ag nanohybrids in the APU coating matrix provided the low antibacterial activity, due to the low content of AgNPs (1.2 wt.% in the ZnO–Ag nanohybrids of the 2.wt.% APU/ZnO–Ag coating).

Viable bacteria counts under visible-light irradiation and in the darkness

In the previous section, the agar-well diffusion method indicated that ZnO–Ag hybrid nanoparticles exhibited high antibacterial activity against *E.coli* at the low concentration (8 mg/mL). Thus, incorporation of ZnO–Ag hybrid nanoparticles (at low content) into the APU coating matrix is expected to provide a good antibacterial activity.

For evaluation the antibacterial activity of coatings in the dark condition, AgNPs might provide a major contribution to the antibacterial activity of the coating, while nano-ZnO might serve only as the supporting oxides for dispersion of AgNPs into the coating matrix. However, under the visible light exposure, the portion of these nano-ZnO could contribute an extra antibacterial activity for the coating.



Figure 10 presented the number of *E. coli* viable cells for the neat APU coating after 24 h of incubation. After incubation, the number of *E. coli* viable cells was 4.3×10^4 CFU/cm², with the value of U_t was 4.63 log. In case of the APU/ZnO (Figure 11) and APU/ZnO–Ag (Figure 12) coatings in the dark condition, the numbers of viable cells was only 9.49 and 3.64 CFU/cm², respectively after 24 h of incubation. Thus, in dark condition, the incorporation of nanohybrids in polymer matrix provided a higher bactericidal efficiency (4.07 log) against *E. coli*, than that of nano-ZnO (3.65 log). Interestingly, under visible light exposure, the higher bactericidal efficiencies were observed



Figure 10. Viable *E. coli* counts for APU neat coating sample, after 24 h incubation: a) 10^{-4} dilution (66 CFU); b) 10^{-5} dilution (9 CFU; 4.3×10^4 CFU/cm²; $U_t = 4.63$)



Figure 11. Viable *E.coli* bacteria counts for APU/ZnO coating sample, after 24 hour incubation: a) in darkness (10^{-1} dilution; 17 CFU; 9.49 CFU/cm²; A_t=0.98); b) under visible light irradiation: (10^{-1} dilution; 13 CFU; 8.58 CFU/cm²; A_t=0.93)



Figure 12. Viable *E.coli* bacteria counts for APU/ZnO–Ag coating sample, after 24 hour incubation: a) in darkness (10^{-1} dilution; 8 CFU; 3.64 CFU/cm²; A_t=0.56); b) under visible light irradiation: (10^{-1} dilution; 5 CFU; 2.90 CFU/cm²; A_t=0.46).



after 24 h for both APU/ZnO–Ag (4.17 log) and APU/ZnO (3.7 log) coating samples.

Table 2 showed the antibacterial activity of neat APU and APU nanocomposite coatings against *E. coli* bacteria. As can be seen in Table 2, the visible light exposure provided more bactericidal efficiency for APU/ZnO–Ag coating (4.17 log), as compared to the dark condition (4.07 log). This increase in the bactericidal efficiency can be attributed to the hybridization of nano-ZnO and AgNPs in the hybrid nanostructures. Besides, the slight increase in bactericidal efficiency was also observed for APU/ZnO coating samples when exposure to visible light (an increase from 3.65 to 3.7 log).

Conclusions

ZnO–Ag hybrid nanoparticles were successfully synthesized by reduction of silver nitrate precursor on the as-received nano-ZnO, using sodium tetrahydridoborate in aqueous solution. TEM photographs indicated that AgNPs (10–30 nm) were attached to the surface of nano-ZnO (< 100 nm). XRD analysis confirmed the presence of both nano-ZnO and AgNPs in the crystal structure of ZnO–Ag hybrid nanoparticles. In addition, data from the diffused reflectance spectra indicated that the deposition of AgNPs on nano-ZnO reduced its E_g from 3.2 eV to 2.75 eV. The agar-well diffusion method indicated that ZnO–Ag nanohybrids exhibited high antibacterial activity against *E.coli* at the low concentration (8 mg/mL). In addition, their larger inhibition zones under visible light exposure were observed when compared to the dark condition.

FE-SEM images indicated that nanohybrids was dispersed homogeneously into acrylic polyurethane polymeric base, when their contents was lower than 2 wt.%. However, at high content (4 wt.%), large agglomerations were found on the surface of nanocomposite coating. Data from mechanical tests showed that addition of nanohybrid at content of 2 wt.% into acrylic polyurethane matrix enhanced significantly the mechanical properties of coatings (impact resistance increased from 180 to 196 kg.cm; abrasion resistance rose from 132 L/mil to 186 L/mil). Data from the antibacterial test of coatings after 24 h indicated that the visible light exposure provided more bactericidal efficiency for APU/ZnO–Ag coating (4.17 log), as compared to the dark condition (4.07 log). This increase in the

Table 2. Antibacterial results of neat APU and APU nanocomposite coat- ings against <i>E. coli</i> bacteria.								
Coating sample	es	After 24 h incubation		Antibacterial activity				
		CFU/cm ²	Log10					
APU neat coati	ng	4.3×10 ⁴	4.63	-				
APU/ZnO coating	Dark Visible light	9,49 8.58	0.98 0.93	3.65 3.70				
APU/ZnO—Ag coating	Dark Visible light	3.64 2.9	0.56 0.46	4.07 4.17				

bactericidal efficiency can be attributed to the hybridization of nano-ZnO and AgNPs in their hybrid nanostructure.

Experimental section

Preparation of ZnO-Ag nanohybrids

The ZnO–Ag nanohybrids were chemically fabricated by reducing of Ag⁺ precusor on the as-received nano-ZnO using sodium tetrahydridoborate (NaBH₄) in aqueous solution. Briefly, nano-ZnO (1.5 g) was firstly dispersed into distilled water (300 mL) under sonication (25 kHz for 60 min). Thereafter, 30 mL of AgNO₃ solution (1.67 mg/mL) was added slowly into the as-prepared nano-ZnO solution, under sonication (25 kHz for 30 min.). In the next step, NaBH₄ solution (0.024 g in 50 mL distilled water) was then added dropwise (1 drop/s) to the above mixture under stirring (120 rpm for 80 min. at 4 °C). The hybrid nanoparticles were collected, then purified.

Fabrication of coating samples

The coating samples with and without ZnO–Ag nanohybrids (at the contents of 0–4 wt.%) were prepared with below formulations as presented in Table 1. Firstly, the nanohybrids were dispersed into toluene and xylene solvent using ultrasonic bath TP-25 (Switzerland) in 2 hr. Then, HSU 1168 resin was added to be further dispersed for 1 hr. These resin samples were then mixed with curing agent using a stirrer in 10 min before being used.

The coatings with a thickness of about 30 μ m were deposited on Teflon sheets, CT3 steel coupons and glass plates by using a Quadruple Film Applicator (Enrichen model 360) for property and structural morphology analysis of coatings. The samples were left to dry out naturally at 30 °C in laboratory for 7 days before testing.

Characterization

Microstructures of ZnO–Ag hybrid nanoparticles and nanocomposite coatings were characterized by a Field emission scanning electron microscope (FE-SEM) (Hitachi, S 4800, Japan) and Transmission Electron Microscopy (TEM) (JEOL, Japan). To analyze the crystalline phases of hybrid nanoparticles, X-Ray powder diffraction (XRD) was used (D8-Advance XRD diffractometer Brucker). The values of bandgap energy (E_g) for ZnO and ZnO–Ag nanoparticles were estimated by using the diffused reflectance spectroscopy (UV-2600 spectrophotometer in reflection mode, UV-Vis-DR, Shimadzu, Japan).

For mechanical tests, the adhesion, impact resistance and abrasion resistance of the coatings were measured according to methods and the standards presented in the previous works. Briefly, the coating adhesion (on steel coupons) was evaluated by cutting test method (under ISO 2409 standard). The values of coating adhesion were classified from level #0 (best value of adhesion) to level #5 (worst value of adhesion). Coating's abrasion resistance was evaluated using the abrasive falling methods (under ASTM D968 standard). The ball impact tests were carried out by using Impact Tester (model 304, Erichsen, Germany) under standardized conditions (ISO 6272).



Antibacterial activity testing methods

Antibacterial evaluation for ZnO–Ag hybrid nanoparticles

To test the antibacterial activity of nanohybrids, the agar-well diffusion method was widely used. Specifically, 100 μ L of 10⁶ CFU *E.coli* bacterial suspension was added to agar plates with the presence of brain heart infusion (BHI) broth as culture medium. In each agar plate, 4 wells (8 mm diameter) were punched, then various amounts of nanohybrids in 50 μ L distilled water (e.g. 8, 16 and 40 mg/mL) were filled to these wells. For the remaining control well, it was filled by 50 μ L distilled water. After this stage, these agar plates were incubated at 37 °C, with or without light irradiation, for 18 hours. Finally, the diameter of inhibition zone was used to evaluate the antibacterial activity of nanohybrids.

Antibacterial evaluation for APU-based coatings in the darkness

To evaluate directly the effect of AgNPs (in nanohybrids) on the antibacterial activity of APU/ZnO–Ag coating, the coating has been added into the log phase growing bacterial culture (in-situ test in the darkness). Briefly, the *E. coli* DH5 α strain was firstly activated from original strain in liquid LB medium overnight. Secondly, the bacteria was inoculated into 20 mL of liquid LB with inoculation rate of 1% (v/v), cultured at shaking speed of 200 rpm at 37 °C until the OD₆₀₀ value of cell suspension (optical density at λ =600 nm) reached 0.3. At this stage, the coatings (APU and APU/ZnO–Ag) were added into the mixed cultures, then cells were continuously cultured and monitored in the darkness. The cell suspensions were collected at various times, from 30 to 300 minutes (with 30 minutes of interval), then their (OD₆₀₀) were evaluated and compared.

Antibacterial evaluation for APU-based coatings in the darkness and under visible-light irradiation

To evaluate the effect nano-ZnO (with or without hybridization with AgNPs) on the antibacterial activity of APU coatings, the viable bacteria counts have been determined on the surface of coatings (after bacterial infection) under visible-light irradiation or in the darkness, This test has been described in the previous works as the ISO 22196:2007 standard. Briefly, 3 types of coatings, such as APU neat, APU/ZnO and APU/ZnO-Ag, have been deposited on glasses (size of $5 \times 5 \times 0.3$ cm). Firstly, the test *E.coli* bacteria (ATCC 25922) were pre-incubated at concentration of $\sim 10^5$ cells/mL in NBnutrient broth. Secondly, each coated glass was infected by adding 0.5 mL of the above pre-incubated inoculum into the sterile Petri dish. Thirdly, after bacterial infection, these Petri dishes were incubated at 35 °C for 24 h. In this stage of incubation, 2 cell-culture chambers have been used, such as one chamber in dark and other one under visible light irradiation (using the same LEDs for above agar-well diffusion method). Fourthly, E.coli bacteria from each Petri dish was recovered just after inoculation or incubation by adding 10 mL of SCDLP broth medium. Fifthly, from these recovered specimens, their viable bacteria counts have been determined by the pour plate culture method with 10-fold serial dilutions for PCA (plate count agar) when incubated at 35 °C for 24 h.

Supporting Information Summary

The Supporting Information includes the detailed experimental section of this work

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Keywords: Acrylic polyurethane · abrasion resistance · antibacterial · impact strength · hybrid nanoparticles

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