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Polymer- Nanoparticles Composites for the Reduction of the Bacterial Adherence to Surfaces

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Abstract: The medical device is one of the sources of nosocomial infections; the adherence of the bacteria on the surface of this device is the first step in the medical device related infection. In this study nanocomposite of polymethyl methacrylate (PMMA)/titanium oxide nanoparticles (1, 5, 10, 20, 30 wt %) and copolymer acrylonitrile butadiene styrene (ABS)/titanium oxide nanoparticles (1, 5, 10, 20, 30 wt %) were used to investigate the ability of the nanocomposite for the reduction of the bacterial adherence to the surfaces. The microorganisms which is used in this study include (*Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Klebsiella pneumonia*). After an incubation of microorganisms with nanocomposites for 2 hours, it was found that the adhered bacterial cells were significantly reduced on all of the TiO_2-containing nanocomposites in the comparison with control polymer. The reduction is reached to 60.82% with10% TiO_2 in ABS for *P. aeruginosa*. The results of the *S. aureus* adherence on nanocomposite are revealed a significant reduction in the number of adhered bacteria and reached to 73.71% with 20% TiO_2 in PMMA. The effect of TiO_2 nanoparticales on the *K. pneumonia* adherence appeared very clear and the reduction uis reached to 90.04% with 10% TiO_2 in ABS.

Key words: nanocomposite; TiO_2, bacterial adherence, nanoparticles

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Introduction

The first step in the initiation of biomaterials-related infection is microbial adhesion to the device and after that the proliferation of adhered bacteria leads to the produce of a biofilm, and infection. The surfaces of the medical devices are the places to the bacterial colonization and the adhesion. So that, the researcher tries to improve the properties of polymers in order to resist the bacterial adhesion for use in the medical devices.

Materials with nanoparticles may have properties which are effective to reduce the microbial adhesion, and biofilm growth. The studies indicated that the incorporating nanomaterial with the medical device can enhance the surface energy, in addition to that increase selected protein adsorption (1). In the nanomaterials, when the material particles become smaller the percentage

of atoms at the surface area are increased. This may lead to the new properties of the nanoparticles. The ratio of the surface to the volume of the materials are increased and their electronic energy states become discrete, and this lead to the unique electronic, optical, magnetic, and mechanical properties of the nanomaterials (2).
Titanium Oxide (TiO_2) is an inert and cheap material, and well known as non-toxicity for the human. So that it is used in cosmetics and toothpaste (4). TiO_2 nanoparticle is insoluble (5). Titanium dioxide (TiO_2) is not classified as hazardous according to the United Nations' (UN) Globally Harmonized System of Classification and Labeling of Chemicals (GHS) (6). TiO_2 is widely used as a self-cleaning and self-disinfecting surface (7).
The polymer is widely used in industries because it has the advantages such as low cost, low weight and good manufacturing flexibility (8). Polymethyl methacrylate (PMMA) is one of the thermoplastic material, and it has many applications in many technological and productive fields. Moreover, it has many advantages of good optical properties, chemical inertness, good mechanical properties, thermal stability, electrical properties, safety, and easy shaping (9). Polymers can be mixed together to produce combinations of properties. The different kinds of monomers are polymerized together and the result was of named as copolymer. Acrylonitrile butadiene styrene (ABS) is an example copolymer (10).
In this study, nanocomposite of polymethyl methacrylate/ titanium oxide nanoparticles and copolymer acrylonitrile butadiene styrene/ titanium oxide nanoparticles are used to investigate the ability of the nanocomposite for the reduction of the bacterial adherence to surfaces.

Materials and Methods

Nano Composites

The pieces of the two nanocomposites polymer, polymethyl methaacrylate (PMMA) and copolymer acrylonitrile butadiene styrene (ABS) with the different concentrations of the nanoparticale of TiO_2 (30-70 nm) are prepared kindly by Dr. Imad Disher college of material engineering, Babylon university in previous study (11).
In the present work, The composites of the polymethyl methacrylate /titanium oxide nanoparticles (1, 5, 10, 20, 30 wt %) are used. Also, the composites of the copolymer acrylonitrile butadiene styrene/titanium oxide nanoparticles (1, 5, 10, 20, 30 wt %) are used. Two sets of the polymer pieces are tested for each concentration. Control pieces which are polymer without TiO_2 also used al Culture
The microorganisms which are used in this study include two clinical isolates of each species of (*Pseudomonas aeruginosa* and *Staphylococcus aureus* and *Klebsiella pneumonia*).
The microorganisms are streaked for the isolation on a nutrient agar plate (oxoid). After that, a single isolated colony is selected and used to inoculate 5 ml of nutrient broth. The bacterial culture is grown on a shaking incubator at 200 rpm for 18 hours at 37°C. Then, it is harvested by centrifugation at 3000 rpm for 10 min. After we removed the supernatant, the cells are washed with phosphate buffer solution (PBS) twice

and resuspended with the PBS solution. The final concentrations of the bacterial cells are diluted approximately 10^6 CFU/mL for each isolated bacteria in 5 ml of PBS.

Adhesion Experiments

Two pieces of the nanocomposites of each weight percentage are used. The nanocomposites pieces are sterilized with three times for 30 second rinses in 70% ethanol (12). The pieces are placed in tube which is contained 5 ml of bacterial suspension. Then the tubes are placed in the incubator at 37°C for 2 h. After that, the pieces of the nanocomposites are removed from the tubes with sterile forceps and washed three times with PBS to ensure the removal of the non-adherent bacteria. The pieces are placed in the tube which is contained 5 ml of PBS and vortex for 120 sec (13), in order to remove all the adherent bacteria from the nanocomposites pieces into the solution. Then, the solution is serially diluted in PBS, cultured on nutrient agar and the numbers of CFU/ml are calculated.

Results and Discussion

The attachment of the bacteria to the surface is a first part in the process of development of the infection. The physicochemical properties of the surfaces play important role in this attachment.

The nanoparticles generally have different physical and chemical properties in comparesion to the fine particles. The smaller size of the nanoparticles means that a large portion of the atoms placed on the particle surface. So that, the properties of the surface, such as energy level, electronic structure is different from the fine particles (14).

The results of the present study are found that adhered bacterial cells are significantly reduced on all of the nanocomposites pieces which containing TiO_2 in comparison with control polymer.

(Figures 1 , 2) demonstrate that the influence of the different concentrations of TiO_2 nanoparticales on the adherence of *P. aeruginosa* to PMMA and ABS respectively. The numbers of adhered bacteria are reduced significantly on all of the nanocomposites which containing TiO_2 and the reduction are reached to 60.82% with10% TiO_2 in ABS.

The results of the *S. aureus* adherence on the nanocomposite had shown in (figures 3,4). Also, the results are revealed the significant reduction in the numbers of the adhered bacteria which is reached to 73.71% with 20% TiO_2 in PMMA.

(Figures 5 , 6) were showed the effect of TiO_2 nanoparticales on the adherence of *K. pneumonia* on PMMA and ABS respectively. The reduction in the number of the adhered bacteria is very clear and it is reached to 90.04% with 10% TiO_2 in ABS.

(Figures 7, 8) are showed the adherence of the different microorganisms on the polymers surface (PMMA and ABS respectively). The results are revealed that the *P. aeruginosa* is showed high adherence on the surface and the *K. pneumonia* is showed low adherence among tested microorganisms.

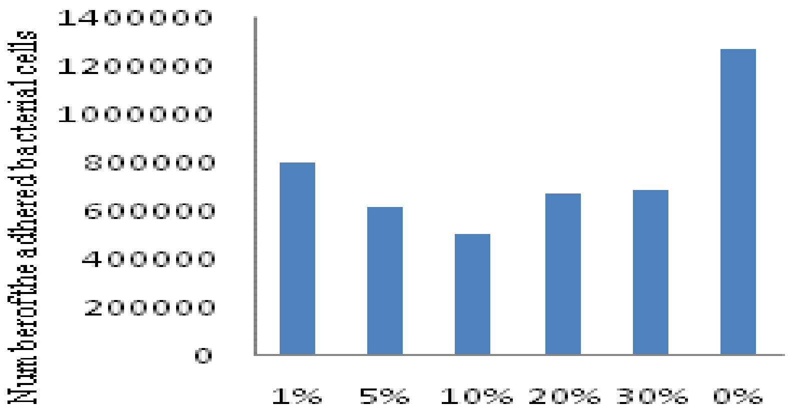

Figure 1 The influence of different concentrations of TiO2 nanoparticles on the adherence of *P. aeruginosa* to PMMA surfaces

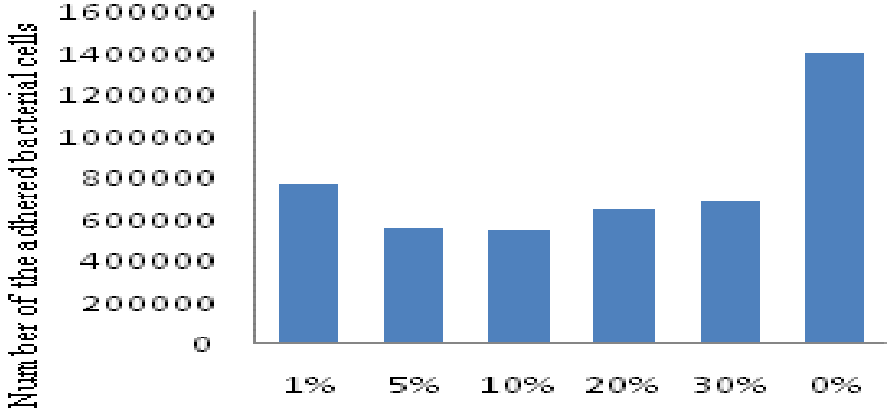

Figure 2 the influence of different concentrations of TiO2 nanoparticles on the adherence of *P. aeruginosa* to ABS urfaces

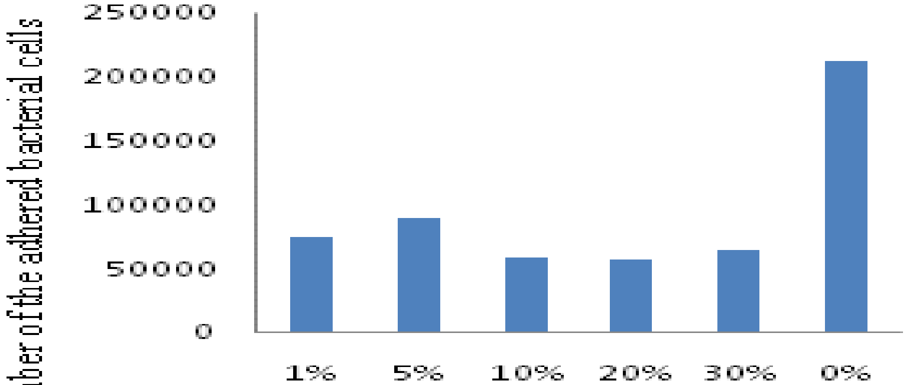

Figure 3 the influence of different concentrations of TiO2 nanoparticles on the adherence of *S. aureus* to PMMA surfaces

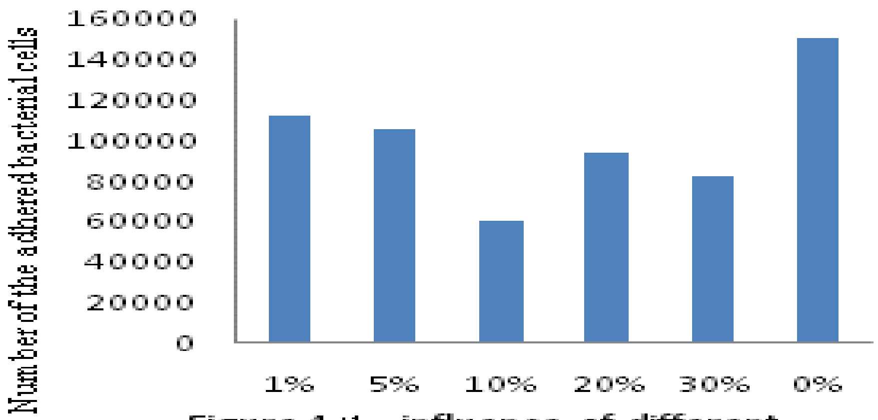

Figure 4 the influence of different concentrations of TiO2 nanoparticles on the adherence of *S. aureus* to ABS surface

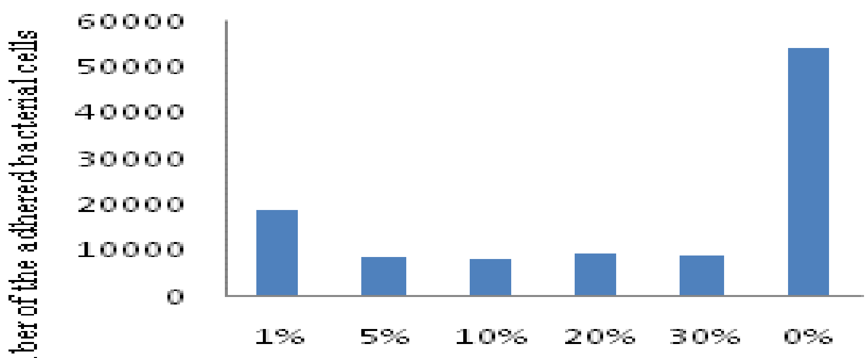

Figure 5 the influence of different concentrations of TiO2 nanoparticles on the adherence of *K. pneumoniae* to PMMA surface

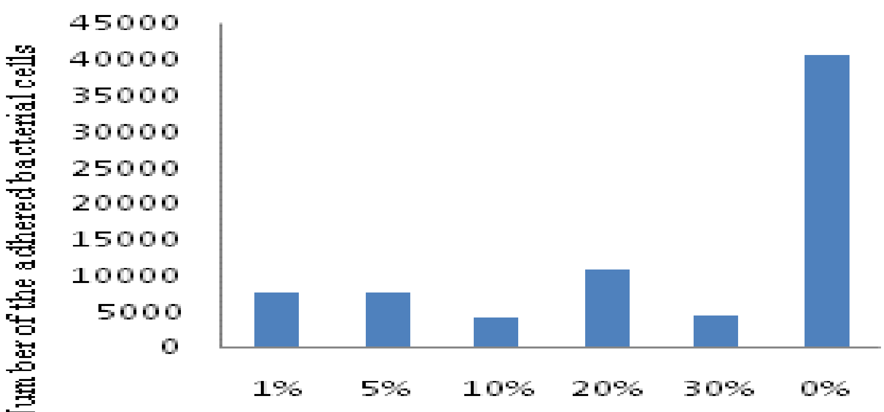

Figure 6 the influence of different concentrations of TiO2 nanoparticles on the adherence of *K. pneumoniae* to ABS surface

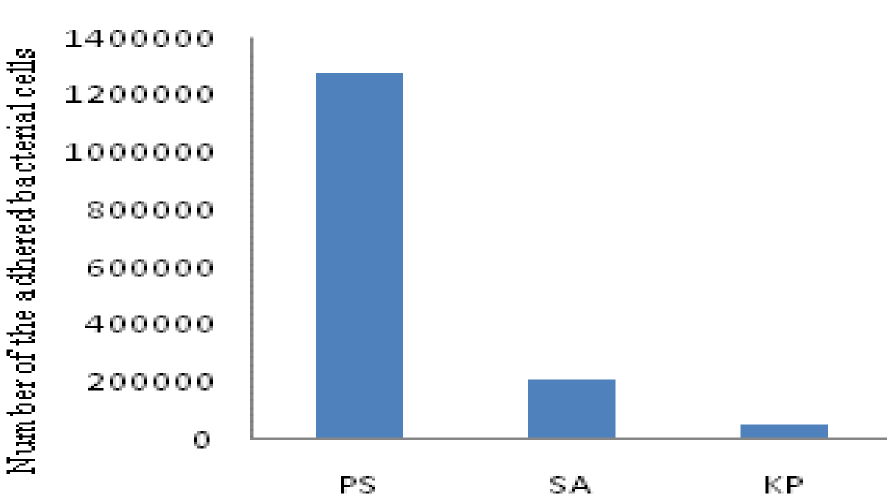

Figure 7 the adherence of different microorganisms on the PMMA surface

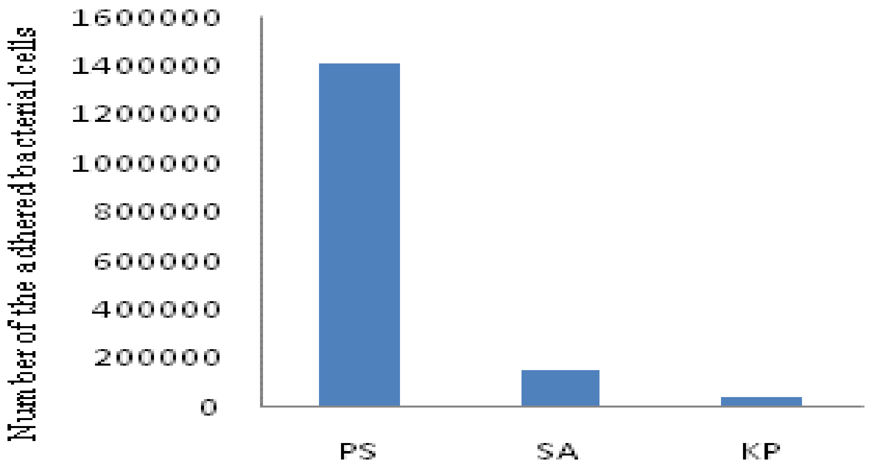

Figure 8 the adherence of different microorganisms on the ABS surface

Many studies were showed that the materials with nanomaterial may have properties which are effective in the reduction of the microbial adhesion (15, 16). Also there are studies revealed that the addition of inorganic nanoparticles to polymers allows the change of the polymers physical properties (16). Nabawia *et al*. ,(17) are showed in their study that the mixing of TiO_2 nanoparticles with PMMA can improve the properties of the polymer which leads to the interesting technological uses. In adition to that, there is a study refered that the mixed nanoparticales with medical device surfaces can enhance surface energy, increase select protein adsorption, and promote protein bioactivity (1). Holmes *et al*. (2009) are tested the modified surface with nanoparticle, the results showed that the coatings with silica nanoparticles significantly reduced adhesion of *Staphylococcus epidermidis* (90%), and *Pseudomonas aeruginosa* (15%) (15). There is no previous studies deal with the effect of TiO_2 nanoparticles on bacterial adhesion, but there is study on Gallium Oxide Ga_20_3 nanoparticles the researchers found that the Ga_20_3 nanoparticles reduced the bacterial adherence and the reduction reached to 46% in *S. aureus*, and 74% in *E coli* (18). Many researchers hypothesize that the rough of the material surface at the nanoscale may minimize flush contact between bacterial cell walls and the surface. This can inhibit electrostatic interactions which is necessary for adhesion to the surface (19). We can conclude that the addition of nanomaterial to polymer can reduce the bacterial adhesion on polymer surface.

References

1. Zhang,L. and Webster, T. (2009). Nanotechnology and nanomaterials: Promises for improved tissue regeneration. Nano Today, Vol.4: 66-80.
2. Ravishankar, R. and Jamuna, B. (2011). Nanoparticles and their potential application as antimicrobials. In: Science against microbial pathogens: communicating current research and technological advances, Volume one A. Méndez-Vilas, Badajoz, Spain, pp: 197- 209.
3. Thomas, H. and Dorothée, V. (2010). Polymer-Nanoparticle Composites: From Synthesis to Modern Applications. Materials, 3: 3468-3517.
4. Gerhard, J.; J¨urgen, L.; Christele, R. and Michael, S. (2007). Grey Goo on the Skin? Nanotechnology, Cosmetic and Sunscreen Safety, Critical Reviews in Toxicology, 37:251–277.
5. Kramer,S.; Gorichev, I. and Lainer, A. (2014). Calculation of the solubility of TiO2 and Titanates in sulfuric acid solution.Russian Metallurgy (metally), Vol. 2014 (9): 704-707.
6. A Guide to the Globally Harmonized System of Classification and Labelling of Chemicals (GHS) https://www.osha.gov/dsg/hazcom/ghs.html
7. Janusz. B.; Agnieszka. J.; Joanna Z. and Joanna. P. (2015). Chances and limitations of nanosized titanium dioxide practical application in view of its physicochemical properties, Nanoscale Research Letters, 10:57-67.
8. Mustafa, A. (2012). History of the development of polymers, IN: Introduction to polymer science and technology. Ireland. Bookboon.com, ISBN 978-87-403- 0087-1, pp: 15.
9. Silva, A.; Dahmouche, K. and Soares, B. (2010). The Effect of Addition of Acrylic acid and thioglycolic acid on the nanostructure and thermal stability of PMMA montmorillonite nanocomposites. Applied Clay Science, 47: 414-420. Cited by: Nabawia. A.; Mohamed. S.; Osiris. W. (2014). Thermal and structural analyses of PMMA/TiO2 nanoparticles composites, Natural Science, 6; 859-870.
10. Mustafa, A. (2012). Microstructure, IN: Introduction to polymer science and technology. Ireland. Bookboon.com, ISBN 978-87-403- 0087-1, pp: 126.
11. Imad,I. (2014).Preparation and characterization of phase-pure Anatase nanoparticles. The Iraqi Journal for Mechanical and Material Engineering. 14(1)
12. Geilich. B. and Webster. T. (2013). Reduced adhesion of *Staphylococcus aureus* to ZnO/PVC nanocomposites, International Journal of anomedicine Vol.8(1): 1177-1184.
13. Onaolapo. J. and Salami. J. (1995). Effect of subminimum inhibitory concentration of ceftriaxone on adherence of *Pseudomonas aeruginosa* to inert surfaces in an experimental model, J. med. med Sci. 24: 275-281.
14. Hongbo. S.; Ruth. M.; Vincent. C. and Jinshun. Z. (2013). Titanium dioxide nanoparticles: a review of current toxicological data, Particle and Fibre Toxicology, 10:15.
15. Holmes, P.; Currie, E.; Thies, J.; Mei, H.; Busscher, H. and Norde, W. (2009). Surface-modified nanoparticles as a new, versatile, and mechanically robust nonadhesive coating: Suppression of protein

adsorption and bacterial adhesion, Journal of Biomedical Materials Research Part A , Vol. 91A, (3): 824–833.

16. Yang, L.; Jin, L.; Xiaofeng, Q. and Clemens. B. (2007). Bactericidal activity of nitrogen-doped metal oxide nanocatalysts and the influence of bacterial extracellular polymeric substances (EPS). Journal of Photochemistry and Photobiology A: Chemistry Vol. 190 (1): 94-100.
17. Nabawia, A.; Mohamed, S. and Osiris, W. (2014). Thermal and Structural Analyses of PMMA/TiO2 Nanoparticles Composites, Natural Science, 6: 859-870.
18. Murth, P.; Venugopalan, V.; Sahoo, P. and Dhara, S.. (2011). Gallium Oxide Nanoparticle Induced Inhibition of Bacterial Adhesion and Biofilm Formation, Nanoscience, Engineering and Technology (ICONSET), 2011 International Conference on, Chennai. pp: 490 - 493.
19. Izano, E.; Amarante, M.; Kher, W.; Kaplan, J. (2008). Differential roles of poly-N-acetylglucosamine surface polysaccharide and extracellular DNA in *Staphylococcus aureus* and *Staphylococcus epidermidis* biofilms. Appl Environ Microbiol. 74(2):470–476. Cited by: Geilich. B. and Webster. T. (2013). Reduced adhesion of *Staphylococcus aureus* to ZnO/PVC nanocomposites, International Journal of anomedicine Vol.8(1): 1177-1184