



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 TiO₂-containing nanocomposites in the comparison with control polymer. The reduction is reached to 60.82% with 10% TiO₂ 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% TiO₂ in PMMA. The effect of TiO₂ nanoparticles on the *K. pneumonia* adherence appeared very clear and the reduction was reached to 90.04% with 10% TiO₂ in ABS.

Key words: nanocomposite; TiO₂, 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 methacrylate (PMMA) and copolymer acrylonitrile butadiene styrene (ABS) with the different concentrations of the nanoparticle 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 (oxid). 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 comparison 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).

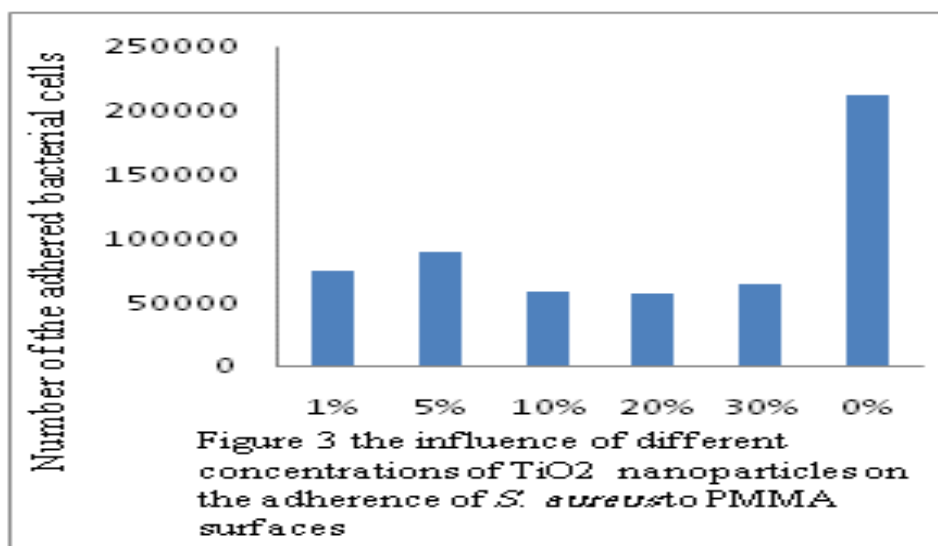
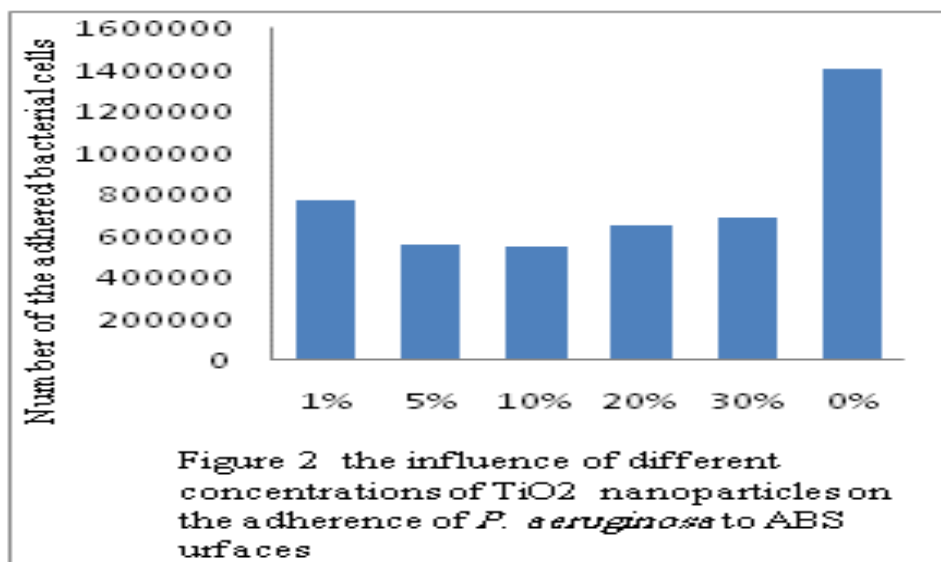
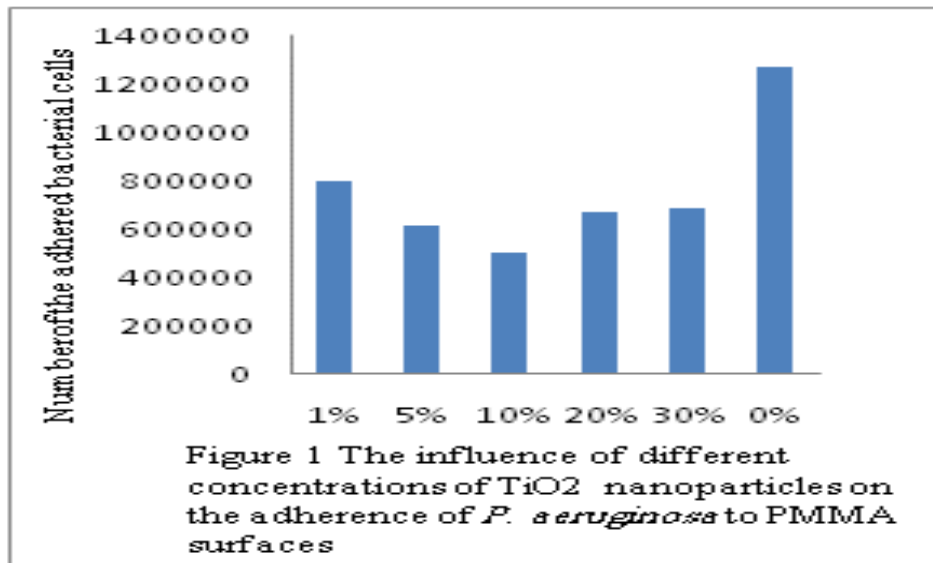
The results of the present study are found that adhered bacterial cells are significantly reduced on all of the nanocomposites pieces which containing TiO₂ in comparison with control polymer.

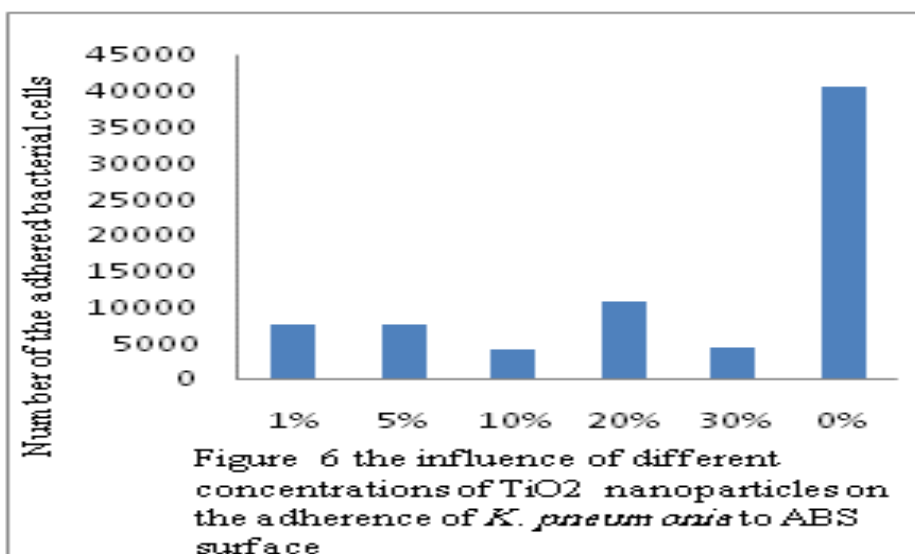
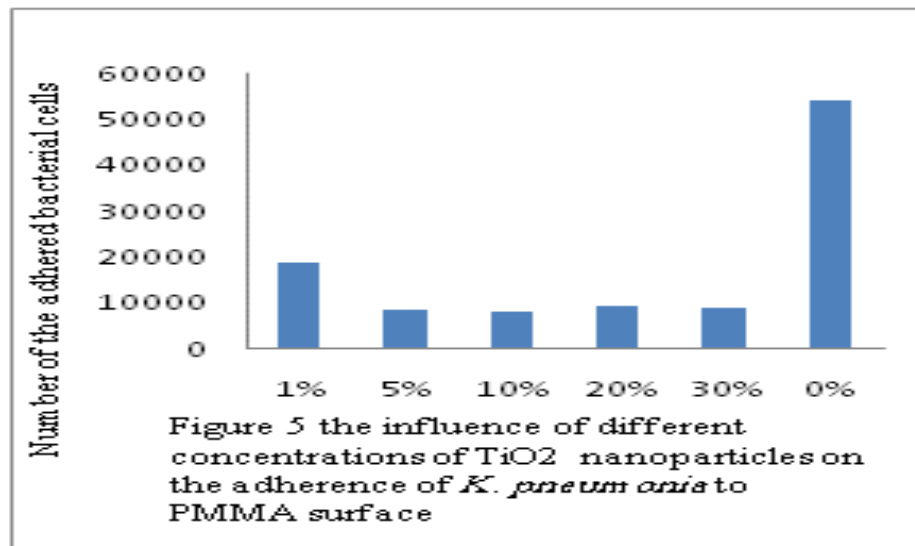
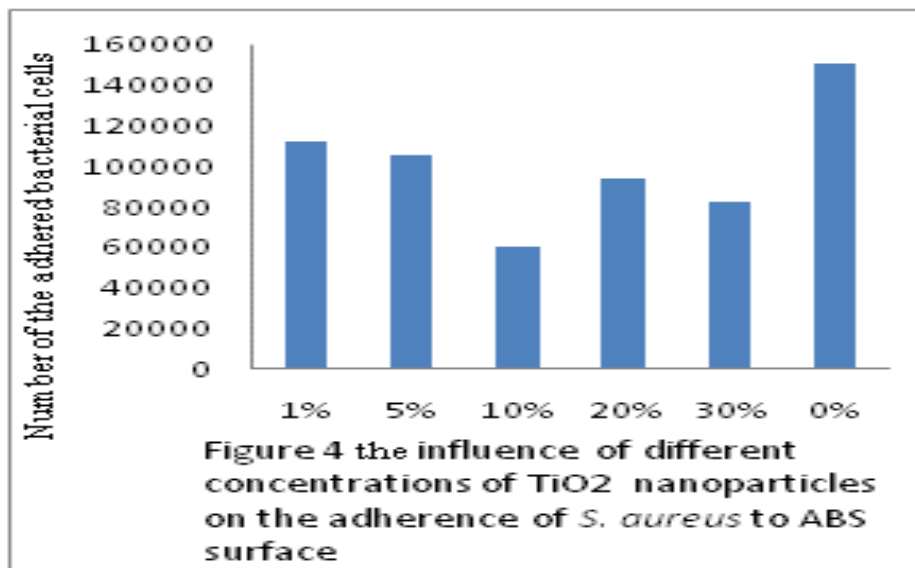
(Figures 1 , 2) demonstrate that the influence of the different concentrations of TiO₂ nanoparticules on the adherence of *P. aeruginosa* to PMMA and ABS respectively. The numbers of adhered bacteria are reduced significantly on all of the nanocomposites which containing TiO₂ and the reduction are reached to 60.82% with 10% TiO₂ in ABS.

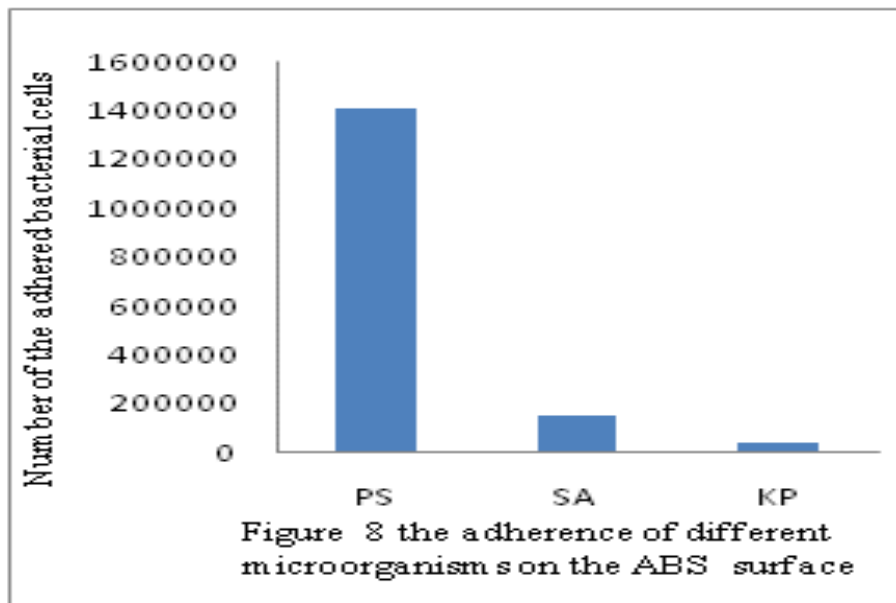
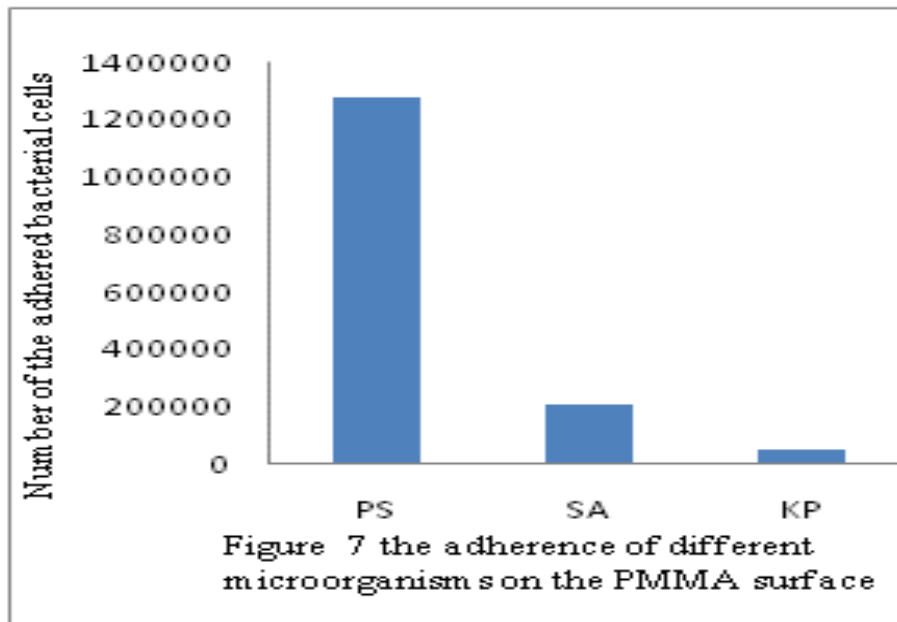
The results of the *S. aureus* adherence on the nanocomposite had shown in (figures 3,4). Also, the results are revealed the significant reduction in the numbers of the adhered bacteria which is reached to 73.71% with 20% TiO₂ in PMMA.

(Figures 5 , 6) were showed the effect of TiO₂ nanoparticules 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% TiO₂ 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.







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 addition to that, there is a study referred 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 TiO₂ nanoparticles on bacterial adhesion, but there is study on Gallium Oxide Ga₂O₃ nanoparticles the researchers found that the Ga₂O₃ 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.

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