

Inhibition of *Pseudomonas aeruginosa* Biofilm Formation on Titanium Dioxide Nanoparticle Coated Catheters and Glasses

Mina Saadat^{1*} and S.R. Mohammadi²

¹Department of Basic Science, Azad University, Research and Science Branch, Tehran, Iran.

²Department of Medical Mycology, School of Medical Sciences, Tarbiat Modares University, Tehran, Iran.

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Bacterial biofilms are complex organized communities of bacteria that surrounded by exo-polysaccharide and grow in association with surfaces. They are thought be responsible for 65% of nosocomial infection and can be found at almost solid-liquid interface including medical implant and catheters. One of the most medically important biofilm forming species is *Pseudomonas aeruginosa* Because of increasing antibiotic resistant, it is necessary to find suitable agent to limit contamination of surfaces and medical devices. In this study Titanium Dioxide (TiO₂) nanoparticles was synthesized from Titanium tetrachloride (TiCl₄) through sol-gel method. Properties of synthesized nanoparticles were evaluated by X-ray diffraction and scanning electron microscopy methods. Catheter and glass chips were coated with TiO₂ nanoparticles. Based on XTT results and scanning electron microscopy data it was showed that *pseudomonas aeruginosa* biofilm formation has effectively decreased on TiO₂ nanoparticles coated catheter and glass chips compared to control group.

Key words: TiO₂ nanoparticles, *P. aeruginosa*, Biofilm formation, Coated surfaces.

Bacterial infection from medical devices is a major problem and accounts for a substantial morbidity as well as causing a sharp increase in health-care costs. As an instance, it is reported that peripheral or central intravenous catheters (CVCs) resulting in bloodstream infections (BSI) occur in about 4–5 out of every 1000 CVC devices inserted patients. In addition implanted devices are also susceptible to infections, resulting in implant failure. When a device must be removed to eradicate an infection, it is often because bacteria have produced a sticky biofilm matrix, forming a strong adhesion to the device surface.¹ Biofilms have been found to be involved in an approximately 80% of all infections.² The main problem with microbial biofilm infections is their tendency to

resist by the host immune system and all antimicrobial agents. Biofilms by pathogenic bacteria like Klebsiella, Pseudomonas, Salmonella and Compylobacter have been reported and these biofilms can be continues sources of contamination to hospital devices.³ *Pseudomonas aeruginosa* as a widespread Gram-negative bacterium with a great ability in attachment to surfaces and biofilm formation has been considered as one of the most important pathogens in hospital infections.⁴ Many different strategies have been developed to decrease the incidence of medical device related infection. One way to prevent infection is inhibition of bacterial adhesion by modifying the surface of the devices. Incorporation of antimicrobial agents in the bulk material or as a surface coating has been considered a viable alternative for systemic application of antibiotics.⁵ As a growing direction in biomaterial design, nanomaterial (materials with at least one dimension less than 100 nm) may be promising agents for antibacterial applications.¹

* To whom all correspondence should be addressed.
Tel.: +982133322842;
E-mail: saadat.mina@gmail.com

Nanoparticles may also have some general mechanism of toxicity toward bacteria but not mammalian cells. Generally nanoparticles bind to bacterial cell walls causing membrane disruption through direct interactions or through free radical production. Mammalian cells are able to phagocytosis nanoparticles, and can subsequently degrade these particles by lysosomal fusion, reducing toxicity and free radical damage.¹ The first publication on the use of titanium dioxide in microbiology for photoelectrochemical sterilization of microbial cells dates back to 1985; since that time, the number of studies devoted to the bactericidal effect of nanodisperse TiO₂ with respect to different pathogenic bacteria is being permanently increased.⁶ Freshly precipitated titanium dioxide dehydrate exhibited high adsorbability with respect to both cations and anions;⁶ There is a wide variety of coating techniques such as PVD,⁷ spin-coating,⁸ or electrospinning.⁹⁻¹⁰ One of the methods for coating surfaces of devices is dip coating. Dip-coating is the most popular method in which the colloids are evaporation-induced and self-assembled on the substrate as it is slowly withdrawn from the colloidal suspension.¹¹

In this study catheter and glass chips were coated by dip coating technique, and biofilm formation of *P. aeruginosa* was investigated using SEM and XTT tests.

MATERIALS AND METHODS

Preparation TiO₂ nanoparticles: sol-gel technique

TiO₂ nanoparticles were synthesized through TiCl₄ hydrolysis. Briefly 1ml TiO₂ pre was dissolved in 58ml deionized distilled water. Prepared solution was shaken for 5 hours without heating. After 24 hours incubation at room temperature, the solution was heated in two heating procedure: 80-100°C for 12 hours and then at 550°C for 2 hours. White TiO₂ nanoparticles were the product of this step. Morphology and the properties of synthesized nanoparticles were analyzed using Scanning Electron Microscopy and X-ray Diffraction tests.

Sample preparation

Catheter and glasses were coated by TiO₂ nanoparticles using dip coating method. Coated samples were heated at 55°C for 10-15 minutes and

then were sterilized under UV radiation for 20 minutes. Uncoated samples were served as control group.

To prepare the microbial suspension, the standard *P. aeruginosa* strain (ATCC 27853) was cultivated in the tryptic soy agar (TSA) medium enriched with 0.2% glucose at 37°C for 24 hours. The grown single colony in the cultivation medium was incubated in the tryptic soy broth (TSB) plus 0.2% glucose. To carry out light spectroscopy, contents of the test tubes were mixed and the light absorbance was set at 0.1 in wavelength of 650 nm for each sample.

Each coated and uncoated catheters and glasses were placed in a well of 12 well plates. 1500 µl of the resultant cell suspension as well as 1500 µl TSB plus 0.2% glucose was added to each well and heated at 37°C for 24 hours. Catheter and glass chips were moved to sterile 12 well plates and washed with sterile 1% PBS for 2 times. Test chips were placed under laminar hood until they were dried. Finally prepared samples were used in scanning electron microscopy and XTT tests.

Biofilm detection

XTT Test

Prepared catheter and glass chips were moved to 12well plates and 100 µl TSB medium plus 0.2% glucose were added to each well. Chips surfaces were collected by scraper and well content was transferred to 96 microplate wells. To determine biofilm formation by XTT test, briefly 100µl XTT solution and 50µl Q coenzyme were added to each sample containing well. After 2-3 hour incubation at 37°C, 100µl DMSO was added to each well. After 15 minutes, light absorbance was read at 490 nanometer (ELISA reader model)

SEM test

Biofilm formation on coated and uncoated catheter and glass chips were investigated by scanning electron microscopy (SEM).

Statistical Analysis

Statistical analyses were conducted using one-way analysis of variance (ANOVA). A significance level of 0.05 was used.

RESULTS AND DISCUSSION

A part of XRD for TiO₂ nanoparticles are showed in (Fig.1). As it is demonstrated, all diffraction peaks show that the ratio between anatas

and rutil phases were 2:1 for TiO₂ nanoparticles. SEM data also showed that synthesized TiO₂ nanoparticles were spherical with 40-65 diameters (Fig. 2).

Reduction of biofilm formation on coated surfaces compared to uncoated counterparts is obviously showed by SEM results in (Fig. 3). Significant inhibition of biofilm formation on coated catheters and glass chips are also showed by XTT results in fig. 4 (p<0.5). Comparison of biofilm formation on coated chips with uncoated counterparts showed 74 and 82% decrease in biofilm formation in the presence of nanoparticles in catheter and glasses respectively (Fig. 4).

This comparison means that the rate of biofilm inhibition on coated catheter was sharper than glass chips which can be explained by the physical and chemical properties of the surfaces. Catheter due to its physical and chemical properties may be a better surface for both bacterial and nanoparticle attachment. It is clear that the

topological and chemical characteristics of a medical device surface are important for the rate of microorganism adhesion. Differences in size, number, and/or distribution of bacterial surface hydrophobins may explain the observed variation among different assays. A perfectly smooth surface will be less likely to be populated than a rough surface, where more surface area is available as well as more adhesive force can be generated by the microorganism per surface area. Also the chemical characteristic is essential for the initial population of a surface by free-swimming pathogens. Hydrophobicity of bacterial cell surfaces has been considered to affect several biological phenomena such as attachment of bacteria to host tissues and adhesion of bacteria to solid surfaces. In reality however, the adhesion of microorganisms is almost always dependent on formation of a protein layer on the surface, possibly exposing high-affinity adhesion sites.^{5,12}

Our results showed that TiO₂ nanoparticles synthesized in a sol gel way can effectively inhibit biofilm formation on catheter and glass chips. TiO₂ nanoparticles are inexpensive and stable. They have great electrical and optical properties. With previously reported antibacterial effects on *E.coli* and *Aspergillus niger*.^{13,14} TiO₂ nanoparticles synthesized in different ways for example Yang *J et al.*, in 2001 Synthesized TiO₂ Nanopowders from Tetra alkyl ammonium Hydroxide in hydrothermal method and Colón *G et al.*, in 2002 Synthesized TiO₂ Nanopowders from Alkoxide Precursor and Using Active Carbon as Additive.^{15,16}

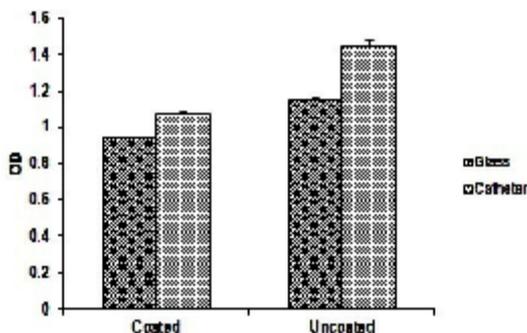


Fig. 1. Biofilm detection with XTT assay on the coated and uncoated glasses and catheter

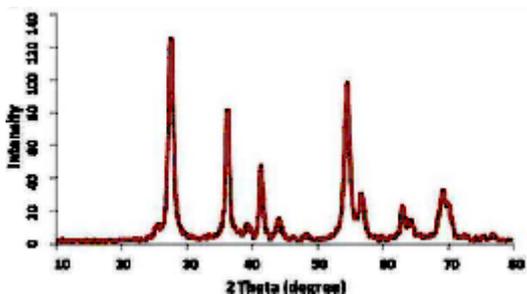


Fig. 2(a). XRD of TiO₂; synthesized TiO₂ nanoparticles were spherical with 40-65 diameters

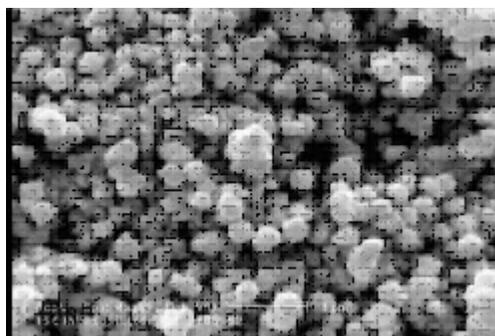


Fig. 2(b) Part of XRD for TiO₂ nanoparticles; The ratio between anatase and rutile phases were 2:1 for TiO₂ nanoparticles

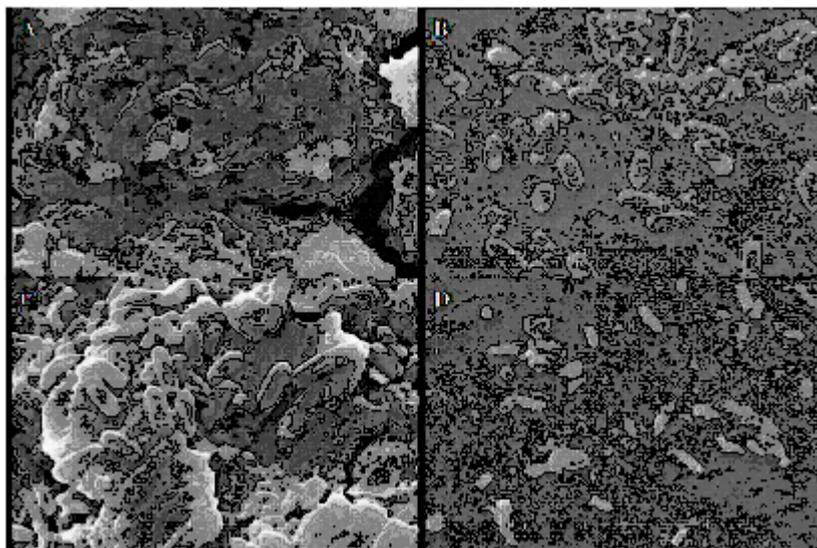


Fig. 3. SEM of biofilm formation on uncoated catheter (A), coated catheter (B), uncoated glass(C) and coated glass (D) are show

CONCLUSION

It is believed that nano featured medical device surfaces enhance surface energy, increase select protein adsorption, promote protein bioactivity, and improve subsequent tissue-forming cell functions, while many researchers now hypothesize that this same action could simultaneously prevent bacterial colonization.¹ Finally it can be concluded that nano TiO₂ coated devices can be promising tools to reduce nasocominal infections and its related costs.

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