

## CuO, TiO<sub>2</sub> and ZnO Nanoparticles for Disinfecting Contaminated Hospital Surfaces

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**Abstract:** Nosocomial infections are infections that are transmitted to people due to hospital admission and medical procedures and usually happen 48 to 72 hours after patient admission and lead to the dissemination of these infectious agents in the society. In this study the various bacteria on the floor, walls and beds of the Afzalipour hospital, Kerman, Iran were determined and the effect of the CuO, TiO<sub>2</sub> and ZnO nanoparticles on these bacteria was investigated. Two hundred and ten (210) samples were taken from the floor, walls and beds of the Afzalipour hospital during 7 months. The samples were transferred to the culture medium, Nutrient Agar. In order to determine the bacteria type, specific culture media and biochemical tests were used. After the type of the bacteria was determined, a uniform suspension of the bacteria with certain density was prepared. Then the suspensions were added to culture mediums containing certain concentrations of nanoparticles and were incubated under proper conditions. The efficacy of the different nanoparticles was determined based on comparing the different growth rates of colonies in the control and the experimental media. The majority of bacteria recognized in this study were *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Escherichia coli*. Results showed that ZnO at the concentration of 1500 mg/L can eliminate *Staphylococcus aureus* and *Escherichia coli* by 100% and at the concentration of 4000 mg/L can eliminate *Staphylococcus aureus*, *Bacillus subtilis* and *Pseudomonas aeruginosa* by 100% and CuO at the concentration of 4000 mg/L can decrease the density of *Staphylococcus aureus*, *Bacillus subtilis* and *Pseudomonas aeruginosa* by 98%. For TiO<sub>2</sub> at 4000 mg/L concentration the bacteria elimination rate for *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Escherichia coli* was 100%, 55%, 80% and 59% respectively. According to these results, the highest antibacterial effect belonged to ZnO, CuO and TiO<sub>2</sub> respectively. The most resistant bacteria to the CuO, ZnO and TiO<sub>2</sub> nanoparticles were *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Bacillus subtilis* respectively. The most sensitive bacteria to all three nanoparticles were gram positive *Staphylococcus aureus*.

**Key words:** Nanoparticles • Nosocomial infections • Disinfecting • Hospital Surfaces

### INTRODUCTION

Nosocomial infections are infections that are transmitted to people due to hospital admission and medical procedures and usually happen within 48 to 72 hours after patient admission and can cause the

dissemination of these infectious agents in the society. The US Center for Disease Control estimates that 2 million nosocomial infections occur each year that leads to 20000 deaths and the annual cost is 4.5 to 11 billion dollars [1, 2]. Nowadays, nosocomial infections are one of the most important problems of hospitals in most world countries.

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The rate of these infections in different hospitals has been reported from a minimum of 1.9% to above 25% which shows the various and high incidence rates of these infections [3]. The rate of occurrence of hospital infections depends on the hygienic and economic characteristics of societies. In developing countries the incidence rate is higher and in developed countries this rate is less [4]. The microorganisms that cause nosocomial infections are more resistant than other microorganisms and the reason is the characteristics they acquire in the hospital environment. These microorganisms have a high virulence and the people who acquire nosocomial infections from hospitals, can transfer the infection to others [5]. Recognizing the most prevalent microorganisms in each hospital and determining the amount of contamination of different surfaces can help find out the source of infection and the right ways of controlling transmission. One way to prevent nosocomial infection and decrease its treatment expenses and mortality is using the right methods of disinfecting hospital surfaces. There are many different ways for disinfecting hospital surfaces and each method has its pros and cons [5, 6]. Nowadays the use of nanoparticles (particles less than 100 nm) for disinfecting has increased in medical sciences and health research. Several studies have used nanoparticles such as ZnO and TiO<sub>2</sub> for eliminating bacteria from different sources [7-9]. The main mechanism of toxicity of Nanoparticles is thought to be via oxidative stress (OS) that damages lipids, carbohydrates, proteins and DNA. Lipid peroxidation is considered the most dangerous effect as it leads to alterations in cell membrane properties which in turn disrupt vital cellular functions [10]. A study done by Mortimer *et al* in 2009 in Estonia, showed that TiO<sub>2</sub>, ZnO and CuO nanoparticles can eliminate 90.6 to 99.5 percent of bacterial contamination [11, 12], while Raffi *et al* in 2010 in Pakistan showed that CuO can eliminate 93% of *Escherichia coli* [13]. Furthermore Shantikumar *et al* in 2009 in India showed that ZnO has a strong disinfecting property [14].

The current study was done in order to determine the types of bacteria on different surfaces at the Afzalipour Hospital, Kerman and then to evaluate the antibacterial effect of TiO<sub>2</sub>, ZnO and CuO nanoparticles, in order to make the best use of nanotechnology for disinfecting surfaces and decreasing nosocomial infections.

## MATERIALS AND METHODS

Two hundred and ten samples were taken by sterile swaps immersed in sterile physiological serum from the floor surfaces, walls and the beds of all of the wards of Afzalipour Hospital in Kerman, Iran during 7 months in 2010. The samples were transferred to the nutrient agar medium. In order to prepare the culture media, 20 grams nutrient agar powder (Merck VM984550) was added to one liter distilled water and after boiling, the media was prepared by the autoclave method at 121° C, 15 psi, during 15 min as general culture media. After incubating in 37° C for 24 to 48 hours, the colonies that grew on the media were identified. Then a loop of the mentioned colonies were cultivated on specific media and after incubating by using biochemical tests the bacteria type in the samples were identified [15]. In order to prepare new media and activate the specific bacteria, the Muller Hinton agar media (Merck, 34 g/L distilled water) was used [11]. One loop of every bacterial strain was added to 25 ml of the Muller Hinton Broth medium in order to prepare a microbial suspension. The turbidity in this suspension created a cellular density of about  $3 \times 10^8$  cells per ml. Then it was diluted using the Muller Hinton Broth medium, until its light density was equal to solution 1 McFarland. Then its turbidity was measured with the spectrophotometric device at 625 nm. Based on the proportions of distillation, the amount of microorganisms needed were prepared [16, 17]. The minimum required density of nanoparticles for determination of the toxicity based on the standard methods for examination of water and wastewater, (20<sup>th</sup> edition) is 5 concentrations. In this study in order to achieve better results 10 different concentrations (10, 100, 250, 500, 750, 1000, 1500, 2000, 3000 and 4000 mg/L) of each nanoparticle were used (Table 1).

In order to determine the effect of the nanoparticles on bacteria, two hundred and ten 15 ml plates containing the Muller Hinton agar culture media were prepared under sterile conditions. As the culture media were cooling, at the temperature of 60 to 70°C, the sterile nanoparticles with different concentrations were added to the media by a 1 to 10 ratio [18]. After the culture media with nanoparticles cooled down, 100 µl of the distilled suspension with bacteria, prepared in the previous steps was cultivated on each of the mentioned plates. After incubating, the number of colonies grown on the media culture (CFU: Colony forming unit) in each ml was determined. A control group was also used during

Table 1: Characteristics of the nanoparticles used in this study

Nanoparticle	Formula	Manufacturer	Size (nm)	Specific Surface (m <sup>2</sup> /g)	% Purity
Titanium dioxide	TiO <sub>2</sub>	Merck	20	40	99
Zinc Oxide	ZnO	Merck	20	90	99
Copper Oxide	CuO	Merck	60	80	98

Table 2: The percent and type of bacteria found in the samples taken from the floors, walls and beds of the Afzalipour Hospital, Kerman.

Bacteria Type	Gram positive or negative	Floors (% positive)	Walls (% positive)	Beds (% positive)
Escherichia Coli	negative	80	55	65
Bacillus Subtilis	positive	30	20	25
Staphylococcus Aureus	positive	67	45	60
Pseudomonas Aeruginosa	negative	78	51	63

the experiments. The controls had all of the characteristics of the other samples except the added nanoparticles. In the next step by comparing the colonies formed on the control samples and the samples containing nanoparticles the bacteria elimination rate was determined. All of the experiments were done based on the methods mentioned in the standard book of experiments on water and wastewater (20<sup>th</sup> edition) [19]. In order to analyze and determine the relationship between the anti bacterial effects and the concentration of nanoparticles, Pearson correlations were calculated.

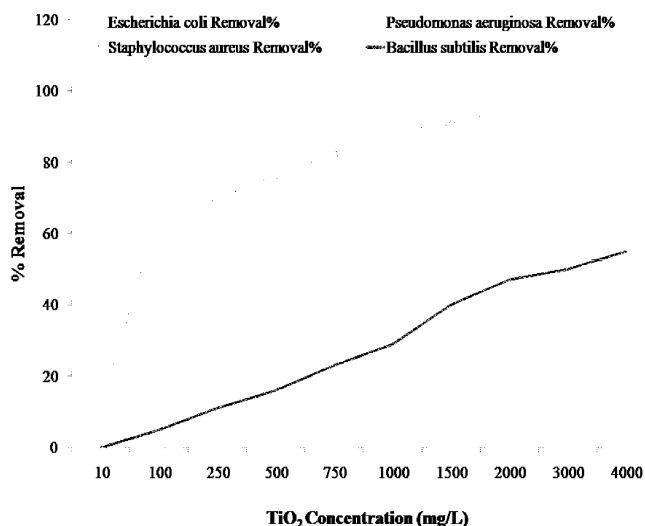
## RESULTS

The type and percent of each bacterium identified in the culture media from the surfaces, walls and the hospital beds of the Afzalipour hospital are listed in Table 2. Seventy samples were taken from the hospital walls, 70 samples from the hospital beds and 70 samples from

the floors. The bacteria most identified was *Escherichia coli* and the bacteria least identified was *Bacillus subtilis*. The other details have been mentioned in table 2.

The elimination rates of gram positive and negative bacteria by TiO<sub>2</sub> are shown in figure 1. As it can be seen the “No Observed Effect Concentration (NOEC)” for *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* is respectively 85, 10, 105 and 210 mg/L. At the concentration of 4000 mg/L the elimination rate of *Staphylococcus aureus* was 100%, *Bacillus subtilis* 55%, *Pseudomonas aeruginosa* 80% and *Escherichia coli* 59%.

Figure 2 shows the elimination rate of different bacteria by ZnO nanoparticles. As the figure shows the NOEC of ZnO nanoparticles for *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Escherichia coli* are 3, 9, 8 and 25 mg/L. At the concentration of 1500 mg/L the elimination rate of *Staphylococcus aureus* and *Escherichia coli* were 100%

Fig. 1: Bacteria elimination rates of TiO<sub>2</sub>

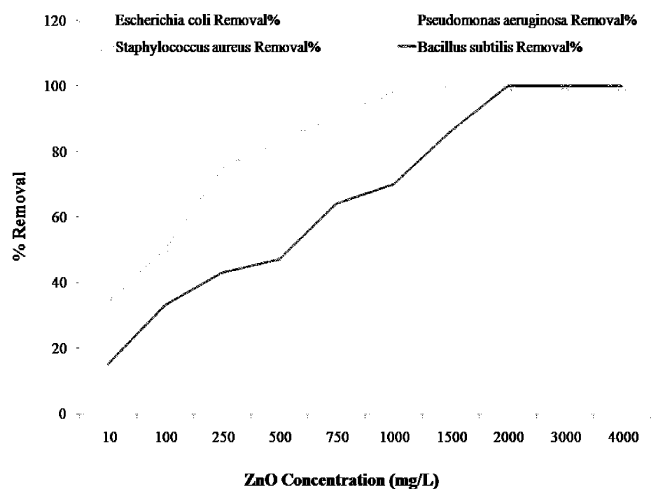


Fig. 2: Bacteria elimination rates of ZnO

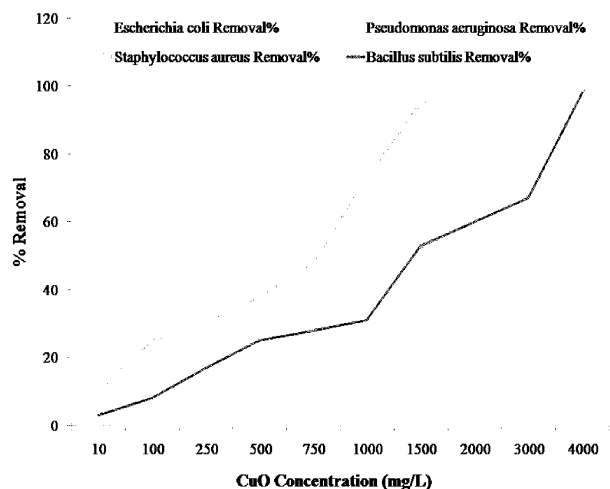


Fig. 3: Bacteria elimination rates of CuO

and the elimination rate of *Bacillus subtilis* and *Pseudomonas aeruginosa* was 86% and 76% respectively. Meanwhile at the concentration of 4000 mg/L, the elimination rate for *Bacillus subtilis* and *Pseudomonas aeruginosa* were 100%.

Figure 3 shows the elimination rates of different bacteria using CuO nanoparticles. As shown in figure 3, the NOEC for *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Escherichia coli* was 3, 10, 10 and 80 mg/L respectively. A 50% elimination rate of all gram positive and negative bacteria happened at a concentration of about 1500 mg/L. At the concentration of 2000 mg/L *Staphylococcus aureus* was completely eliminated and at this concentration the elimination rate of *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Escherichia coli* was 60%, 77% and 79% respectively. At the concentration of 4000 mg/L the elimination rate of

*Bacillus subtilis*, *Pseudomonas aeruginosa* and *Escherichia coli* were 100%. The results from this study show that there is a strong and direct association between concentration of nanoparticles and the elimination rate of the bacteria.

## DISCUSSION

Our study confirms other studies [20-22] that nanoparticles can be used for disinfection. In our study the elimination rate was different for different nanoparticles and the toxicity or the anti bacterial effect of the nanoparticles depended on the bacteria type. The most sensitive bacteria in our study were gram positive *Staphylococcus aureus*, which was more sensitive than *Escherichia coli* and gram negative *Pseudomonas aeruginosa* and was sensitive to all three nanoparticles

and was eliminated at lower concentrations of nanoparticles (figure 1, 2 and 3). The results of this study are in line with the results from Zhou *et al* done in 2002 in Japan [2]. In our study *Bacillus subtilis* in most circumstances showed more resistance than other bacteria to nanoparticles, which was not in line with the results from Young *et al* [23]. The fact that in some studies gram positive bacteria are more sensitive and in other studies the opposite is reported can be because of the individual and intricate genetic differences between bacteria. In our study ZnO nanoparticles at concentrations of 1000 mg/L could eliminate 65 to 100% of all the gram positive and negative bacteria, but this result was different from the results of Adams *et al* in which the elimination rate at the 1000 mg/L concentration was 48%. The reason for the different results of the two studies can be difference in the size of nanoparticles, the bacteria culture circumstances and the bacteria strains. It has been shown that the size of nanoparticles and the strain of bacteria can affect the toxicity of the nanoparticles [24].

Meanwhile the CuO nanoparticles were able to eliminate 98 to 100% of *Staphylococcus aureus*, *Bacillus subtilis* and *Pseudomonas aeruginosa* at 4000 mg/L concentrations but the TiO<sub>2</sub> nanoparticles could eliminate only the *Escherichia Coli* bacteria completely at 4000 mg/L concentration (figure 1). This different elimination rate is more likely due to differences in cell membrane, the presence or absence of resistant forms of the bacteria such as spores and ligand-receptor interaction, photocatalytic factors, reactive oxygen species and the capacity and electrical charges on the cell surface and the characteristics of the nanoparticle itself [21, 23, 25].

The least concentration of ZnO nanoparticles with disinfecting abilities for all bacteria under study was 10 mg/L and for CuO and TiO<sub>2</sub> nanoparticles were 100 and 250 mg/L respectively. This fact shows the stronger antibacterial effect of the ZnO nanoparticles in comparison to the other nanoparticles. These results were similar to the results of Jiang *et al*'s study done in 2009 in America and showed that ZnO nanoparticles at the 20 mg/L concentration have a stronger elimination effect than the other nanoparticles [20, 21].

In the present study the most resistant bacteria to the CuO, ZnO and TiO<sub>2</sub> nanoparticles were *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Bacillus subtilis* respectively. The most sensitive to all three nanoparticles was gram positive *Staphylococcus aureus*. Our results showed that the weakest and strongest association between bacteria elimination rate and nanoparticle

concentration happened respectively with ZnO and CuO nanoparticles. The results also showed that at a specific concentration of TiO<sub>2</sub>, ZnO and CuO nanoparticles, the strongest antibacterial effect happened with ZnO. The gram positive bacteria without spores, probably due to their high nanoparticle absorption, were the most sensitive bacteria.

## CONCLUSION

The nanoparticles ZnO, CuO and TiO<sub>2</sub> can be used in appropriate doses as strong disinfecting agents in hospitals and can probably reduce nosocomial infections and their consequences.

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