Evaluation of the resistance of CuO-coated contact lenses to bacterial contamination

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Abstract: The main aim of this research was to prepare an antibacterial coating on the surface of Contact Lenses (CL) by using copper oxide nanoparticles (CuO NPs) and assessment the activity of these nanoparticles to reduce the attachment of bacteria on the lenses. For coating we used an immersion method with three different solutions Ethyl alcohol 99%, Oil and lenses solution and dispersed CuO nanoparticles at different concentrations (100ppm, 200ppm and 300ppm). Two immersion methods were used to prepare coating 1- immersion for 5 minutes in ethanol and contact lens solution. 2- double dipping method for 5 seconds for each turn used with oil solution. The coated lenses was examined by scanning electron microscope and also was incubated with two different bacteria: Staphylococcus aureus and Pseudomonas aeruginosa for 24hrs, 72hrs and 30 days at 37°C. In this study another aim was preceded by testing the ability of the two bacterial types to form biofilm by tube and Congo red agar methods.

Key Words: Biofilm, Contact lens, Copper oxide nanoparticles, Immersion

1. Introduction
The contact lens was first designed by Leonardo DaVinci in 1508, and the first glass contact lenses designed for vision correction for very short periods (hours) of wear in the 19th century; Soft contact lens as we know it today was made of biocompatible and transparent hydrophilic hydrogel polymers[1]. Over 120 million people around the world wearing contact lenses for refractive correction and cosmetic purposes [2].

The use of Contact lens is a major risk factor for developing microbial keratitis (MK). It has been reported that contamination of contact lenses is associated with contact lens peripheral ulcer (CLPU), contact lens acute red eye (CLARE), and infiltrative keratitis. Microorganisms including bacteria, yeasts, molds, and amoebas have been involved in ocular infections and have the ability for adhesion to contact lenses, growth, and biofilm formation on its surface by encountered by antimicrobial proteins on the eye surface, including lysozyme, lactoferrin, and antimicrobial peptides, and by phagocytic cells. Microbial infection of the cornea (microbial keratitis) induces damage to corneal tissues and could potentially result in vision loss (blindness).

The most common eye infections are bacterial infections which may be caused by one of the following strains: Ps.aeruginosa, S.aureus, E.coli, S.pneumoniae and other strains [3].
**Pseudomonas aeruginosa** is one of the most common pathogens responsible for contact lens-related microbial keratitis. Pseudomonas isolated from ocular infections has exhibited an ability for biofilm formation and express antimicrobial resistance genes [4]. Keratitis is an eye-threatening disease, with the majority of cases are related to infective causes such as bacteria, viruses, fungi and protozoa. Within infectious keratitis, bacteria are the most common causative pathogens [5]. Also conjunctivitis is commonly occurring infection. It’s basic symptoms include redness of eye, increased amount of tears, mucous discharges, burning eyes, increased light sensitivity, itchy eyes and blurred vision. In most cases, it self-cures within one to two weeks. However, in the case of other infections (such as viral or fungal infection) or chronic inflammation, antibiotic therapy is required [3].

Many procedures that have been used to decrease the risk of eye infection. These procedures include lens , storage case cleanliness, using lens care solutions that provide effective disinfection, and constant replacement of lens cases. The development of antimicrobial surfaces for contact lenses or contact lens storage cases may provide an effective strategy to decrease the risk of MK [6]. some nanoscale materials have used as new antimicrobial agents to replace antibiotics in certain applications, for example Silver nanoparticles (AgNPs) exhibits strong antimicrobial activity and have been proven to interrupt adhesion and biofilms formation by bacteria on solid surfaces [7].

**2. Materials and Methods**

**1- Materials**

**A- Bacterial strains**

In this study two bacterial strains obtained from the bacterial bank unit in Biology department/College of Science have been used .

- *Staphylococcus aureus* (gram positive).
- *Pseudomonas aeruginosa* (gram negative).

**B- contact lenses**

Many contact lenses (top vision) made in Korea have been used in this research.

**C- Copper oxide nanoparticles (CuO NPs)**

25 gm of CuO nanoparticles 30-50 nm produced by VCN Materials Company have been used.

**D- Dispersion solutions**

Three different solutions with different density have been used for dispersion of CuO nanoparticles, these include:

- Fresh look contact lens solution 150 ml.
- Ethyl alcohol 99%.
- Oil solution called baby Johnson.

**E-Media**

The following media were used after preparation followed the instruction of the company ,the media are: Nutrient agar (Neogen) , Nutrient broth (Neogen) , MacConkey agar (Neogen) , Mueller-Hinton agar(Biomark) , Mannitol salt agar (Neogen) and Brain Heart Infusion Broth(Biomark)

**F- solutions and stains**

The following solutions and stains were used: Crystal Violet 0.1%(B.M.L) , Congo red (Fluka Garantie) ,Buffer saline solution (BSS) ,Ethyl alcohol99%, Oil solution (Johnson & Johnson) Normal saline ,Glycerol (Panreac), BaCl₂ and H₂SO₄.

**2- Methods**

**A- Investigation of the ability of bacterial strain to form biofilm**

Two methods were used to screen the ability to biofilm formation:

**1- Tube method:**

The bacterial types *S.aureus* and *Ps.aeruginosa* were grown in nutrient broth for 48hrs at 37°C .The broth was discharged and the tubes were rinsed gently with buffer saline solution to remove unbound bacterial cells, then tubes were placed upside down and left to dry. The tubes were filled with crystal
violet stain 0.1% for 20 min then the stain was discharged and tubes were rinsed gently with BSS to remove excess stain and put upside down to dry [8].

2- Congo red agar method:
Both *S. aureus* and *Ps. aeruginosa* were inoculated on MSA and MacConkey agar media respectively and incubated for 24hrs at 37° C. After incubation a loopful of growth of each bacterium was inoculated separately on previously prepared Congo red agar and incubated for 24hrs at 37° C [9].

B- Investigation of the effects of CuO nanoparticles on Bacteria by using agar wells diffusion method
All the following steps were performed according to [10].
- 1.5x10^8 cfu/ml bacterial suspensions of each *S. aureus* and *Ps. aeruginosa* were prepared in normal saline by comparison with McFarland standard number 0.5.
- Muller Hinton agar plates were inoculated with bacteria.
- 300µg/ml of CuO nanoparticles suspensions were prepared in deionized water.
- 6mm wells were punched in Muller Hinton agar plate and 100µl of each CuO nanoparticle suspensions were transferred to separated wells.
- The plates then were incubated for 24hrs at 37° C.

C- preparation of CuO nanoparticle coating
- 0.01, 0.02 and 0.03 gm of CuO nanoparticles were added to 100 ml of each of Ethanol 99%, lens solution and oil solution separately to prepare suspensions of 100ppm, 200ppm and 300ppm respectively.
- Six lenses were immersed in each suspension with dispersed nanoparticles (alcoholic, lens solution and oil solution)

Two immersion methods were used to prepare coating
1- Immersion for 5 minutes in ethanol and contact lens solution .
2- Double dipping method for five seconds for each turn used with oil solution.
Each lens then was removed from dispersion solution and washed by deionized water to remove the remaining solution and unbound NPs [11].

D- Assessment of CuO-coated lenses’ resistance to bacterial contamination
- 1.5x10^8 cfu/ml suspensions of *S. aureus* and *Ps. aeruginosa* were prepared in BSS by comparison with McFarland standard number 0.5.
- 1ml of bacterial suspension was transferred to 19 ml of nutrient broth tubes (10 broth cultures were prepared) [12].
- Three coated lenses from each suspension were transferred to each broth culture (9 broth cultures tubes).
- three uncoated lenses were transferred to the remaining (10th) broth culture tube to be used as control.
- Culture tubes were incubated at 37° C
- After 24hrs, 72hrs and 30 days one lens from each culture tube was transferred, rinsed gently with BSS to remove unbound cells and then placed in sterile nutrient broth separately and incubated for 24hrs at 37° C.
- The turbidity then was measured by spectrophotometer at wavelength of 600nm to assess the ability of CuO nanoparticles to reduces bacterial adhesion to the lenses [13].

E- Statistical analysis
The results were reported as Mean±SEM using SPSS (version 23) and variance between groups were made using one way ANOVA followed by Duncan multiple range test

3. Results and Discussion:
1-Biofilm formation:
The results of tube and Congo red agar methods (figure1a,b) and (figure2 a,b) showed production of thin layers of cells on the tube's walls and formation of black colonies on Congo red agar indicates that both *S. aureus* and *P. aeruginosa* which indicates that the two types of bacteria have the ability to form biofilm
Figure 1a: On the lift tube with *S.aureus* biofilm. On the right control tube.

Figure 1b: On the lift tube with *Ps.aeruginosa* biofilm. On the right control tube.

Figure 2a: *S.aureus* colonies on congo red agar

Figure 2b: *Ps.aeruginosa* colonies on Congo red agar

This result agree with many studies that refered to the positive result of biofilm formation which is represented by development of violet layer in the bottom and on the walls of tube, while by using the Congo red agar method the positive result is represented by the formation of black colonies on the surface of agar plate [14][9].

There was a variation in the ability of both bacteria to form biofilms using the tube method and the Congo red agar method as we encountered bacterial isolates that gave a positive result with the tube method and negative result with the Congo red agar method for the same isolate.

Halim *et al* [15] mentioned that the proportion of biofilm formation by tube method or the Congo red agar method varies from one bacterium to another. The reason may be due to the difference in sensitivity and specificity of each method.
**Effect of CuO NPs on bacteria:**

The results of the effects of CuO nanoparticles suspension on bacterial growth by agar wells diffusion method revealed that the CuO nanoparticles suspension at concentration of 300µg/ml exhibits an inhibition zone of 23mm and 29mm in diameter for *Ps.aeruginosa* and *S.aureus* respectively (figure 3a & b).

![Figure 3a: inhibition zone produced by CuO nanoparticles against *Ps.aeruginosa*](image1)

![Figure 3b: inhibition zone produced by CuO nanoparticles against *S.aureus*](image2)

The result of inhibitory action of CuO nanoparticles against *S.aureus* was agreed with the results given by Raheem *et al* [16] who found that CuO NPs is highly recommended as an alternative anti-MRSA agent with significant inhibitory and antibacterial effect. As well as Kawahara *et al* [17] mentioned that CuO nanoparticles were effective in killing a range of hospital-acquired Pathogens. The results of our study also agree with results given by Chauhan *et al* [18], who confirmed that copper oxide nanoparticles have an inhibitory effect on the growth of *Ps. aeruginosa*.

Antibacterial effects of Nano-metal oxide include, physical disruption and oxidative stress [19]. Reactive oxygen species (ROS) including superoxide anion (O$_2^{-}$), hydrogen peroxide (H$_2$O$_2$), hydroxyl radicals (HO$^*$) and organic hydro peroxides (OHP), deposition of NPs on the surface of bacterial cell and accumulation of NPs in the cytoplasm/perioplasmic area can be resulted in bacterial death [20]. ROS can result in damage of cellular constituents peptidoglycan, lipids, DNA and proteins through releasing from NPs and penetration into bacteria [21].

**Preparation of CuO NPs coating**

The immersion of contact lenses in different CuO nanoparticle suspensions resulted in creation of coating on the surface of lenses comparing with uncoated once as shown in scanning electron microscope images (figure3-4a,b,c) but at different efficiency as the results revealed in tabel1.
Figure (4a): SEM image of uncoated lens.

Figure (4b): SEM image of CuO nanoparticles-coated lens

*SEM: Scanning Electron Microscope

Figure (4C): uncoated contact lens on the left and CuO NPs coated lens on the right

**Assessment of CuO-coated lenses’ resistance to bacterial contamination**

In this experiment the result was the appearance of turbidity in all tubes in comparison with broth tube is an evidence of the formation of a biofilm on the surface of the lenses as shown in (figure 5)
Figure (5): CuO-coated and uncoated lenses transferred from bacterial culture to sterile nutrient broth after rinsing with BSS and incubated for 24hr at 37°C. Turbidity indicates the formation of biofilm on the lenses.

From these results we conclude that higher concentrations of CuO NPs may be needed for complete prevention of bacterial adhesion and biofilm formation on the surface of CL or perhaps some NPs were detached during incubation.

The turbidity of each tube was measured spectrophotometrically (EMCLAB) to assess the activity of different concentrations of CuO NPs in preventing bacterial adhesion to the surface of lenses, these results are summarized in table (1).

Table 1: the absorbance of media containing bacteria forming a biofilm on the surface of CuO NPs coated lenses at different concentrations along with an uncoated control after incubating the lenses for 24, 72 hrs and 30 days.

| Con. | Dispersion solution | S.aureus Incubation period | Ps.aeruginosa Incubation period |
|------|---------------------|---------------------------|-------------------------------|
|      | 24 hrs              | 72hrs                     | 30days                        | 24hrs                          | 72hrs                          | 30days                        |
| Control | Alcohol            | 0.65±0.01                  | 1.18±0.00                     | 1.41±0.00                     | 0.97±0.01                     | 1.81±0.00                     | 1.82±0.00                     |
|        | L.S                 | 0.39±0.02                  | 0.71±0.00                     | 0.74±0.01                     | 0.68±0.01                     | 0.79±0.00                     | 0.81±0.02                     |
|        | Oil                 | 0.64±0.04                  | 0.75±0.00                     | 0.76±0.00                     | 0.81±0.04                     | 0.87±0.01                     | 0.91±0.00                     |
|        | 100 ppm             | 0.65±0.05                  | 0.79±0.02                     | 0.82±0.01                     | 0.96±0.04                     | 1.73±0.05                     | 1.74±0.01                     |
|        | Alcohol             | 0.38±0.01                  | 0.62±0.01                     | 0.64±0.01                     | 0.66±0.02                     | 0.75±0.00                     | 0.75±0.00                     |
|        | L.S                 | 0.61±0.00                  | 0.68±0.00                     | 0.68±0.00                     | 0.88±0.00                     | 0.78±0.01                     | 0.78±0.00                     |
|        | Oil                 | 0.61±0.01                  | 0.68±0.01                     | 0.70±0.00                     | 0.94±0.01                     | 1.50±0.04                     | 1.50±0.01                     |
|        | 200 ppm             | Alcohol                    | 0.35±0.01                     | 0.61±0.00                     | 0.62±0.00                     | 0.54±0.01                     | 0.71±0.00                     | 0.71±0.00                     |
|        | L.S                 | 0.52±0.02                  | 0.64±0.00                     | 0.65±0.00                     | 0.65±0.02                     | 0.74±0.01                     | 0.74±0.00                     |
|        | Oil                 | 0.60±0.03                  | 0.65±0.02                     | 0.67±0.00                     | 0.94±0.04                     | 1.24±0.04                     | 1.24±0.00                     |
|        | 300 ppm             | Alcohol                    | 0.35±0.01                     | 0.61±0.00                     | 0.62±0.00                     | 0.54±0.01                     | 0.71±0.00                     | 0.71±0.00                     |
|        | L.S                 | 0.52±0.02                  | 0.64±0.00                     | 0.65±0.00                     | 0.65±0.02                     | 0.74±0.01                     | 0.74±0.00                     |
|        | Oil                 | 0.60±0.03                  | 0.65±0.02                     | 0.67±0.00                     | 0.94±0.04                     | 1.24±0.04                     | 1.24±0.00                     |

Similar letters refers to no significant differences at the P≤0.05.

From the previous table the results showed that there are significant differences between most groups as a result of reduction in the number of bacterial cells attached to the surface of the lenses in the presence of the CuO nanoparticles. The group of lenses coated with CuO nanoparticles at a concentration of 300ppm using alcohol as a dispersion medium for nanoparticles showed a stronger effect in prevention of bacterial adhesion and biofilm formation on the surface of CL (0.35,0.61 and
0.62 for *S. aureus* and 0.54, 0.71 and 0.71 for *Ps. aeruginosa* after 24, 72hr and 30 day of incubation respectively compared to the control group and other groups, while the group of lenses coated with nanoparticles at a concentration of 100ppm using the oil solution as a dispersion medium for nanoparticles showed little differences compared to the control group especially at CuO concentration of 100ppm whereas no significant differences were noticed in comparison with the control lenses. A decrease in absorbance indicates a reduction in the number of bacterial cells [13].

Our results showed that the number of bacterial cells attached to the surface of the lenses to form biofilms increases over time, and this result agree with the study of [22]. Chae and Schraft [23] concluded that the different strains of same species may have different behavior in biofilm formation and thats may be why *Ps. aeruginosa* gave higher turbidity than *S. aureus* during each incubation period, *Ps. aeruginosa* may have greater tendency for attachment and biofilm formation than *S. aureus*.

In our study three solutions with different density were used for dispersion of CuO nanoparticles and preparation of coatings on the surface of contact lenses. The results show that CuO nanoparticles have potential activity against bacterial adhesion in comparison with the control uncoated lenses. The aim of using three different solutions was to obtain good distribution of CuO nanoparticles, and as a result better coating. Better coating can be obtained by using Ethanol 99% as a media for dispersion of CuO nanoparticles while in using oil the nanoparticles were accumulated together and gave weak dispersion and coating. These results disagree with the results given by [11].

The increased amount of nanoparticles has greater bactericidal effects hence greater ability to prevent bacterial adhesion and biofilm formation, this result agreed with the result given by [6] but other properties of lenses like transparency and flexibility must be considered as they may be affected by the high concentration of nanoparticles.

### 3. Conclusion

- Copper oxide (CuO) nanoparticles have an antimicrobial activity against both gram positive and gram negative bacteria.
- It is not easy to handle with and distributing metal nanoparticles homogeneously as they are insoluble.
- It is possible to prepare an effective antimicrobial coating on the surface of contact lenses by using nanoparticles like copper oxide nanoparticles.

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### 5. References

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