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In vitro evaluation of nickel oxide-based nanocomposite as wound dressing material against the bacterium isolated from burns

Azadeh Basiri[1], NasrinTalebian[*2], Monir Doudi[1], Maryam Tayebani[2]

1. Microbiology Department, Falavarjan Branch, Islamic Azad University, Falavarjan, Isfahan, Iran.
2. Science Faculty, Chemistry Department, Shahreza Branch, Islamic Azad University, Shahreza, Isfahan, Iran.

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ABSTRACT

The introduction of newly devised wound dressing has been a major breakthrough in the management of wounds or infections. The aims of this paper are to isolate and identify bacterial species causing burn wound infections from a University-related Iranian hospital as well as determination of the antimicrobial susceptibility of the isolated microorganisms to newly devised nanocomposite materials for developing efficient wound dressing. The NiO nanoparticles were generated in situ and subsequently impregnated on the surface of cotton fabrics using ultrasound irradiation. Then, surface modification was performed to reduce initial bacterial attachment using polyethylene glycol. Cotton fabric was characterized by measuring scanning electron microscope (SEM), X-ray diffraction (XRD) and antibacterial properties. Disk diffusion method was used to quantify the efficacy of NiO-based wound dressing against the most common burn wound pathogen, *Pseudomonas aeruginosa*, isolated from burns and wound swabs patients of Emam Burn and Accidents hospital in Isfahan province, Iran. All isolates showed high resistance to the commonly used antibiotic (Ampicillin, Gentamicin, Cephalexin, Co-trimoxazole and Amoxicillin). In vitro evaluation showed that the modified cottons exhibited excellent biocidal action against high-resistant isolated Gram-negative bacteria compared to unmodified ones. The results suggested that NiO nanoparticles may be considered as an effective component of therapy for burn infections and in the combination with different antibacterial agents to overcome the resistance of the microorganisms and to obtain synergic antibacterial activity.

1. Introduction

Despite significant improvement in the survival of burn patients, infectious complications continue to be a major cause of morbidity and mortality. Burn patients are at high risk of wound infection, sepsis, and ventilator-associated pneumonia because of reduced immune responses. In the ‘Ebers and Smith’ papyri (1500-1600 B.C.) are descriptions of dressing materialsused in ancient Egypt, including bandages with a variety grease which can beseen as the precursors of tullegras. For centuries silver has been known to have bactericidal properties (Ebell et al., 1937; Bryan et al., 1930). As early as 1000 B.C., the antimicrobial properties of silver in rendering water potable were appreciated (Richard et al., 2002; Russell et al., 1994). More recently, silver has re-emerged as a treatment option for infections in burn wounds and particularly sulfadiazine cream is used to manage burns

[*]Corresponding author. Dr. NasrinTalebian
Tel.: 0913 328 8163
E-mail address: talebain@iaush.ac.ir

(Singh et al., 2012). However, the prolonged topical application of silver sulfadiazine cream can induce argyria (Chaby et al., 2005), toxic effect on skin cells (Hussain et al., 2006), traumatise new epithelised surface and delay wound healing (Sawhney et al., 1989). Systemic antibiotics treatment can be used (David et al., 2013) but they are less favorablebe cause of the low penetration rate into dead tissue and the risk of resistance development (Yildirim et al., 2005).

Due to an increasing number of patients suffering from wounds and burns, intrinsically difficult treatment and healing, and increasingly pathogenic infections caused by bacterial strains resistant to antibiotics, health care professionals faced with developing a new generation of biocides for wound dressing (Mihai et al., 2014). Recently, nontraditional antibiotic agents have been of tremendous interest in overcoming resistance that is developed by several pathogenic microorganisms against most of the commonly used antibiotics. Especially, several classes of antimicrobial nanoparticles (NPs) and nanosized carriers for antibiotic delivery have proven their effectiveness for treating infectious diseases, including antibiotics resistant ones in vitro, as well as in animal models (Allaker et al., 2008).

The impregnation of inorganic nanoparticles on textiles can open up a new opportunity for multi-functional modification of fibers. Amongst inorganic materials, the use of noble metal antimicrobials of which the most prevalent is silver and metal oxides have elicited a great deal of interest for use as antimicrobial agents for wound dressing (Mdhumathi et al., 2010; Shalumon et al., 2011; Gouda et al., 2010).

Despite aggressive local and systemic treatment to minimize infection, severe burn wounds continue to become infected with environmental and nosocomial pathogens at relatively high rates. Among these, *Pseudomonas aeruginosa* (*P.aeruginosa*) is paramount, accounting for over half of all severe burn infections (Hodle et al., 2006). This gram-negative bacterium is well adapted to the environment, utilizing biofilm colony growth, which provides a tremendous survival advantage for the pathogen and effectively prevents eradication by the host immune system or antimicrobial drug treatment. A recent review of burn trauma patients who acquired secondary infection with *P.aeruginosa* reported that mortality was approximately four fold greater than in those without *P. aeruginosa*, with an average of 23 ventilator-assisted days in *P.aeruginosae* infected patients (Armour et al., 2007). Historically, mortality in burn patients with *P.aeruginosa* bacteremia has been as high as 77% over a 25-y period (McManus et al., 1985).

In Iran, high incidence of hospital-acquired infections constitutes a major problem on health care systems especially in burn units due to the spread of resistance to antibiotics commonly used in clinical practice (Khorasani et al., 2008). There are a few epidemiologic burn studies in the different geographical regions limited to South West, North and South of Iran to report burn incidence and burn mortality rates as well as the descriptive epidemiology of burns to patients of different aged years and etiological factors of burn injuries in Iran society (Soltani et al., 1998). Over the past 30 years, *P.aeruginosa* has been found to be the most prevalent bacteria in burn centers in Iran (Shahcheraghi, 2003).

In this study, 25 bacterial isolates were collected from burns and wound swabs of patients of Emam hospital, the only Teaching-University related hospital and subspecialty referral center located in Isfahan province. The isolates' susceptibility patterns to selected antibiotics were determined by the disk diffusion method. Nearly all isolated strains showed high antibiotic resistance. We have developed a new method for preparing cotton bandages with antibacterial properties by coating fabric surface with NiO nanoparticles via ultrasound irradiation. Then, surface modifications to reduce initial bacterial attachment and to improve their functionality were performed by pre-treating the fabric surface with poly (ethylene glycol). Then, the antibacterial activity treated bandages were evaluated against antibiotic resistance isolated strains. The results illustrated that organic/NiO nanohybrid treated bandages have significant antimicrobial activity as combination therapy to combat drug resistance isolated bacteria compared to single drug therapy. We proposed the main strategy to combine different antibacterial agents to over come the resistance of the microorganisms and to obtain synergic antibacterial activity for future studies.

2. Materials and Methods

2.1. Sample collection and Microbiology

This study was conducted on patients with deep and full thickness burns who had been hospitalized at Emam University related Burn and Accidents hospital, located in Isfahan, Iran. The samples including wound swabs and tissue biopsies were collected from hospitalized patients of burn and surgical units. For the isolation of *P. aeruginosa*, sheep blood agar and EMB (Eosin Methylene Blue) were used. Subsequently growth at 42°C in brain heart infusion, the oxidative test and the Oxidative-fermentation (OF) test for carbohydrate utilization were used for identification of *P.aeruginosa* (Gilard et al., 1991).

2.2. Antibiotic sensitivity

The isolates susceptibility patterns to antibiotics were determined by the disk diffusion method of Bauer (Bauer et al., 1966). Using Muller Hinton agar (Merck, Germany) and commercially available paper discs (Hi-Media, India). The antibiotics tested included Ampicillin (AM), Gentamicin (GM),Cephalexin (CN), Co-trimoxazole (SXT) and Amoxicillin (AMX).

Briefly, an inoculum from Muller–Hinton agar was taken and then suspended in Nutrient Broth. The suspension was incubated for 2 h at 37°C and its turbidity was adjusted to a 0.5 McFarland standard. The microorganisms were inoculated on Muller–Hinton agar plates and then disks containing drugs were applied on plates. The growth of colonies was examined after incubation of the plates for 24h at 37°C. The antibiotics sensitivities were tested by Disk Diffusion Method. A zone of inhibition becomes apparent and its size provides some indication of the potency of the antimicrobial activity.

2.3. Sol preparation and Treatment of cotton fabrics

Sol–gel technology provides a new way to functionalize fabrics by improving their chemical composition and physical properties (Alongi et al., 2011). In textile materials, this technique is able to modify the surface and then, to improve antibacterial finishing.

In a typical experiment, using nickel nitrate Ni(NO3)6.6H2O as asource of Ni and methanol as solvent, 3.32 g of former was added to 40 ml of solvent and stirred vigorously at 60°C for 1 h, leading to the formation of light green colored sol, Fig.1. Antibacterial-coated bandages were prepared as follows: the cotton fibers were washed first by water and detergent at 50°C for 30 min to remove the impurities such as wax and fat and then washed several times by a large amount of de-ionized water. They were further cleaned in acetone (Merck) for 10 min and dried at room temperature. Then, a cotton bandage (0.7 g, 10 × 10 cm) was added to above sol in a 100 ml sonication flask and then irradiated for 30 h with a high intensity ultrasonic Ti-horn (20 kHz, 750 W at 70% efficiency, Sonics and Materials VCX600 Sonifier). The flask was placed in an ice-water cooling bath maintaining a constant temperature of 30°C during the sonication. The treated samples were then placed in 70°C pre-heated oven to remove the solvent from the fiber and then heated at 150°C for 15 min, to complete the formation of nickel oxide from the precursor. Then the unfixed nanoparticles were removed by washing with sodium lauryl sulfate solution and the fabric was dried under vacuum.

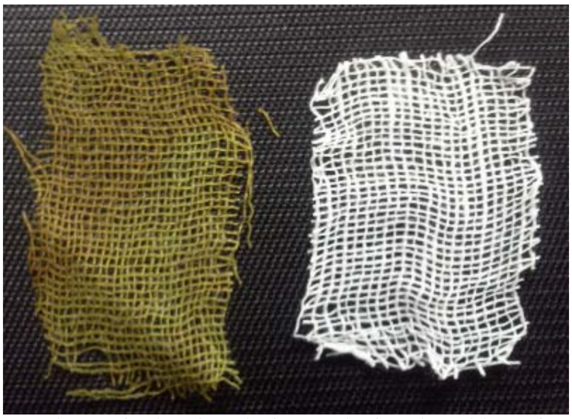


Fig. 1. Photo of a bandage before and after deposition of NiO nanoparticles.

In other experiment, the pre-scoured samples were first modified with 10% aqueous poly (Ethylene glycol) (PEG) solution at 25°C for 60 min and finally dip-coated in the NiO sol as previous procedure. Three more samples were independently prepared including untreated cotton fabrics, single PEG pretreated and PEG/NiO pretreated without ultra sound irradiation, as control.

2.4. Characterization

The surface morphology of cotton fibres and NiO nanoparticles loaded cotton fibres were studied using a Philips, XL30 Scanning Electron Microscope (SEM). The crystalline and average particle size of prepared nanoparticles was analyzed by XRD pattern using X-ray diffractometer (Bruker, D8 Advance, Cu Kα).

2.5. Antibacterial activity

The antibacterial efficiency of the all fabrics was assessed according to pour-plate method as follows: Bacteria cells were precultured at 37°C for 24 h. All the culture media were sterilized with normal autoclaving before being used.

The inoculum was made by direct suspension of colonies insaline solution with concentration of 8.9 g/L. It was adjusted to the turbidity of McFarland standard 0.5 or approximately $1–2\times10^8$ CFU/ml. The treated cotton fabrics and control sample were cut into small pieces and transferred into a vial. 10 mm diameter discs of the test fabrics and control fabrics were thengently pressed on to the surface of the plate.1 ml of inoculum was added to each test tube. The test tubes were loosely capped to prevent evaporation. The plates were incubated at 37°C for 24 h and the inhibition zone was measured around as the potency of the antimicrobial activity.

2.6. Minimum inhibitory concentration (MIC) assay

The minimum inhibitory concentrations (MICs) for selected antibiotics and PEG/NiO powder sample were also determined using the microdilution broth methods as recommended by NCCLS (Pietro, 2002).

3. Results

3.1. Epidemiology study

There are a few epidemiologic burn studies in different geographical regions of Iran to report burn incidence and burn mortality rates as well as the descriptive epidemiology of burns to patients of different aged years and etiological factors of burn injuries in Iran society (Rastegarlari et al., 2000; Askarian et al., 2000). As reported in these studies, the most frequent organism isolated was *P.aeruginosa*, followed by *Staphylococcus aureus* (*S.aureus*). Other organisms included mix *pseudomonas* and, *S.aureus*, *E.coli*, *Acinetobacter* and *Citrobacter* spp.

Emam Burn Hospital in Isfahan is the only Teaching-University related hospitaland subspecialty referral center located in Isfahan province. 95% of patients in the hospital grounds deprived groups of society. Nearly half of the country's burns victims are annually treated in this hospital. 60% of patients were male and 40% were female. The most common age group was≤ 10 years group admitted (42%), second-degree burns (81%) and the mean body surface area burned (30%). The most common etiology of burns was due to flame (60%), and scalds (24%), respectively. However, other injuries related to electricity, ultraviolet radiation, chemicals and respiratory damage due to smoking are also considered. The mean length of hospitalization was 15±3 days. The overall mortality rate was 14%. Most of the injuries requiring hospital admission occurred during the winter months. The prevalence of resistant strains varies geographically (Yildirim et al., 2005). As the spectrum of microbiota causing infections in burn patients varies with geographical location, the comparison of bacterial isolates with other studies may be difficult. However, *P. aeruginosa* was the most common pathogen causing wound infection and bacteremia in Emam burn center, and was also most common in other reports, especially from developing countries (Pandit et al., 1993). The second most common isolate was *S.aureus*, which is similar to other studies (Shankowsky et al., 1994). This is in contrast, however, to some other studies especially from developed countries which report *S. aureus* as the most predominant organism in burn infection (Pegg., 1992). This may be explained by the fact that this opportunistic microorganism grows mainly in moist body areas, such as burn wounds (Song et al., 2001), and also by prolonged hospital stays and the administration of broad-spectrum antibiotics in burn cases. A burn represents a site susceptible to opportunistic colonisation. The situation of burn victims with *P.aeruginosa* infection is particularly problematic, since this organism is inherently resistant to many drug classes and is able to acquire resistance to all effective antimicrobial drugs (Livermore, 2002).

The infection control programme for burn centers in Iran is similar to that of other countries. The patients in different centers have a programme with at least thrice weekly washing, dressing and culture of wound surface and other parameters. Due to the presence of multidrug resistance bacteria, the treatment is combination therapy for example including Aminoglycosides, Ceftazidime and Ciprofloxacin. In some reports, antibiotic treatment and sensitivity were studied against the most bacterial pathogen strains isolated from the infected wounds of burn patients hospitalized (Rastegarlari et al.,2000; Khorsani., 2008; Shahcheraghi et al., 2003).

3.2. Antibiotic susceptibility pattern

Antibiogram of *P.aeruginosa* to 5 antimicrobial agents is presented in Fig. 4. All bacterial isolates showed high resistance to the commonly used antibiotic which could be as a result of prolonged hospital stays, used antibiotics over-usage; unavailability and the high cost of preferred antimicrobial agent choices. The most effective agent against *P.aeruginosa* was Gentamicin (GM, ~ 52% sensitive) and the highest resistance was observed for Cephalexin (CN, 100% resistance).

The emergence of antibiotic- and/or multidrug-resistant bacteria is recognized as a crucial challenge for public health. Killing of antibiotic-resistant bacteria requires multiple expensive drugs that may have side effects. NPs can offer a new strategy to tackle multidrug-resistant bacteria (Leid et al., 2012).

3.3. Cotton bandages characterization

Fig. 2 shows the X-ray diffraction (XRD) patterns of untreated and treated NiO bandages. The diffraction pattern for untreated cellulose fiber showed the characteristic intense peaks at 16˚, 22˚ and 26˚ which coincides with reported literature values, as shown in Fig. 2(A) (Mathew et al., 2005). The XRD pattern of NiO treated bandage presented single-phase NiO with a cubic structurein good agreement with JCPDS no. 44-1159, Fig. 2(B) (Kim et al., 2006).

The surface morphology of the untreated and treated cotton fabricscanbe seen in Fig. 3(A) and (B). An obvious difference between theuntreated and NiO treated cotton fabrics was observed. These images demonstrated the presence of NiO nanoparticles on the coated cotton fabrics.

3.4. Antibacterial activity assessment

The influence of different treatments based on single NiO coating and NiO/PEG nanocomposite with or without ultrasonicon the antibacterial activity of the cotton fabrics is given in Table 1. The zone of inhibition of the test sample was measured in mm, and it was a measure of the antimicrobial activity of the treated bandages. No bacterial reduction was found on the untreated cotton sample even after 3h contact time, indicating *P. aeruginosa* use cotton as a nutrient. The finished fabrics revealed different antibacterial activity depending on the treatment conditions. However, all the treated fabrics almost showed good antibacterial property with higher activity by the fabrics ultrasonically treated with NiO/PEG nanocomposite.

Ultrasonic irradiation has been proven to be an effective method of deposition of nanoparticles on the surface of various substrates including textiles. This process results in a smooth and homogeneous layer of coating and is capable of projecting nanoparticles towards the fabric surface at a very high speed. The high impact speed causes them to adhere strongly to surfaces (Abramov et al., 2009). Moreover, highly hydrophilic surface coatings utilizing PEG chains are well known to reduce both protein adsorption as well as bacterial attachment (Roosjen et al., 2004).

The obtained results showed that cotton bandage surface modification provides a way to impart some functional properties to textiles including antibacterial activity, self-cleaning etc. These properties increased in the modified fabrics by eco-friendly polymer modifications.

3.5. Minimum inhibitory concentration

Dilution methods are used to determine the minimum inhibitory concentrations (MICs) of antimicrobial agents including five selected antibiotics and NiO nanopowder. Determining the MIC gives a quantitative assessment of the potency of an antimicrobial agent and is widely used in the comparative testing of new agents. As shown in Table 2, it was found that the NiO nanopowder antibacterial efficacy is almost the same order of magnitude the efficacy of free tested antibiotics.The different MIC values for biocide samples indicated different bacteria toxicities.

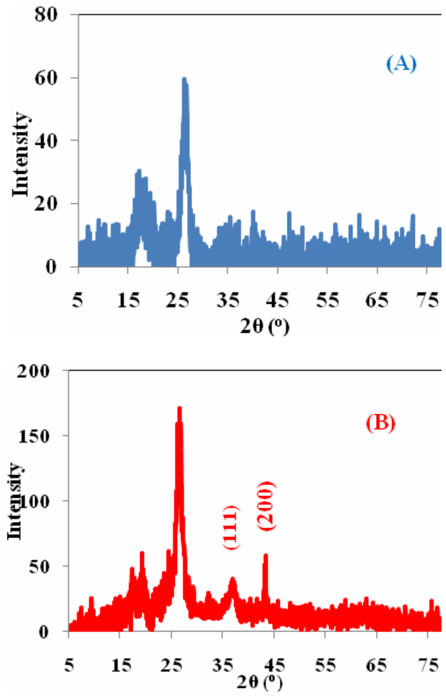


Fig. 2. XRD of (A) untreated and (B) NiO/treated cotton bandage.

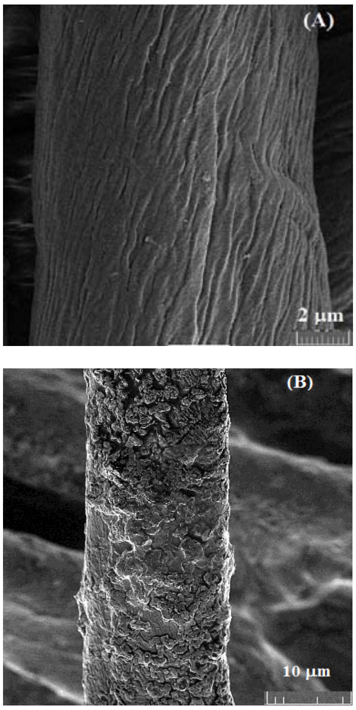


Fig. 3. SEM photographs of (A) untreated cotton, and (B) PEG/NiO treated cotton

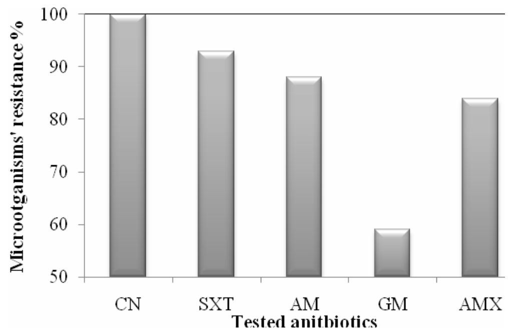


Fig. 4.Distribution of isolated microorganisms' antibiotic resistances

Table 1. Photographs of inhibition zones for different bandage samples as antibacterial ctivity

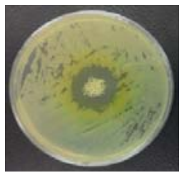
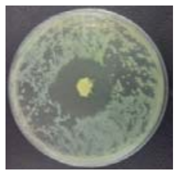
Condition	*P. aeruginosa*
Untreated	
Ultrasonically PEG treated	
Ultrasonically NiO treated	
NiO/PEG treated	
Ultrasonically NiO/PEG treated	

Table 2. MICs for control organisms on Mueller-Hinton

Antimicrobial agent	MIC (µg/ml) *P. aeruginosa*
AM	17-19
GM	0.18-0.21
CN	29-30
SXT	25-28
AMX	8-15
NiO powder	20-24

4. Discussion

P.aeruginosa was the most common pathogen causing wound infection and bacteremia in Emam burn center, Isfahan, Iran, and was also the most common in other reports,especiallythose from developing countries. *Pseudomonas* is very resistant to most antibiotics and antibiotic resistance in these organisms would develop very rapidly. Therefore, use of antibiotics to control acquisition of infections is not cost-effective and so not reasonable. Our results from isolated strains antibiotic resistance suggest that, in order to reduce burn wound infection rate and decrease *P. aeruginosa*. infection rate and also mortality rate in burn patients, the combination of bactericidal NiO nanoparticles with protein-repelling chemical moieties, such as chains of poly (ethylene glycol) should be performed. Then, NiO nanoparticles are uniformly deposited onto the surface of a cotton bandage by the sonochemical method. The present study provides evidence for the antimicrobial effect of the NiO nanoparticles against common burn wound pathogen, *P.aeruginosa*. We propose that future approaches should combine the nanoparticles and bacteria-repellent property of PEG-coatings with potential applications in wound dressing, bed lining and as active bandages. However, more accurate testsare required for clinical management of burn infections.

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Conflict of interest

There are no conflicts of interest to declare.

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