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Bactericidal Action of *E. coli* Mediated Nanoparticle Coated Medical Dressings

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Abstract

The aim and objective of the present work is to synthesize silver nanoparticles and to study the antimicrobial activity of silver nanoparticle coated medical dressings against wound causing organisms. In the future, this can lead to wound care management through nanotechnology. The nanoparticles were synthesized by using *E. coli* in silver nitrate solution (10^{-3} M) as a commonly available strain, and activity was observed against *S. aureus* as a test culture. Dressings were prepared and coated with silver nanoparticles and routine antimicrobial tests were performed in microbiology laboratory. It was observed that a synthesized nanoparticle was effective against the gram negative organism. Such *E. coli* induced silver nanoparticles showed a good bactericidal activity. This is the first reported study of *E. coli* coated medical dressings in the area of nanotechnology. The results are discussed in detail with photographs, along with further study designed to improve the range of bacteria which enable the control of antibiotic resistance in wound care management.

Keywords: Wound care management, silver nanoparticles, silver coated dressings, antibacterial activity

Introduction

Pathogens resistant to multiple antibiotics are rapidly emerging, entailing important consequences for human health. Increasing rate of mortality has significant economic impact on developing countries [1]. There are tools to fight these threats. Among them, nanotechnology is a promising field in the area of medical research in therapeutics using natural resources [2].

Nanoparticles have dynamic molecular effects on surfaces and are easy to use in the phenomenon called surface technology. Biological, chemical and physical methods of nanoparticle synthesis already exist, and have been under investigation by various laboratories, with the aim of reducing particle size and increasing the effects and biocompatibity. This dynamic property could open new arenas in the near future to crack difficult interruptions in the fields of posology and drug development. Currently the World Health Organization (WHO) list silver sulfadiazine as a powerful anti infective topical medicine. However, although silver nanoparticles (AgNp's) have such particular properties, the mechanism has not yet been properly described [3].

To evaluate the efficacy of such silver nanoparticles against the pathogens present on wounds in burn care patients, the work was initiated primarily with a few organisms, so that they could be modified economically and manufactured easily. Multiple resistance pathogens were challenged in terms of clinical importance with silver nanoparticle coated dressings.

The antiquity of the work shows that a gram of negative bacteria itself plays a role in killing another gram of positive pathogen by means of reduction of nitrate, and is found to be successful. Various database have been available regarding microbial production of silver nanoparticles including *Pseudomonas stutzeri* [4], *Lactobacillus* [5], *Klebsiella* [6], and in plants, Vitex negundo [7] etc. In the present study, *E.coli* as a clinical isolate has been chosen.

Materials and methods

Biogenesis of silver nanoparticle

Various biological protocols have been established and quoted in literature. Among them, chemical reduction is a common process [17]. Apart from this, some investigators have reported that phytonanoparticles from easily available plants, also microbial source, are the most important, but cost effectiveness is the main issue to be solved.; Keeping this in mind, frequently available bacterium *E. coli* have been isolated from clinical specimens and have been subjected to cultures in normal LB broth under laboratory conditions for 24 h at 37 °C [8].

Silver nitrate solution of 10^{-3} M. was prepared and stored in brown bottles. A 5 ml biomass of *E. coli* was then added to 100 ml of $AgNo_3$ solution [9,10]. The color change of the solution from pale yellow to reddish brown was observed periodically. The reddish brown color indicated that the silver nanoparticles were synthesized.

Preparation of coated dressings

One centimeter of dressings was prepared in an aseptic manner in a laboratory and was stored in sterile vials. 10 ml of silver nanoparticle solution was then poured in the vials to coat it for 3 h. After exposure, it was then removed and aseptically dried in a hot air oven for 1 h at 50 °C. Similarly, dressings without inoculation were also prepared for control.

Bactericidal assays

In the bactericidal assay, Muller-Hinton Agar plates were plated in triplicate, test organisms of *S.aureus* were inoculated by spread plate and prepared with dried coated medical dressings wetted by distilled water, and were placed in the centre of the petridish and subjected to incubation for 24 - 48 h, at 37 °C for observation of bactericidal effect. A control plate containing dressings with silver nitrate was also incubated for comparison of efficacy of microbial mediated nanoparticles.

Results

Confirmation of metal-microbe interaction

It was found that, the metal silver reduced when exposed to the bacterial cell biomass, thereby leading to the formation of silver nanoparticles by changing the color of the solution from pale yellow to brownish red. It was suggested that the *E. coli* was able to synthesize silver nanoparticles (**Figure 1**). Dressings also showed some absorbing capacity, and changes were observed in dressing texture.

Figure 1 Left: control, Right: test.

Antibacterial efficacy

The dressings in the plates indicated zones of inhibition in and around the dressings, and no growth of *S.aureus* was seen in the AgNp coated dressings, whereas the control showed more growth. **Figures 2 - 4** shows the activity in the silver nitrate solution dressing.

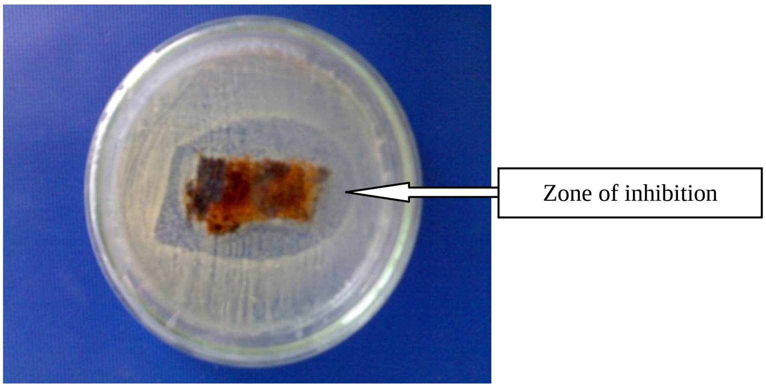

Figure 2 *E. coli* AgNp coated medical dressing

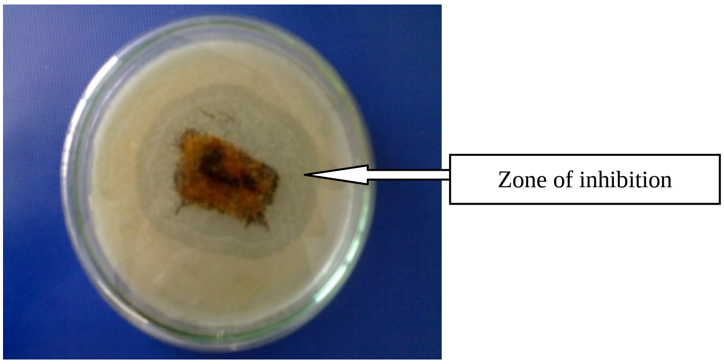

Figure 3 *S. aureus* inhibited zone of inhibition.

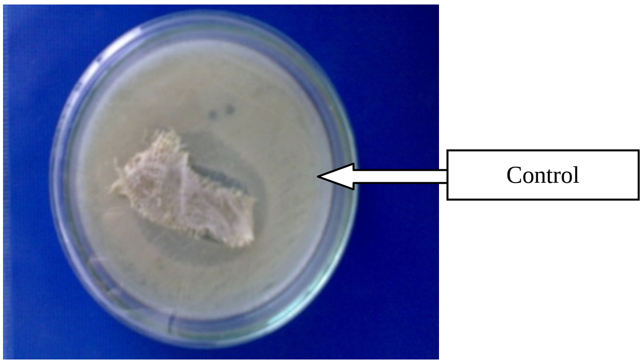

Figure 4 Control with silver nitrate solution dressings.

Discussion

Various investigations have been done so far to evaluate the antimicrobial action of silver nanoparticles, but the focus of the present study was on gram negative organism *E. coli* [11,12]. The findings show that the nanoparticles formulated by *E. coli* are cheaper compared to other methods. The mechanism behind the healing is a reduction in healing time, as well as showing bactericidal activity against commensal pathogens such as *S. aureus*. It has already been said that silver nanoparticles exert the same effect on both gram negative and gram positive organisms [13]. It is also well known that silver ions and other metal salt have an inhibitory effect, but there are still some problems in delivering drugs with it. This study can overcome the problem by using reduced silver salt [14]. Understanding the mechanism and delivery of drugs is a tedious job for researchers; hence a hypothesis has been made to coat medical dressings and to monitor bactericidal activity against pathogens. Surprisingly, it has been found to be effective. Several separate activities of silver nanoparticles were tested earlier, but this is a first time that *E. coli* mediated silver nanoparticles were used to coat medical dressings against pathogenic strains found in wounds in tertiary hospitals. Chemically synthesized nanoparticles require prevention of aggregation of the particles, but biologically synthesized nanoparticles are harmless, eco-friendly and stable [15].

Conclusions

This study suggests that there is a need to improve technology and to use natural easily available reducing agents which reduce effort but are potentially strong. In the future, different microbial biomasses can be used which may be more effective and less resistant. This type of clinical based basic study can be performed without complicated devices.

Currently, preliminary synthesized nanoparticles are subjected to further analysis using MALDI-MS for better resolution and surface studies. It is assumed that, this research will explore more ideas for future drug discovery & development in nanotechnology. Last but not least, an easily available negative gram of *E. coli* commensally works against harmful positive gram pathogens. Any unexplored organism that works effectively against pathogens should therefore be further investigated.

References

[1] J Omololu-Aso1, DO Kolawole, OO Omololu-Aso and SO Ajisebutu. Antibiotics sensitivity pattern of *staphylococcus aureus* from fomites in the Obafemi Awolowo University Teaching Hospital Complex (OAUTHC) Nigeria. *Int. J. Med. Med. Sci*. 2011; **3**, 32-6.

[2] D Montefiore, VO Rotini and FAB Adeyemi-Doro. The problem of bactericidal resistance to antibiotics among strains isolated from hospitals peatiens in Lagos. *J. Antimicro. Chemother*. 1970, **23**, 641-61.

[3] AI Wertheimer and TM Santella. Innovation and the WHO's essential medicines list: Giving credit where credit is due. *Res. Social. Adm. Pharm*. 2007; **3**, 137-44.

[4] K Tanja, J Ralph and O Eva. Silver based crystalline nanoparticle, micribially fabricated. *Proc. Natl. Acad. Sci. USA* 1999; **96**, 13611-4.

[5] B Nair and T Pradeep. Coalescence of nanoclusters and formation of submicron crystallites assisted by Lactobacillus strains. *Cryst. Growth. Des*. 2002; **2**, 293-8.

[6] A Ahmed, P Mukherjee, D Senapati, MI Khan and R Kumar. Extracellular biosynthesis of silver nanoparticles using the fungus Fusarium. *Colloid. Surface. B* 2003; **28**, 313-8.

[7] N Prabhu, DT Raj, KG Yamuna, SS Ayisha and DIJ Puspha. Synthesis of silver phytonanoparticles and their antibacterial efficacy. *Dig. J. Nanomater. Bios.* 2010; **5**, 185-9.

[8] M Ip, SL Lui, VKM Poon, I Lung and A Burd. Antimicrobila activity of silver dressings an *invitro* comparison. *J. Med. Microbiol*. 2006; **55**, 59-63.

[9] JS Kim, E Kuk, KN Yu, JH Kim, SJ Park, HJ Lee, SH Kim, YK Park, YH Park, CY Hwang, YK Kim, YS Lee, DH Jeong and MH Cho. Antimicrobial effects of silver nanoparticles. *Nanomed. Biomed.* 2006; **3**, 95-101.

[10] V Kostenko, J Lyczak, K Turner and RJ Martinuzz. Impact of silver-containing wound dressings on bacterial biofilm viability and susceptibility to antibiotics during prolonged treatment. *Antimicrob. Agents Chemother*. 2010; **54**, 125120-31.

[11] F Furno, KS Morley, B Wong, BL Sharp, PL Arnold, SM Howdle, R Bayston, PD Brown and PD Winship. Silver nanoparticles and polymeric medical devices; a new approach to prevention of infection *J. Antimicrob. Chemother.* 2004; **54**, 1019-24.

[12] PL Darke and KJ Hazelwood. Exposure- related health effects of silver nanoparticles compounds; a review. *Ann. Occup. Hyg*. 2005; **49**, 575-85.

[13] H Kong and J Jang. Antibacterial properties of novel poly (methyl methacrylate) nanofiber containing silver nanoparticles. *Langmuir* 2008; **24**, 2051-6.

[14] L Zhang, D Pornpattananangkul, CMJ Hu and CM Huang. Development of nanoparticles for antimicrobial drug delivery. *Current Med. Chem.* 2010; **17**, 585-94.

[15] K Vithiya and S Sen. Biosynthesis of nanopaticles: A review. *Int. J. Pharm. Sci. Res.* 2011; **2**, 2781-5.

[16] V Deepak, K Kalishwaralal, SRK Pandian and S Gurunathan. An insight into bacterial biogenesis of silver nanoparticle, industrial production and scale-up. *Metal Nano. Micribiol*. 2011; **183**, 12-6.

[17] MG Guzmán, J Dille and S Godet. Synthesis of silver nanoparticles by chemical reduction method and their antibacterial activity. *Int. J. Chem. Biol. Eng*. 2009; **2**, 104-11.