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Evaluation of Antibacterial Properties of Leather Treated with Silver Nanoparticles

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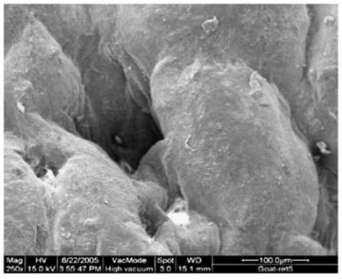
Graphical abstract



Abstract

The objective of this study is to treat leather with silver nanoparticles so as to develop antibacterial properties in the leather. The biological activity on leather shoes resulted in bacterial growth and caused the unpleasant smell. Silver nanoparticles have been known to possess some antibacterial effects and were added to the oil used in the leather processing step. A small amount of silver nanoparticles (6 and 8%v/v) were added to oil and its antibacterial properties have been evaluated using the immersion and capacity test methods. The bacteria suspension, a turbidity equivalent to a 0.5 McFarland standard was prepared for the *S. aureus* and *E. coli* bacteria. Results revealed that antimicrobial tests on samples containing silver nanoparticles showed suitable antibacterial properties indicated by the International Standard (ISO 22196) tenderness of suspension of less than $3x10^8$ CFU/ml. Thus leather treated with silver nanoparticles has antibacterial properties with the tendency to reduce the unpleasant odors.

Keywords: Leather; antibacterial properties; nanoparticle, silver

Abstrak

Objektif kajian ini adalah untuk merawat kulit dengan nanopartikel perak untuk membangunkan ciri-ciri antibakteria di kulit. Aktiviti biologi ke atas kasut kulit menyebabkan pertumbuhan bakteria dan menyebabkan bau yang tidak menyenangkan. Nanopartikel perak telah diketahui mempunyai beberapa kesan antibakteria dan telah ditambah kepada minyak yang digunakan dalam langkah pemprosesan kulit. Sedikit nanopartikel perak (6 dan 8%v/v) telah dimasukkan ke dalam minyak dan ciri-ciri antibakteria telah dinilai menggunakan rendaman dan kaedah ujian kapasiti. Larutan bakteria, bersamaan kekeruhan McFarland piawai sebanyak 0.5 telah disediakan untuk *staphylococcus aureus* dan bakteria *Escherichia coli*. Hasil kajian menunjukkan bahawa ujian antimikrobial ke atas sampel yang mengandungi nanopartikel perak menunjukkan ciri-ciri antibakteria yang indikasi oleh Piawai Antarabangsa (ISO 22196) kelembutan penggantungan kurang dari $3x10^8$ CFU/ml. Oleh itu kulit yang dirawat dengan nanopartikel perak mempunyai ciri-ciri antibakteria dengan kecenderungan untuk mengurangkan bau-bau tidak menyenangkan.

Kata kunci: Kulit; sifat anti-bakteria; nanopartikel; perak

1.0 INTRODUCTION

One of the basic needs for human beings since ancient times has been clothing and footwear in the form of animal skins. Today, despite the passing of centuries, the leather industry still maintains its widespread appeal and strong market presence. However, the unpleasant smell during use is a major problem in shoes made of leather. Studies have shown that the unpleasant smell is related to bacterial growth and their metabolism [1–4]. Research shows that the hot and humid environment inside shoes, especially athletic shoes increase bacterial over-growth and accentuate the problem [5, 6].

Successful elimination of this malodor depends on bacterial eradication. As a result, foot odor was found to be derived from isovaleric acid, which is produced when Staphylococcus epidermidis, a resident species of the normal cutaneous microbial flora, degrades leucine present in sweat. In addition, Bacillus subtilis was detected in the plantar skin of subject with strong foot odor, and this species was shown to be closely associated with increased foot odor [7]. In order to solve this problem, either aroma materials have been injected into leather or odor absorbencies have been placed inside shoes [8]. However, these odor absorbency materials, such as activated charcoal placed inside the shoe or foot powders and other materials cannot solve

the major problem, which is to completely remove the unpleasant smells [9]. Other problems include the preparation of leather that require the adding of heavy metals such as cobalt, chromium, copper, nickel, and selenium during leather processing [10] which is quite a hazardous process and therefore should be eliminated due to human toxicity and environmental constraints. Silver is known to have antibacterial properties and thus seems to be a good alternative for these heavy metals. The advantages of using silver nanoparticles are due to its high specific surface, fine size and its ability to disperse well in many medium. Many researchers have studied about the antibacterial properties of silver in polymers and nanocomposites fibers with silver nanoparticles exhibiting good antibacterial properties [11] but no work on leather has yet been performed.

Thus the objective of this study is to determine if silver nanoparticles with its antibacterial properties can be a suitable substitute in leather preparation. One of the most important steps in leather production is the oil phase [12]. The oil phase consists of dipping and coating the impregnated fiber surface with a thin layer of oil. Before the leather is covered in oil, it is dry and rigid; its fibers cannot slide over other and it cannot stand up against the tensile force of the friction and traction of a sewing needle [8]. The use of additives in the oil phase aids in the stability and sustainability of the leather against environmental conditions and usage [12]. Due to the limitations of placing silver nanoparticles directly onto leather, this study focused on the use of silver nanoparticles in the oil phase which is applied to the leather during processing so as to prevent germs and bacteria growth and increase the life span of leather.

■2.0 MATERIALS AND METHOD

2.1 Materials

Nano particles of 10 nm in size and concentration of 0.1 g/L was obtained from Nano star company. The tanning oil was purchased from Vatan Leather Company. The bacteria having features mentioned in Table 1 was obtained from BoAli research center of Mashhad, Iran.

2.2 Preparation of Antibacterial Leather

100 mL of silver nanoparticles with 10nm size was added into the oil and dissolved at a temperature of 67^{o}C. This mixture was then applied to the leather in the leather processing step in order to prevent unsavory odor in leather used in shoes and to increase the antibacterial property.

2.3 SEM

In this study, EVO Series Environmental Scanning Electron Microscope was used. The leather surface that was treated under the corona process was analyzed using the SEM. The scanning electron microscope (SEM) uses a focused beam of high-energy electrons to generate a variety of signals at the surface of solid specimens. The signals that were derived from the electron-sample interactions reveal information about the sample including external morphology (texture), chemical composition, and crystalline structure and orientation of materials making up the sample.

2.4 Preparation Of Suspension

Staphylococcus aurous ATCC 6538 and *Escherichia coli* ATCC 8739 were the strains used in evaluating the antibacterial properties also known as the Capacity test. They are streaked onto the plates separately. After 24 hours, the bacteria grew and bacterial colonies were observed in the plates. Some of the colonies were dissolved in the physiology serum (salt serum) until it reached 0.5McFarland turbidity standard (this is equal to 1.5 × 10^6 CFU/ml). On the other hand, to prepare the bacterial suspension, a turbidity equivalent to a 0.5 McFarland standard was prepared for the *staphylococcus aurous* and *Escherichia coli* bacteria and these were then diluted by a diluted concentration equal to 1.5 × 10^6 CFU/ml (containing Mueller Hinton medium and bacteria) [13].

Table 1 Bacteria used for antibacterial test

Genealogy	Bacteria
ATCC1 6538p,PTCC2 1112 CIP3 53.156, GCMC4 346 NBR5 12732, NCIB6 8625	*Staphylococcus aureus*
ATCC 8739p,PTCC 1330 CIP 53.126, GCMC 1576 NBR 3972, NCIB 8545	*Escherichia coli*

2.5 Evaluation of Microbial of Leather with Nanoparticles

In this study *Staphylococcus aureus* ATCC 6538 and *Escherichia coli* ATCC 8739 were used and the test methods were based on immersion and capacity tests to evaluate the antibacterial properties. According to this method, processed leather samples with the control sample size of 1 × 2 cm^2 were sterilized. Since leather is sensitive to the high temperature that is used in sterilization techniques, a low heat sterilization method was used in this study by immersing the leather and control samples in 70% ethanol for 20 minutes.

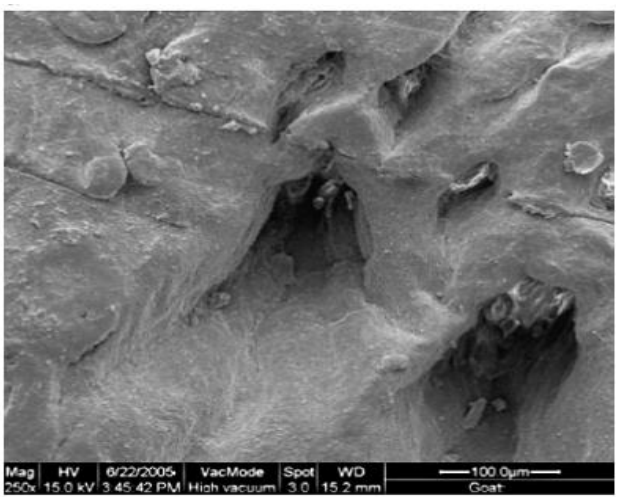
The samples were placed individually in test tubes and 1 ml of the prepared suspensions was added to the test tubes that were then sealed and placed in an incubator for 24 hours. Next, 10 µl of the test tube suspension was used for the culture on the Mueller Hinton Agar and the plates were placed in the incubator for 24 hours. The bacterial colonies were counted and compared with the control suspensions. Subsequently, in order to evaluate the capacity of the processed leather with silver oxide nanoparticles, the four highest concentrations of suspension (1.5 × 10^6, 1.5 × 10^7, 1.5 × 10^8, 3 × 10^8) CFU/ml from all of the stages were selected for analysis [13].

■3.0 RESULTS AND DISCUSSION

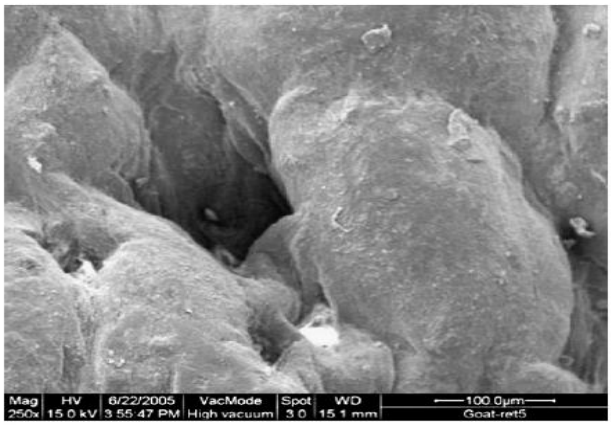
3.1 SEM Analysis

Figure 1(a) and (b) illustrate the SEM images of the leather samples before and after exposing to oiling process. As can be observed from Figure 1(a) the leather samples which are not exposed to oiling process with silver nanoparticles have less pores compared to the sample that were exposed to oiling process with silver nanoparticles. The presence of more pores could be due to the use of heat during the oiling process which is a requirement,

because silver nanoparticles cannot dissolve in oil at room temperature. The increase in the pores allows the silver nanoparticles to be absorbed more easily onto the leather surface and remain intact in the pores even after a long a time.



(a)



(b)

Figure 1 SEM analysis of the leather a) before adding oil and silver nanoparticles (raw leather) b) after adding oil and silver nanoparticles

3.2 Antibacterial Properties

An important parameter that must be considered during the production of materials, fabrics and fibers with new properties, are the rates of their dilutions. In other words, up to what percentage dilution can be performed on them, as high dilution rates indicate improvement and increased properties [13].

Table 2 illustrates the results of the capacity tests at tenderness of suspension of 1.5×10^6 CFU/ml and 1.5×10^7 CFU/ml for the various samples. The microbial tests results on the leather samples containing the nanoparticles in the lubricate period revealed no growth of *Staphylococcus aureus* and *Escherichia coli* compared to suspension and evidence samples and this is illustrated Table 2. In fact no growth for the two bacteria *Staphylococcus aureus* and *Escherichia coli* was observed in the lubricated leathers containing 6% (v/v) and 8% (v/v) silver nanoparticles.

However when the tenderness of suspension was increased to 1.5×10^8 CFU/ml and 3×10^8 CFU/ml, some bacterial colonies were observed for the lubricated leathers containing 6 and 8% (v/v) silver nanoparticles but the amount of bacterial growth was lesser compared to the suspension and evidence samples as exhibited in Table 2.

The results indicated that leather with silver nanoparticles can preserve silver nanoparticles, so silver nanoparticles in the leather treated in the lubricate method can remain in the leather even in high tenderness (Table 2). The results revealed that the leather has antibacterial property because according to the International Standard (ISO 22196) tenderness of suspension, 3×10^8 CFU/ml is the highest tenderness for polymer and plastic materials [13]. On the other hand, if polymer and plastic materials in this tenderness do not have growth level higher than suspension and evidence sample, in this case that polymer or plastic materials have antibacterial property.

Table 2 Capacity test results at low and high tenderness of suspension

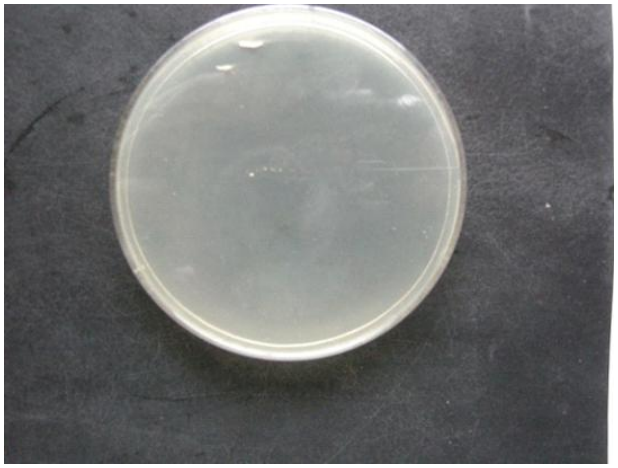
Sample	**Tenderness**			
	Low		**High**	
	1.5×10^6 CFU/ml	**1.5×10^7 CFU/ml**	**1.5×10^8 CFU/ml**	**$3*10^8$ CFU/ml**
	E. coli *S. Aureus*	*E. coli* *S. Aureus*	*E. coli* *S. Aureus*	*E. coli* *S. Aureus*
Suspension	5000 5800	1400 1100	10000 9600	10000 10000
Evidence	490 270	150 300	8500 9000	9200 9800
Sample with 6% of silver	No growth No growth	4 6	8000 8800	8400 9000
Sample with 8% of silver	No growth No growth	No growth No growth	7500 8000	8200 8500

Figure 2 depicts the agar plate test results of the various leathers which include suspension of *Escherichia coli*, evidence leather and leather with 6% and 8% (vv) silver nano particles with tenderness of suspension 1.5×10^6 CFU/ml. It was observed that the leather with 6 and 8% v/v) silver nano particles can preserve antibacterial properties completely because no bacterial growth was observed in the plates.

■4.0 CONCLUSIONS

Leather products especially shoes have long consumption life. The inner part of the leather shoes contribute to disagreeable odor due to the growth factor of the bacterial products. This is one of the problems faced in this industry. The purpose of using silver oxide nanoparticles on leather is to eliminate unpleasant foot odor. In this study, using silver nanoparticles in the oil phase not only made leather products odor free but give them antibacterial properties as well. From this leather, shoes such as leather hiking boots, which are typically worn for a long time and provide a suitable environment for fungi production, can be successfully produced on an industrial scale with antibacterial and anti odor properties.

A



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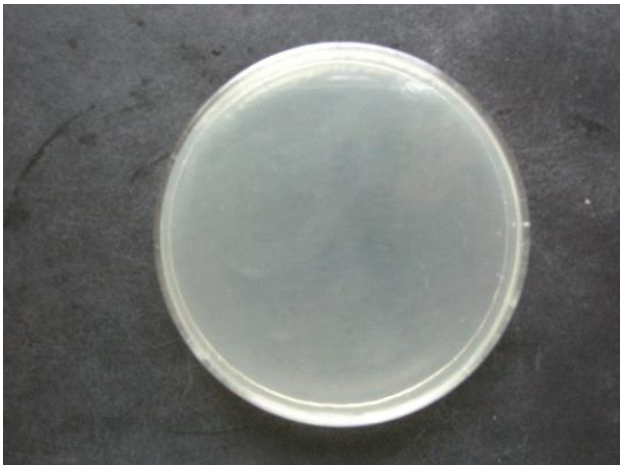

C

Figure 2 Agar plates for *Escherichia coli* with tenderness 1.5×10^6 a) suspension sample b) sample with 6% v/v silver nanoparticles c) sample with 8% v/v silver nanoparticles

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