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Anti-biofilm and cytotoxicity activity of impregnated dressings with silver nanoparticles

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ABSTRACT

Infections arising from bacterial adhesion and colonization on chronic wounds are a significant healthcare problem. Silver nanoparticles (AgNPs) impregnated in dressing have attracted a great deal of attention as a potential solution. The goal of the present study was to evaluate the anti-biofilm activities of AgNPs impregnated in commercial dressings against *Pseudomonas aeruginosa*, bacteria isolated of chronic wounds from a hospital patient. The antimicrobial activity of AgNPs was tested within biofilms generated under slow fluid shear conditions using a standard bioreactor. A 2-log reduction in the number of colony-forming units of *P. aeruginosa* was recorded in the reactor on exposure to dressing impregnated with 250 ppm of AgNPs, diameter 9.3 ± 1.1 nm, and also showed compatibility to mammalian cells (human fibroblasts). Our study suggests that the use of dressings with AgNPs may either prevent or reduce microbial growth in the wound environment, and reducing wound bioburden may improve wound-healing outcomes.

1. Introduction

Infections that develop into chronic conditions are a fast-growing problem in the developed world. The underlying biology is thought to be the ability of bacteria to form biofilms [1], biofilms are microbial consortia embedded in self-produced exopolymer matrices composed mainly of exopolysaccharides (EPS), which obtain numerous advantages such as passive resistance, metabolic cooperation, by product influence, quorum sensing systems [2], an enlarged gene pool with more efficient DNA sharing, and many other synergies, which give them a competitive advantage [3]. Multiple studies have documented that such aggregated communities are more resistant to a variety of antibiotics and that the action of the immune system compared to their planktonic counterparts [2,4]. In the biofilm, the microorganisms exhibiting an altered phenotype with respect to growth rate and gene transcription, in comparison with the planktonic form [5]. Several Gram negative pathogens use quorum sensing (QS) to coordinate specific gene expression, thereby synchronizing the expression of particular phenotypic features between the individual cells [6]. QS is thought to play an important role during the initial event of infection for the common opportunistic Gram negative human pathogen *Pseudomonas aeruginosa*, which is associated with nosocomial, medical devices and wound infections [7]. A wound is an ideal environment for bacterial colonization and biofilm formation, around 60% of chronic wounds contain biofilms [8]. The increase in the incidence of chronic wounds is due to both the aging of populations (longer lifespan) and the concurrent increase in comorbidities, such as diabetes, obesity, venous hypertension, and peripheral vascular disease [9]. Chronic wounds including chronic venous leg ulcers (CVLUs), diabetic foot ulcers (DFUs) and pressure ulcers are a major cause of distress and disability for patients, as well as being a drain on the resources of the health service, costing over £1 billion per annum [10] and in the USA at $US 6–15 billion [9].

A principal component of wound care is the choice of dressing. Many modern wound dressings have a variety of different attributes, with the aim of creating a supportive wound-healing environment. Such dressings are designed to absorb exudate, provide an optimum moisture balance at the wound surface, prevent maceration of surrounding tissue, and control bacterial colonization [11,35]. In addition to the treatment for wound infections, several antibiotic drugs such as chloramphenicol, gentamicin, neomycin, and bacitracin are applied topically on open sores; however, the routine use of topical antibiotics leads to the progressive decline of therapeutic efficacies of these antibiotics due to antimicrobial resistance [12]. Therefore, there is a strong need for the development of new types of drugs for the infected wound treatment.

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The advance in nanotechnology has driven the development of AgNPs with a nanometer size scale that yields many interesting properties. The key synthesis aspects of silver nanoparticles focus on the ability to control their size, shape and dispersity [13]. AgNPs show the highest antimicrobial efficacy against bacteria, viruses, and eukaryotic microorganisms [14,15,16]. AgNPs can react with sulfur-containing proteins inside or outside the cell membrane, which in turn affects bacterial cell viability. It was also proposed that silver ions (particularly Ag^{+}) released from silver nanoparticles (Ag^{0}) can interact with phosphorus moieties in DNA, resulting in inactivation of DNA replication [17]. Due to these novel properties, the incorporation of silver nanoparticles into various matrices has been intensively investigated in order to extend their utility in materials and biomedical applications [18]. Since the election of dressing is an important factor for wound care, antimicrobial finishing for textile fibers has become necessary in the production of protective textiles. Selection of antimicrobial agent depends on several criteria, such as its effectiveness for bacteria and fungi, toxicity and application method. AgNPs are well-investigated antimicrobial agents, and in a large number of investigations [19], as well as their pharmacokinetics, which depends on the particle type, size, surface charge, surface coating, protein binding, exposure route, dose, and animal species [20].

Hence the present study was designed to enhance our knowledge of anti-biofilm activity of impregnated dressings with AgNPs against bacteria isolated in wound of hospital patient used a standard method, as well as their effect upon human dermal fibroblasts as the main cell type involved in the wound healing repair [21].

2. Methodology

2.1. Silver nanoparticle preparation

The preparation started with a 0.01 M $AgNO_3$ solution placed in a 250 ml reaction vessel. Under magnetic stirring, 10 ml of deionized water containing gallic acid (0.1 g) was added to 100 ml of silver nitrate solution. After the addition of gallic acid, the pH value of the solution was immediately adjusted to 11 with NaOH 1.0 M. After the synthesis and in order to purify the silver nanoparticles, the obtained dispersions were dialyzed using dialysis membrane (12 kDa molecule weight) during 48 h [22].

2.2. Characterization of silver nanoparticles

Silver nanoparticles were characterized using dynamic light scattering (DLS) analysis in triplicate by using a Malvern Zetasizer Nano ZS (Instruments Worcestershire, United Kingdom) operating with a He–Ne laser at a wavelength of 633 nm, and a detection angle of 90°; all samples were analyzed for 60 s at 25 °C. To confirm the shape, each sample was diluted with deionized water and 50 μl of each suspension was placed on a copper grid for transmission electron microscopy (TEM) (JEOL JEM-1230, Tokyo, Japan) at an accelerating voltage of 100 kV [22].

2.3. Bacterial strains and culture media

The clinical strains used in this study were isolated from a patient with diabetes type II and DFU in the left feet dorsal face by post-traumatic burn, with thirty years of evolution and two years with ulceration, the patient was hospitalized at the Central Hospital Dr. Ignacio Morones Prieto, San Luis Potosi, Mexico. The sample collection was performed following the guidelines of the American Society for Microbiology standards and according to the ethics guidelines. All the clinical strains were isolated in conventional cultures solid medium and the identification and antimicrobial test were realized using a VITEK® system. All strains were cultured in trypticase soy broth medium (TSB, Sigma-Aldrich), and maintained at 4 °C in solid medium obtained by supplementation of 1.5% (w/v) agar (BD Biosciences).

2.4. Dressings with silver nanoparticle preparation

Commercial, non-adhesive and also non-occlusive dressings were used in this study. The sterile wound dressings tested did not contain an antimicrobial agent, and the composition reported by the manufacturer was: the sample A composed with 100% cotton and the sample B composed with cotton and nylon. Both samples enclosed in a perforated sleeve of polyethylene terephthalate. Both commercial dressings were aseptically divided into 1 × 1 cm test squares and stored until use. The dressings were impregnated by immersion in solutions of AgNPs using four different concentrations: 1000, 500, 250 and 125 ppm. The squares were immersed in the solution, and carried to sonication for 15 min, and this time were obtained by observing the minimum time to achieve a homogeneous dispersion in the color of the solution over the dressing. After 15 min, the squares were placed on a sterile Petri dish and dried in an oven at 37 °C for 4 h.

2.5. Characterization of dressings with silver nanoparticles

The characterization of both dressings treated with silver nanoparticles was determined by three different methods: i) low resolution images, ii) SEM micrographs tested with energy-dispersive X-ray spectroscopy (EDX) detector and iii) atomic absorption spectroscopy (AAS).

The shape and structure of dressing treated with AgNPs were observed in a three-dimensional image perception using stereomicroscopy SteREO Discovery V8 (Carl Zeiss, Germany).

Scanning electron microscopy (SEM) micrographs of dressing treated with AgNPs were taken with a SEM JEOL 6510 operated at an accelerating voltage of 15 kV, the study allowed us to know the dispersion of the AgNPs over the dressings. The chemical composition of both dressing was recorded using an energy-dispersive X-ray spectroscopy (EDX) detector.

AAS measurements were performed to determine the total amount of immobilized AgNPs in each sample. To quantify the silver loading the sample was calcined at 600 °C for 15 min in a muffle using a porcelain crucible, which ensures the elimination of any component of the dressing except silver. The obtained ash was washed with 1 ml of nitric acid to recover the silver from the crucible. The resulted solution was taken to a volumetric flask, and it was gauged at 25 ml. The solution was placed in a dark flask and was allowed to settle the remaining ash. Finally, from the flask was taken a sample from the surface and again was placed on other dark flasks until the sample was analyzed. AAS was performed using a Spectrometer (PerkinElmer 3110), with a hollow cathode silver lamp, with a wavelength of 328.1 nm slit of 0.7 nm.

2.6. Anti-biofilm evaluations of the treated dressings

This study used an in vitro drip flow reactor (DFR) model, which was approved by the USA standard setting organization American Society for Testing and Materials International (ASTM), ASTM E2647-13 [23]. This model mimics the environmental conditions of the chronic wound in the skin; by growing the biofilms at a liquid/solid/air interface and slow flow [24]. The DFR (BioSurface Technologies Corp) comprises four individual, parallel test channels. For each experiment we used dressing with AgNPs and dressing without AgNPs. The CDC reactor, used to grow up the microorganism, was filled with sterile 800 ml of TSB, and it was inoculated with 1 ml of TSB diluted overnight culture (optical density of 0.08 at 600 nm) of clinical strain. The medium with microorganism was grown at 23 ± 1 °C and stirring of 800 to 1500 Reynolds number, the reactor was maintained in a batch mode (mixed, no flow) for 24 h. At the end of the first 24 h, CDC reactor was then attached to a medium reservoir with 2.5 l of medium (10% TSB), as well as to the DFR, which contented the sterile dressing sample in each channel, the flow was started and maintained for another 24 h at continuous slow laminar flow (Reynolds number between 12 and 20) rate of 0.82 ml/ min per channel and stand with a 10° angle [25]. After