Antimicrobial characteristics and biocompatibility of the surgical sutures coated with biosynthesized silver nanoparticles

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ABSTRACT

Surgical sutures play important role during the wound healing of the surgical sites which are known to be sensitive to microbial infections. Silver nanoparticles (AgNPs) have been recently used as promising agents against multiple-drug resistant microorganisms. This study was designed to coat the sutures with silver nanoparticles obtained via a green synthesis approach. Microbial-mediated biological synthesis of AgNPs were carried out ecofriendly using Streptomyces sp. AU2 cell-free extract and deposited on silk sutures through an in situ process. Sutures coated with biosynthesized AgNP (bio-AgNP coated sutures) were characterized using Scanning Electron Microscopy (SEM) and elemental analysis were carried out using Energy Dispersive X-ray Spectroscopy (EDS). The silver amount released by the bio-AgNP coated sutures was calculated by Inductively Coupled Plasma-Mass Spectroscopy (ICP-MS) throughout a degradation process. Antimicrobial potential of the bio-AgNP coated sutures was determined against common pathogenic microorganisms Candida albicans, Escherichia coli and Staphylococcus aureus. To determine the biocompatibility/cytotoxicity of the bio-AgNP coated sutures, the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium) assay was used through an indirect test method; that the elutions obtained by the extraction of the sutures at 1, 4, 8 and 10. days and were placed in contact with 3T3 fibroblast cell culture. To best of our knowledge, this is the first report about coating of the nonabsorbable silk sutures with silver nanoparticles biosynthesized using a microbial extract.

1. Introduction

Sutures account for the 57% of the global surgical equipment market as the most widely used surgical implants [1,2]. They are natural or synthetic fibrous biocompatible materials and can be classified as multifilament, monofilament, braided and twisted [3]. Besides being able to lead foreign body reaction, surgical sutures are highly susceptible to microbial colonization and biofilm formation [1,2]. Surgical sutures that are used to close scared tissues have a potential effect on causing a microbial infection [4,5]. The healing of the post-operative wound infection is a complicated process, that may retards normal wound healing, and induce life-threatening situation. Surgical site infections (SSI) that are associated with severe mortality and morbidity [6] are mostly localized at the incision [7,8]. In order to minimize the risk factors for wound infections, recent studies have been focusing on designing antibiotic-coated sutures. Antibiotic-coated sutures are demonstrated to have effective antibacterial efficacy using in vitro laboratory tests, in vivo animal experiments, as well as clinical studies [9–12].

Nevertheless, the long-term use of antibiotics are known to lead to bacterial resistance and increase the virulence of the organism [13,14]. To avoid antimicrobial-resistance, researchers are nowadays focusing on the invention of new antimicrobial agents.

With the help of the improving technologies, it is now possible to incorporate functional bioactive substances to enhance the potential capability of the sutures. Silver has recently been used as an effective antimicrobial agent that is thought to be a potential solution to the problem of multidrug-resistant microorganisms. When compared to current antibiotics, silver performs against microorganisms by attaching the cell membrane and penetrate inside. The nanoparticles preferably attack the respiratory chain of the microorganisms, cell division and cause to cell death [15].

With the emergence of the nanotechnology, its now possible to synthesize nano scale particles from conventional materials, which
gains the material to exhibit new physical, chemical, biological and biomedical properties [16]. In recent years, silver nanoparticles (AgNPs) have been most intensively researched metallic nanoparticles. Broad-spectrum antibacterial effects of the AgNPs have long been reported in many studies [15,17–19].

Products designed by nanotechnology and coated with nano-silver particles are the most growing sector for most of the industries. Especially the antimicrobial feature of those particles led to increasing demand of its medical applications. Some of such products already available in the market that include biomedical devices, wound dressings, contraceptive devices, surgical instruments and body prostheses [20–24].

Green nanosynthetic routes that provide appreciable results for nanotechnology are accepted as environmentally safe, non-toxic, cost and time saving applications that [25]. Novel green synthesis applications utilize diverse biological organisms (microorganisms, plants etc.) and are known to be a promising tool for reducing metals via specific metabolic pathways [26].

In the present study, silver nanoparticles biologically synthesized from a cell-free extract of a microorganism, Streptomyces sp. AU2, have been impregnated on the surface of the nonabsorbable silk sutures using an in situ process. Bio-AgNP coated sutures have been characterized microanalytically using SEM and EDS. The silver release amount has been determined using ICP-MS. The antimicrobial potential of the sutures were determined against pathogenic microorganisms. The biocompatibility/cytotoxicity of the bio-AgNP coated sutures have also been determined using 3T3 fibroblast cell culture.

2. Materials and methods

2.1. AgNP biosynthesis

Green synthesis process was applied according to the previously reported method published by us [27]. The Streptomyces sp. AU2 strain was grown in ISP (International Streptomyces Project)-2 broth and the biomass was used to obtain the cell-free culture supernatant for the biosynthesis of AgNPs.

2.2. AgNP immobilization to sutures

Nonabsorbable 3.0 silk sutures (Dogsan, Turkey) were used for bio-AgNP coating. For coating process of the sutures; 10 suture fragments of approximately 1 cm, 10 mL of cell-free supernatant and 50 mL AgNO₃ solution (1 mM) (filtered through a 0.22 µm filter) were incubated at 28 °C and 130 rpm for 48 h. Visual colour change was observed and the colour change (from opaque white to brownish yellow) of flasks that contained suture fragments indicated the presence of AgNPs. Biosynthesized AgNP-coated sutures (bio-AgNP coated sutures) were taken from the flasks and kept dried until the characterization assays.

2.3. Morphological and microanalytical characterization

For elemental analysis and monitoring the SEM images, the bio-AgNP coated sutures and noncoated sutures (control group) were put onto the specimen stubs using a double-sided adhesive carbon tape. In order to obtain the current EDS peaks, sutures were not sputter-coated with a conductive element such as gold or carbon. SEM images were taken using a JSM 7600F Field Emission Scanning Electron Microscope (JEOL, Japan) at an accelerating voltage of 15 kV. To determine the presence of metallic silver coated onto the bio-AgNP coated sutures, samples were analyzed by energy dispersive x-ray spectroscopy system (EDS) (Oxford Instruments, UK) combined with SEM.

2.4. Inductively coupled plasma-mass spectrometry (ICP-MS) analysis

In order to determine silver release from the bio-AgNP coated sutures, sutures were incubated in 50 mL phosphate buffered saline (PBS) solution at 37 °C for 21 days. Required amount of eluates were extracted (at days of 1, 7, 14 and 21) and silver concentration was measured using ICP-MS analysis performed at the Central Laboratory in Middle East Technical University using Perkin Elmer Scienx ELAN DRCII model equipment. Analysis were performed in triplicate.

2.5. Antimicrobial activity

Antimicrobial activity of the bio-AgNP coated sutures were determined against Candida albicans ATCC 10239 (a fungi), Escherichia coli ATCC 25922 (a gram-negative bacteria) and Staphylococcus aureus ATCC 25923 (a gram-positive bacteria) using a standard agar plate method [28]. Briefly, C. albicans was grown in Saboraud Dextrose Broth (SDB); E. coli and S. aureus were grown in Nutrient broth (NB). Inoculums were prepared by adjusting the turbidity of the medium to match the 0.5 McFarland Standard Dilutions. 20 mL of Saboraud Dextrose Agar (SDA), and Nutrient Agar (NA) were sterilized in separated flasks and cooled to 45–50 °C. After injecting the microorganism cultures to sterile plates (1000 µL), appropriate media was distributed and mixed homogenously. When the inoculated media solidified, bio-AgNP-coated sutures and control sutures were placed onto the agar surfaces. Plates incubated with C. albicans were incubated at 30 ± 0.1 °C for 24–48 h; E. coli and S. aureus strains were incubated at 37 ± 0.1 °C for 24–48 h. After the incubation periods ended, antimicrobial activities were monitored against the tested microorganisms. Studies were performed in triplicate.

2.6. Biocompatibility/cytotoxicity

Biocompatibility of bio-AgNP coated sutures was assessed using mouse embryonic fibroblast cell line NIH 3T3 obtained from American Type Cell Culture (ATCC) (Manassas, VA, USA). Cells were maintained in Dulbecco’s minimal essential medium (DMEM) (Sigma Aldrich) supplemented with 10% fetal calf serum (FCS), antibiotics (100 IU/mL streptomycin and 100 IU/mL penicillin) and 2 mM L-glutamine.

2.6.1. Extraction method

The in vitro cytotoxicity of bio-AgNP coated sutures was assessed by an indirect extract method [29,30]. Based on the standard protocols explained by ISO 10993-5, suture fragments (1 cm length/mL) were immersed in fresh cell culture medium at 37 °C for 5 days to obtain the extracts [31]. After 5 days of incubation, extract products were collected. 3T3 fibroblasts from the first passage were seeded in 96-well tissue culture plates (1.0 × 10⁴ cells/each well) and incubated for 24 h. After removing the culture medium, each well was replaced with extract medium. The cells were incubated with the extract medium for 1, 4, 8 and 10 days at 37 °C. Extract mediums in culture plates were changed at every 2 days. Extract medium was discarded at the end of the incubation period and the cell viability was determined by MTT assay. Briefly, the wells were rinsed with dPBS, and 20 µL of MTT (5 mg/mL, prepared in phosphate-buffered saline) (Sigma, Aldrich) was added to each well. The medium containing MTT was poured off after a 2 h incubation at 37 °C and 100 µL of DMSO (dimethylsulfoxide) (Sigma, Aldrich) was added to dissolve in the insoluble formazan crystals. Fresh culture medium was used as a negative control. Plates were put in an orbital shaker for 15 min and the absorbances were measured at 570 nm using a microplate reader (Thermo Scientific Multiskan FC, Thermo Fischer, Vantaa, Finland). The % cell inhibition was determined using the following formula:

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\% = \left[\frac{100 \times (\text{Sample}_{\text{abs}}) - \text{(Control}_{\text{abs}})}{(\text{Control}_{\text{abs}})}\right]
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2.7. Statistical analyses

The mean values ± standard deviation (SD) of the tests (n = 3) were calculated. The results of the MTT assay is expressed as the
To avoid the microbial colonization on the sutures, antimicrobial coating technology has been considered to reduce the risk of infection [32]. Recently, nanosilver is actively being considered for application in suture materials [33]. Within this study, its aim is to design and characterize an antimicrobial suture material developed by using green nanotechnology.

Scanning Electron Microscopy (SEM) images and Energy Dispersive X-ray Spectroscopy (EDS) results are reported in Fig. 1a-b. SEM images of the both control group (non-coated) and bio-AgNP coated sutures revealed the typical multifilament structure of the sutures. Silver nanoparticle deposition onto the bio-AgNP coated sutures were also visible and the presence of the silver ions were detected by EDS analysis (Fig. 1.a). There were no silver peak in the EDS spectrum of the non-coated suture (Fig. 1.b). Similar results were also obtained by De Simone et al. [34] and Gallo et al. [35] who coated the silk and polyglactin 910 PGLA sutures with silver using a process based on the photoreduction of silver solution, respectively.

Silver is known to have great antimicrobial activity when incorporated with biomedical equipments [15,36]. Green synthesized silver nanoparticles that have advantages over conventional methods involving chemical agents associated with environmental toxicity have been proved to have great antimicrobial capabilities [37]. Antimicrobial characteristics of the bio-AgNP coated sutures were determined against three pathogenic microorganisms using agar diffusion test (Fig. 2). The clear growth inhibition area around the bio-AgNP coated sutures indicates an effective antimicrobial capability. It was reported that, when the zone of inhibition measurement is higher than 1 mm, a material is considered to have good antimicrobial potential according to the Standard Antibacterial test "SNV 195920–1992 [38–40]. The highest zone of inhibition was observed against C. albicans which is an important pathogenic fungi that isolated from infected medical devices and responsible for almost 15% of hospital-acquired cases of sepsis [41]. Other tested strains are known to be multi-resistant bacteria that are responsible for nosocomial infections [42]. According to the National Nosocomial Infections Surveillance System (NNIS), the most frequently isolated pathogens from SSI are Staphylococcus aureus (20%) and coagulase-negative staphylococci [43]. As an expected result, no inhibition zone was observed for the non-coated sutures. The obtained results clearly figure out that coating sutures with biosynthesized AgNPs might be a preventive application to protect the surgical site from microbial biofilm formation. SSI occur when pathogenic organisms grow in the surgical wounds, so the wound healing slows down and the wound edges separate [44]. Pratten et al. [45] who coated silver-doped biactive glass (AgBG) onto the silk sutures resulted that AgBG coating limited the Staphylococcus epidermidis attachment. In another study, silk sutures were coated with silver nanoparticles by an in situ photoreduction of a silver solution using ultraviolet (UV) lamp and their antibacterial activity analysis indicated the good efficacy of the sutures against Gram positive (S. aureus) and Gram negative (E. coli) bacteria [34]. It is reported that the antimicrobial potential of AgNPs, that is directly related to their shape, size and concentration, possibly exhibit activity on reactive oxygen species (ROS) formation, protein-AgNP interaction, inhibition of DNA replication and disrupting the microbial cell wall [46–48].

To determine the silver release amount at the 1, 7, 14 and 21 days of degradation, ICP-MS measurements were applied for bio-AgNP coated sutures. Silver amount increased throughout the degradation period of the bio-AgNP coated sutures (Fig. 3). There were no silver at day one while the measured silver amounts were 0.50 ± 0.04 µg/L, 1.31 ± 0.05 µg/L and 2.93 ± 0.08 µg/L at day 4, 7 and 21, respectively. According to Schierholz et al. [49], silver ion concentration that is > 10 mg/L is not considered to be toxic to certain human cells, so our results remained below the acceptable limit for silver release. Similar results were reported by Gallo et al. [35] who coated absorbable multifilament polyglactin 910 PGLA sutures with silver nanoparticles and determined silver release increased in the silver coated sutures over time passing from 0.28 ± 0.03 ppm to 0.54 ± 0.06 at day one and at
lead to bacterial resistance and also increase the virulence of micro-organism, silver nanoparticles as a potential antimicrobial agent may be a promising solution to the problem of multidrug-resistance of microorganisms [52].

4. Conclusion

Wound healing after a surgical operation is directly related to the hygiene of the surgical site. Prevention of the microbial accumulation plays an important role for the rapid management of the patient which is also important to avoid high economical costs for antibiotic use. This study was aimed to modify the nonabsorbable silk sutures to provide an antimicrobial functionality that is effective on multidrug-resistant microorganisms. Sutures were coated with bio-synthesized silver nanoparticles that were obtained via an ecofriendly, non-toxic and cost-efficient method. The results revealed out that silver nanoparticles strongly adhered to the sutures. Antimicrobial activity of the bio-AgNP coated sutures figured out strong antimicrobial capacity against pathogenic strains. Despite the increasing silver ion release from the degradation process of the suture, silver amounts measured by ICP-MS were below the toxicity limits reported in the literature. Cytotoxicity test applied on 3T3 murine fibroblasts using the extracts of the bio-AgNP coated sutures demonstrated that they do not effect the cell viability. This study resulted that coating the sutures with biosynthesized silver nanoparticles may provide antimicrobial and antibiofilm functionality that is related to the success of the clinical applications and wound healing process.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2018.12.034.

References

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