ISSN 1846-6168 (Print), ISSN 1848-5588 (Online)
https://doi.org/10.31803/tg-20180131161141

Original scientific paper

GREEN SYNTHESIZED SILVER NANOPARTICLES LOADED PVA/STARCH CRYOGEL SCAFFOLDS WITH ANTIBACTERIAL PROPERTIES

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Abstract: In this study, Polyvinyl alcohol/Starch (PVA/Starch) cryogel scaffolds were combined with antibacterial silver nanoparticles (AgNPs), and the antimicrobial properties of composite scaffolds were determined for potential in tissue engineering applications. The porous PVA/Starch scaffolds were prepared by cryogelation technique. The nanoparticles were prepared by green synthesis from Aloe barbadensis leaf extract and characterized. The antibacterial, antifungal and antiyeast properties of AgNPs and AgNPs loaded PVA/Starch cryogel scaffolds were investigated. The highest antimicrobial activity of composite scaffold was found against Pseudomonas aeruginosa. Based on our studies, the results indicate that biodegradable, biocompatible and antimicrobial AgNPs loaded PVA/Starch scaffolds have potential to be used at an infection site in tissue engineering applications.

Keywords: aloe barbadensis; antimicrobial activity; green synthesis; PVA/Starch scaffold; silver nanoparticles

1 INTRODUCTION

Over the past few decades, green process for the synthesis of nanoparticles has attracted wide interest because of its inherent features such as rapidity, simplicity, being eco-friendly and cheap [1]. A wide variety of applications include the use of nanoparticles due to their unique optical, electrical, mechanical, magnetic and chemical properties [2-4]. Due to their unique properties, metal nanoparticles, such as Ag, Au, Pt and Pd, are extensively used in pharmaceutical industry, clothing, cosmetics, optics, catalysis, mirrors, photography, electronics, food industry, and many other fields [5, 6]. Green biosynthesis has significant importance in the progress of nanotechnology. Several physical and chemical methods have been previously proposed to be used to synthesize nanoparticles. However, biosynthesis is an easy, alternative, eco-friendly and inexpensive approach compared to the previous methods. In addition, green synthesis method provides the production of nanoparticles with a well-defined shape and controllable size [1]. There are three main green synthesis perspectives in the production of AgNPs including the selection of solvent medium, reducing agent and nontoxic stabilizers [5]. One of the green methods of nanoparticle synthesis is the utilization of various plants and their parts [1]. The plant extract mediated synthesis of nanoparticles does not involve specific media and culture conditions and the reaction time is very short compared to other synthesis methods [7]. Gardea-Torresdey et al. reported the possibility of the synthesis of nanoscale metals by using plants. Later, various plants have been used to synthesize silver nanoparticles, such as *Azadirachta indica, Delonix elata, Tephrosia purpurea, Melia dubia, Tribulus terrestris, Artemisia nilagirica, Boerhaavia diffusa, Ficus religiosa, Piper pedicellatum* and *Melia azedarach* [1, 8].

The fabrication of scaffolds for tissue engineering applications with antibacterial properties is essential during implantation, surgery and wound healing process. Ideally, the scaffolds should have the ability to regenerate new tissue, treat the infection by delivering an antibacterial agent, and could provide a targeted treatment for the infection site [9]. Silver nanoparticles are loaded in different kinds of scaffolds (such as bacterial cellulose nanoparticles and chitosan-nanohyroxyapatite scaffold) to be applied as biomaterials with capability of reducing the bacterial and fungal infections [10, 11]. The incorporation of AgNPs into cryogels would attract a great deal of attention because of the resulting scaffolds' antimicrobial activity.

Cryogels are one the most promising types of scaffolds due to their interconnected and homogeneous macroporous structure which leads to three-dimensional cell growth, diffusion of nutrients and waste transfer during tissue regeneration [12]. Therefore, in this study we synthesized and characterized AgNPs by green synthesis approach with antimicrobial (antibacterial, antifungal and antiyeast) properties from aloe barbadensis leaf extract, combined them with PVA/Starch cryogel scaffolds, and demonstrated their antibacterial potential for tissue engineering applications.

2 MATERIALS AND METHOD

2.1 Chemicals

Silver nitrate was obtained from Merck, Germany. Aqueous solution of silver nitrate and other diluted solutions were prepared with distilled water. For the antimicrobial studies, nutrient agar (NA) and potato dextrose agar (PDA) were obtained from Merck, Germany. For the synthesis of scaffold, PVA with a molecular weight of 89,000-98,000 g/mol (99% hydrolyzed) was purchased from Sigma Aldrich, USA. Starch was obtained from Emir Chemicals, Turkey. Sodium dodecyl sulfate (SDS) was obtained from Merck, Germany. Both powder and liquid AgNPs, and AgNPs loaded PVA/Starch scaffolds were tested against bacterial and fungal species. The list of microorganisms used is presented in Table 1. Bacterial and yeast species were obtained from ATCC bacterial culture guide, and fungal species were provided by the Department of Genetics and Bioengineering, Faculty of

Engineering and Architecture, Yeditepe University (Istanbul, Turkey).

Table 1 The list of microbial species tested in antimicrobial studies

Bacteria	Yeast	Fungi
Pseudomonas aeruginosa ATCC 27853	*Candida albicans* ATCC 102312	*Aspergillus niger*
Escherichia coli ATCC 25922		
Staphylococcus aureus ATCC 29213		

2.2 Preparation of *Aloe Barbadensis* Leaf Extract

The freshly collected leaves of *Aloe barbadensis* were washed with tap water to remove the dust particles present on the surface and then rinsed with distilled water. The cleaned leaves were chopped into small pieces and dried for 2 days at 40 °C in the oven. The completely dried leaves were powdered with mortar for further usage in the preparation of leaf extract. 10 g of the powdered leaves were mixed with 100 ml of distilled water at 60 ± 1 °C for 30 min. After cooling, the mixture was filtered with a cheese cloth and the aqueous filtrate was centrifuged. The clear leaf extract of *Aloe barbadensis* was used for synthesis of AgNPs.

2.3 Synthesis of AgNPs

In order to synthesize AgNPs, 50 mL of the leaf extract was added drop by drop to 500 mL of 10 mM silver nitrate solution with stirring magnetically at room temperature for 30 min, as shown in the experimental set-up in Fig. 1. After incubation of the solution for 48 hours at dark conditions, the yellow color of the mixture of silver nitrate and leaf extract was changed to deep brown, which designates the formation of AgNPs. After the synthesis of AgNPs, the solution containing colloidal nanoparticles was centrifuged at 45x100 r/min for 5 min to separate AgNPs. The collected particles were subsequently dispersed in distilled water. Finally, AgNPs were dried at 110 °C in the oven for 24 h, and were stored at +4 °C in the refrigerator for further usage.

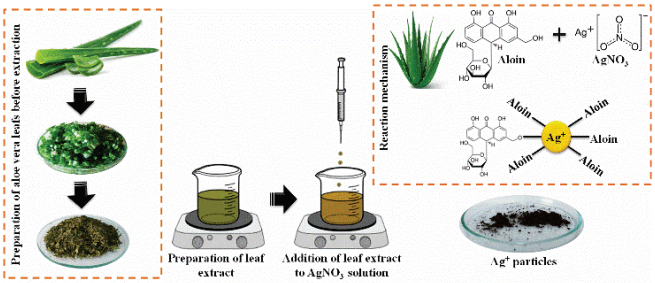

Figure 1 Experimental set-up of AgNPs synthesis from Aloe barbadensis leaf extract

2.4 Preparation of PVA/Starch Cryogel Scaffold

PVA/Starch cryogels were prepared by cryogelation technique as described in our previous study [13]. Briefly, PVA/Starch solution was fixed at 8% (w/v) polymer concentration at 90:10 (w:w) polymer ratio. SDS was added to the prepared polymer solution and homogeneous mixture was incubated at cryostat and freezer, then lyophilized before use. Synthesis, characterization, cytotoxicity and genotoxicity evaluation of PVA/Starch cryogels were investigated in previous study [13].

2.4 Characterization Studies of AgNPs

The synthesized AgNPs were characterized by UV-Vis Spectrophotometer (Chebios Optimum-One UV-VIS Spectrometer, Italy), which is the most widely used technique for structural characterization of metal nanoparticles. Fourier Transform Infrared Spectrometer (FTIR, Perkin Elmer, USA) for *Aloe barbadensis* leaf extract and AgNPs was obtained at a resolution of 4 cm^{-1} in the wavelength range 4000 to 450 cm^{-1}. The particle size distribution of AgNPs was performed by Zeta Sizer (Malvern, Nano ZS90, England) using Dynamic Light Scattering (DLS) technique.

2.4 Antimicrobial Activity of AgNPs and AgNPs Loaded PVA/Starch Scaffolds

Inhibition of microbial growth by the AgNPs and AgNPs loaded PVA/Starch scaffolds were tested against selected microorganisms. Antimicrobial activity tests were carried out using disc diffusion assay as described previously [14]. The blank discs were impregnated with 19 µL of liquid nanoparticles and blank disks were wetted with 19 µL of sterile distilled water and impregnated with powder nanoparticle samples. Liquid and powder AgNPs loaded blank disks were placed on the inoculated agar. PVA/Starch scaffolds were cut in 0.1 cm height and 50 µL of AgNPs solution was dropped on the scaffolds. AgNPs solution embedded scaffolds were placed into the inoculated plates. Ofloxacin (5 µg/disc) for bacteria and nystatin (100 µg/disc) for fungi were used as positive control. The inoculated plates were incubated at 36 ± 1 °C for 24 h for bacterial and yeast strains and at 27 ± 1 °C for 72 h for fungal isolate.

3 RESULTS AND DISCUSSION

3.1 Synthesis and Characterization of AgNPs

AgNPs were synthesized using *Aloe barbadensis* leaf extract as a reducing agent according to the method described in the previous section. Synthesis of AgNPs was proven with a color change from yellow to deep brown after 48 h of the addition of *Aloe barbadensis* leaf extract to silver nitrate solution. This color change is due to the excitation of surface plasmon resonance (SPR) vibrations of the AgNPs. The SPR vibrations of synthesized AgNPs were recorded by UV-Vis spectrum analysis of the reaction medium at different time points (12, 24 and 48 h). As shown in Fig. 2, a characteristic and well-defined SPR vibration was obtained at 430 nm, which is in agreement with the findings from previous studies [15, 16]. In addition, it was observed that increasing the reaction time increased the intensity of absorbance.

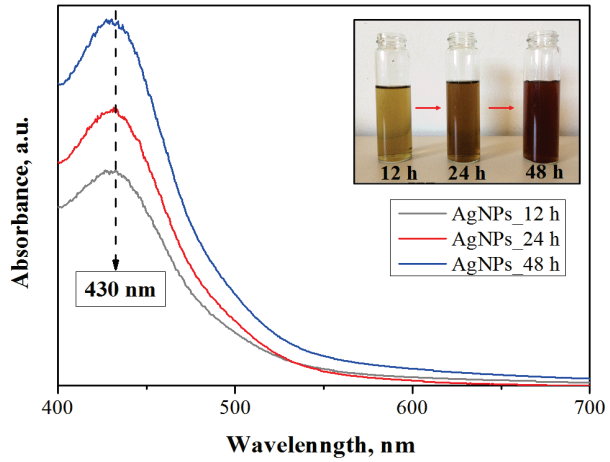

Figure 2 UV-Vis spectra of AgNPs solution at different reaction time points

FTIR analysis can be used for characterization of the synthesized AgNPs [17]. FTIR analysis of leaf extract and synthesized AgNPs is presented in Fig. 3. The absorption peaks of *Aloe barbadensis* leaf extract located at 1040, 1415, 2854 and 2919 cm^{-1} correspond to the presence of various compounds including proteins, amino acids, organic acids, vitamins, flavonoids, alkaloids, polyphenols, terpenoids, and polysaccharides. FTIR spectrum of AgNPs demonstrated sharp absorption peaks at 1619 and 3320 cm^{-1} which corresponds to amide and alcoholic hydroxide groups, respectively [6]. FTIR results confirmed that the *Aloe barbadensis* leaf extract can reduce and stabilize Ag^{1+} ions into zero valent Ag^0 nanoparticles. The flavonoid compounds in the extract of *Aloe barbadensis* might be responsible for this reduction [18, 19].

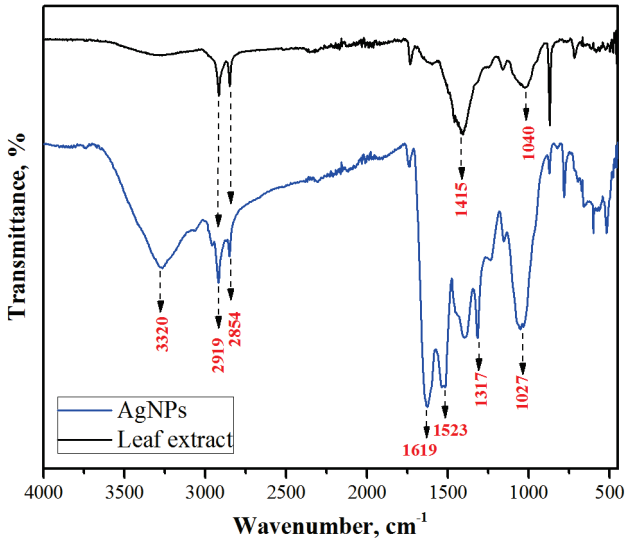

Figure 3 FTIR spectra of leaf extract and AgNPs

DLS measurements were done to determine the hydrodynamic size of the nanoparticles. Before DLS measurement, final reaction mixture containing the AgNPs was diluted with three fold distilled water. The particle size distribution curve (Fig. 4) demonstrated the various sizes of the particles ranging from 5.848 to 25.37 nm with an asymmetric distribution. The average particle size of the nanoparticles was 8.84 nm. In previous reports, the particle size of green synthesized AgNPs from various plant extracts increased from a nanometer to a hundred of nanometers. These studies proposed that by changing the composition of a reaction mixture or reaction conditions, the characteristic properties of nanoparticles such as size, shape, morphology and surface charge, could be controlled. In addition, the hydrodynamic diameter is presented as an important parameter, for understanding the size of nanoparticles and their performance in biological assays [20].

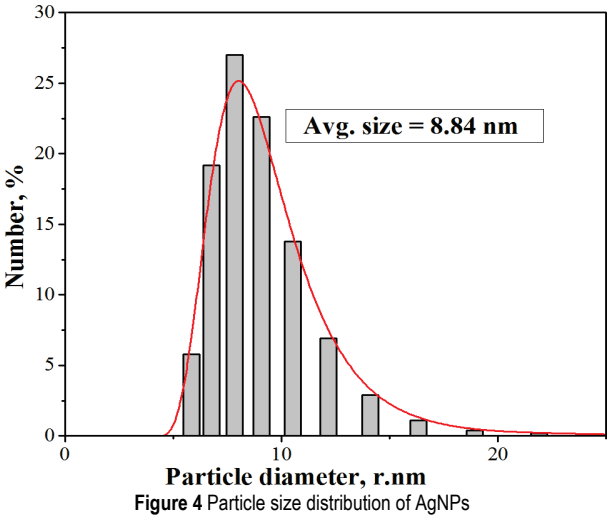

Figure 4 Particle size distribution of AgNPs

3.2 Antimicrobial Activity of Synthesized AgNPs and AgNPs Loaded PVA/Starch Cryogel Scaffolds

Antimicrobial activity of the powder and liquid nanoparticles, and AgNPs loaded scaffolds were investigated both quantitatively and qualitatively based on disc diffusion assays by evaluating the presence of inhibition zones and zone diameters. Antimicrobial activity test results were shown in Tab. 2.

Fig. 5 and Fig. 6 show the results of the antimicrobial tests regarding microorganisms. Disc diffusion assays revealed that both powder and liquid nanoparticles displayed remarkable antimicrobial activity on tested gram positive and gram negative bacterial and fungal species.

PVA/starch cryogel scaffolds which are promising materials for bone tissue engineering or wound dressing applications were synthesized and characterized in our previous study. Fourier transform infrared spectroscopy and scanning electron microscopy (SEM) were used to investigate the chemical structure and pore morphology of the scaffolds. Degradation profile and swelling ratio of the scaffolds were also determined. 3-(4,5-dimethylthiazoyl-2-yl)-2,5-diphenyltetrazolium bromide assay and SEM were used to investigate the biocompatibility of the scaffolds and cell morphology. In order to evaluate DNA fragmentation, genotoxicity test was also performed [13]. They were found to be biocompatible and have ability to enhance the attachment and growth of Mouse Embryonic Fibroblast cells. In the current study, the PVA/Starch cryogel scaffolds were impregnated with green synthesized AgNPs in order to

enhance their functions by adding antimicrobial properties. The composite PVA/Starch/AgNPs cryogel scaffolds were found to be effective against tested bacteria (both gram-positive and gram-negative), yeast and fungus (Tab. 2, Fig. 6). The composite cryogel scaffolds with antibacterial properties would have potential in preventing contamination risks in tissue engineering applications.

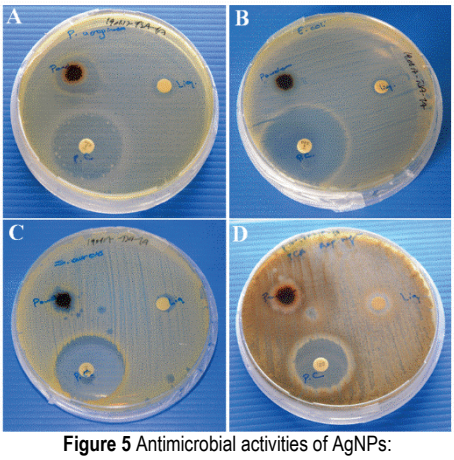

Figure 5 Antimicrobial activities of AgNPs:
A) *P. aeruginosa,* B) *E. coli,* C) *S. aureus,* D) *A. niger*

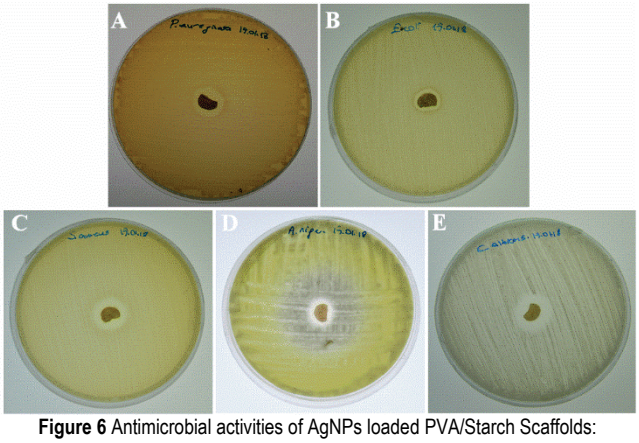

Figure 6 Antimicrobial activities of AgNPs loaded PVA/Starch Scaffolds:
A) *P. aeruginosa,* B) *E. coli,* C) *S. aureus,* D) *A. niger,* E) *C. albicans*

Antimicrobial activity of various scaffolds, including AgNPs impregnated, against gram-positive and gram-negative bacteria including *E. coli, S. aureus, and P. aeruginosa* and yeast (*C. albicans*) have been stated [21-25]. The selected bacteria, except *A. niger*, are responsible for nosocomial infections [21-26]. Moreover, *Candida* species are among the most opportunistic fungal pathogens responsible for invasive fungal infections. The antimicrobial activity of wound dressing or bone transplantation material is a favorable parameter for preventing surgical contaminations. AgNPs usage in tissue engineering scaffolds were reported previously [27, 28]. Jiang et al. produced nanohydroxyapatite/ polyurethane composite scaffolds with different amounts of silver phosphate particles for bone regeneration and tested them with *S. aureus* and *E. Coli*. Their results revealed that incorporation of silver phosphate particles into the scaffolds could impart excellent antibacterial activity to commonly existing bacteria [29]. Polycaprolactone and gelatine were also used to produce fibrous scaffolds and the scaffolds were coated with silver in different concentrations of silver nitrate aqueous solution. Scaffolds demonstrated antibacterial effects towards *Bacillus cereus* (B. cereus) and *E. coli* [30]. Moreover, nanosilver particles-collagen/chitosan hybrid scaffolds were produced for wound healing applications and applied in full-thickness skin defects in Sprague-Dawley rats to investigate the therapeutic effects of the scaffolds. The studies demonstrated that nanosilver particles-collagen/chitosan scaffolds promoted wound healing by regulating fibroblast migration and macrophage activation [31].

Table 2 Antimicrobial activity of powder and liquid nanoparticles and AgNPs loaded PVA/Starch scaffolds on microorganisms determined based on disc diffusion assay

Microbial Species	Zone of inhibition (mm)			
	Liquid	Powder	Scaffold	PC
Pseudomonas aeruginosa	12	22	20	30
Escherichia coli	10	14	12.5	30
Staphylococcus aureus	9	12	15	40
Candida albicans	NT	NT	17.5	24
Aspergillus niger	12	17	12	25

NT: not tested

As a result, the synthesized AgNPs loaded PVA/starch scaffolds could be helpful to treat both the development of infections due to microbial contamination of the scaffold throughout the surgery and the development of latent infections for biomedical applications.

4 CONCLUSION

The green synthesis of AgNPs using plant extracts is an eco-friendly method when compared to chemical and physical synthesis. The present study shows that leaf extract of *Aloe barbadensis* can be used efficiently for the synthesis of AgNPs. Green synthesized AgNPs loaded PVA/Starch cryogels were synthesized as novel tissue engineering scaffolds. Antimicrobial properties of the composite scaffold demonstrated its potential for preventing contamination risks in tissue engineering applications.

Acknowledgement

This work was supported by The Scientific Research Projects Unit of Mersin University (2018-1-TP3-2731). Authors would like to thank Yeditepe University Genetics and Bioengineering Department for providing microbial species.

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