Current Applied Physics 14 (2014) S172–S179

Contents lists available at ScienceDirect

Current Applied Physics

journal homepage: www.elsevier.com/locate/cap

Gelatin-based sponge with Ag nanoparticles prepared by solution plasma: Fabrication, characteristics, and their bactericidal effect

Seong Cheol Kim [a], Sung Min Kim [a], Gook Jin Yoon [b], Sang Woo Nam [b], Sang Yul Lee [a,**], Jung Wan Kim [b,*]

[a] Center for Surface Technology and Applications, Department of Materials Engineering, Korea Aerospace University, Goyang, Republic of Korea
[b] Center for Surface Technology and Applications, Division of Bioengineering, University of Incheon, Incheon, Republic of Korea

ARTICLE INFO

Article history:
Received 2 August 2013
Received in revised form
16 November 2013
Accepted 2 December 2013
Available online 25 January 2014

Keywords:
Solution plasma
Ag nanoparticles
Gelatin
Biocomposite
Bactericidal effect

ABSTRACT

A solution plasma process was established for fabrication of gelatin-based sponge incorporated with Ag nanoparticles (AgNPs) in this study. Without using any chemical reducing agent, colloidal AgNPs were formed in gelatin medium via the reduction reaction of Ag precursors ($AgNO_3$) and the hydrogen radicals generated during discharge. The gelatin/AgNPs sponges fabricated by lyophilization had a 3D scaffold structure of micropores with diameters of about 20 μm. TEM analysis showed that the spherical AgNPs in the thin layer of gelatin were evenly well dispersed and had mean particle sizes of about 10–15 nm. The gelatin/AgNP solutions and sponges prepared in this study exhibited excellent bactericidal activity against two well-known bacteria, *Escherichia coli* and *Staphylococcus aureus*. All of them inhibited growth of *E. coli* more efficiently than that of *S. aureus*. Minimal inhibition concentration of gelatin/AgNP formed with 3% gelatin and 5 mM $AgNO_3$ was <20 and 40 μg/ml for *E. coli* and *S. aureus*, respectively. The sponge of 3% gelatin and 5 mM $AgNO_3$ discharged for 740 s showed the strongest bactericidal activity toward both bacteria.

1. Introduction

Recently, much attention has been paid to manufacturing materials with minimal risk of environmental problem. Solution plasma, a novel technology, has potentials for advancement in production of nano-materials and decomposition of organic-based compounds as well as control of microorganisms, and has been expected to be developed as a green strategy due to its unique characteristics [1–5].

Plasma can be created in liquid under atmospheric conditions and it is beneficial in the aspects of minimal usage of chemical agents, short process time, and easy handling [6,7]. Since plasma is generated in aqueous solution by supplying high voltage, a variety of radicals (H•, O•, OH•), gases (H_2, O_2, H_2O_2), electrons, and radiation (UV rays) could be generated via association, excitation, and ionization of water molecules during discharge [8–11]. The outputs of solution plasma can be controlled by changing various factors, i.e. physical parameters of power supply (voltage, frequency, duty ratio, and electrode geometry) and chemical parameters of discharge media (polarity and pH). With high energetic and active outputs, reaction rate can be enhanced, making it superior to commercial chemical reactions. Although various active species are generated physically, selective reaction can be achieved. For an example, hydrogen radicals play a crucial role in the formation of nano-metallic particles by reducing metal ions to nanoparticle precursors, making usage of chemical reducing agent unnecessary.

Until now, various novel nanometals have been synthesized using solution plasma without the addition of a reducing agent. Au nanoparticles (AuNPs) were first synthesized by the method using aurate chloride ($HAuCl_4$) as a precursor in the presence of various stabilizers, such as sodium dodecylsulfate, cetyl tetraammonium-bromide [3]. The shape and size of AuNPs could be easily controlled by adjusting the plasma and chemical conditions [3,5,12–15]. Other novel nanoparticles of Ag and Pt also have been successfully synthesized using solution plasma [16]. Incorporation of Ag nanoparticles (AgNPs) in the mesoporous matrix by discharging the solution of $AgNO_3$, P123 block copolymer, and silica powder was reported to be effective in shortening the reaction time and minimizing the usage of chemical agents. In this approach, solution plasma was beneficial not only for the formation of Ag but also for the calcination to remove the organic template instead of the commercial thermal calcinations [17]. The nano-composite of Pt nanoparticles (PtNPs) and carbon nanoballs was also prepared

* Corresponding author. Tel.: +82 10 9286 8641; fax: +82 32 835 0804.
** Corresponding author. Tel.: +82 10 7350 0166; fax: +82 2 3158 3770.
E-mail addresses: sylee@kau.ac.kr (S.Y. Lee), kjw5864@incheon.ac.kr (J.W. Kim).

1567-1739/$ – see front matter © 2014 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.cap.2013.12.032

using the advantages of solution plasma. In short processing time, carbon nanoballs impregnated with PtNPs could be synthesized [18].

Gelatin is one of the well-known biomaterials, a polypeptide commercially derived from the hydrolysis of collagen with triple helix structure [19,20]. Collagen, gelatin, and their derivatives are non-toxic, biocompatible, and biodegradable. Therefore, the molecules have potential in synthesizing biocomposites with various substances incorporated, e.g. organic molecules, drugs, and nanoparticles. Especially, AgNPs, DNA, drugs, and cells have been incorporated in the gelatin/collagen matrix in attempts to develop bactericides, fungicides, artificial skin/bone substitutes, wound healing dressing, drug and cell carriers, and bio-responsible sensors using various chemicals [21–23].

Herein, we focused on the advantages of solution plasma for the formation of AgNP in the gelatin by minimizing the usage of chemical agent. The biopolymer, gelatin, was selected to stabilize the colloidal AgNPs synthesized during discharge and to use as the biomatrix for fabrication into sponge by lyophilization. After discharge, UV–Vis spectroscopy was carried out to evaluate the formation of AgNPs in the gelatin solution via the response of surface plasmon absorption. The size and shape of AgNPs in the gelatin matrix and the sponge texture in the microscale were also investigated. In addition, the bactericidal effects of gelatin/AgNP sponge against a gram positive bacterium, *Staphylococcus aureus*, and a gram negative bacterium, *Escherichia coli*, were examined.

2. Materials and method

2.1. Conditions for the synthesis of AgNPs in gelatin medium

In this study, a simple process for the preparation of gelatin/AgNP suspension was established. Typically, various amounts of granule gelatin (1–3%, w/w; Samchun, Korea) and $AgNO_3$ flake (1–5 mM; Junsei, Japan) as Ag precursor were dissolved in deionized water and the mixtures were stirred until they became homogeneous. Then, the solutions were poured into a glass reactor for plasma generation.

A bipolar pulsed high voltage power supply (MPS-06K06C, Kurita-Nagoya, Japan) was employed to generate plasma. The parameters of the power supply were fixed at 1600 V, 20 kHz, 2 μs, 1.0 mm, and 180 s of voltage, frequency, pulse width, electrode gap, and discharge time, respectively. Time dependence of discharge on the properties of AgNPs was also studied by increasing discharge time to be 740 s. By discharging, suspensions of AgNPs in gelatin medium were obtained. The resulting samples were designated as Ag***a***G***b***, where ***a*** represented the concentration of $AgNO_3$ and ***b*** that of gelatin.

2.2. Fabrication of gelatin/AgNP sponge by lyophilization

The gelatin/AgNP suspensions were fabricated in the form of sponge using a lyophilizer (MCFD8508, Ilshin Co., Korea). Five milliliters of each suspension was poured into a polystyrene dish and solidified by freezing at −80 °C for 2 h. The frozen samples were lyophilized at −40 °C with a pressure below 6.38×10^{-4} MPa for 24 h to dried sponges. At the final step, the dried samples were fixed by UV–crosslinking ($\lambda = 254$ nm) for 15 m to reduce solubility in water.

2.3. Characterization of gelatin/AgNP sponge

AgNP formed in gelatin medium was examined using an UV–Vis spectrophotometer (UV–3600 with scanning wavelength from 200 to 1200 nm, Shimadzu, Japan). Viscosity of the gelatin/AgNP suspensions was measured using a viscosity meter (LVDV-1 prime, spinodal S34 at 100 rpm, BROOK FIELD, USA). AgNPs in the sponge samples were observed by a transmission electron microscope (TEM; JEOL JEM-2010 microscopes at 200 kV, JEOL, Japan). Microstructure of the gelatin/AgNPs biocomposite sponges was observed by a field emission scanning electron microscope (FE-SEM; JEOL JSM-6700F at 5 kV, JEOL, Japan). The reduction yield of $AgNO_3$ was confirmed with various discharge times using a X-ray Photoelectron Spectrometer (XPS; Multilab-2000, XPS by scanning binding energy from 0 to 1000 eV, Thermo Fisher Scientific, UK).

2.4. Agar diffusion analysis

The gelatin/AgNP sponges prepared in this study were tested for their capability inhibiting bacterial growth. Their bactericidal activity was investigated using a Gram positive bacterium, *S. aureus* (KCTC1916), and a Gram negative bacterium, *E. coli* (MC1061). The fabricated gelatin/AgNP sponges were sterilized using ethylene oxide gas. First, bactericidal activity of the sponges was initially tested by the Kirby–Bauer agar diffusion method [24]. About 10^4 colony forming units (CFUs) of the bacterial cells were spread on Műller–Hinton agar plates (beef extract 0.2%, acid digest of casein 1.75%, starch 0.15%, Agar 1.7%; Difco Co., USA) and small disks of gelatin/AgNP sponge (6 mm in diameter) with varying concentrations of AgNP (1, 3, and 5 mM) were placed on them aseptically. The resulting zones of growth inhibition were measured to assess the anti-bacterial effect after incubation at 37 °C for 24 h.

2.5. Determination of minimal inhibitory concentration (MIC)

MIC of AgNPs in the gelatin/AgNP sponge for each bacterium was also determined. For the test, 0.05 ml of overnight bacterial cultures ($\sim 10^6$ CFU/ml) and gelatin/AgNPs solutions with various AgNP concentrations (0, 20, 40, 80, and 160 μg/ml) were mixed in test tubes containing 5 ml of nutrient broth (tryptone 1%, yeast extract 0.5%, sodium chloride 0.5%). Then, all tubes were incubated at 37 °C with gentle shaking (120 rpm). The turbidity of each tube was observed with naked eyes in 24 h of incubation.

2.6. Quantitative analysis of bactericidal activity

Bactericidal activity of gelatin/AgNP sponge was examined quantitatively by determining CFU remained in the culture after treatment with the sponge. Certain amounts of gelatin/AgNP sponges with AgNP concentrations of 1, 3, and 5 mM were added to bacterial broth ($\sim 10^6$ CFU/ml) and incubated at 37 °C for 1 h. Successively, aliquots of the culture (10 μl) were spread over nutrient agar plates and bacterial colonies formed in 16 h of incubation at 37 °C were counted.

3. Result and discussion

3.1. Synthesis of gelatin/AgNP solution

In this study, various factors such as concentrations of gelatin and $AgNO_3$ precursor, and discharge time were considered for the synthesis of gelatin/AgNP solutions. Under the controlled discharge conditions, plasma was continuously generated in the solution of $AgNO_3$ precursor and gelatin in the gap between the electrodes. Consequently, the color of the solution turned to gray–black from transparent light yellow probably due to the formation of AgNPs that generally response the surface plasmon absorption in the ranges of UV–Vis wavelengths. This confirmed that the gelatin/AgNP solutions could be formed in one-step process using solution plasma without additional usage of chemical reagent in this study.