Journal of Photochemistry & Photobiology, B: Biology 159 (2016) 229–236

Contents lists available at ScienceDirect

Journal of Photochemistry & Photobiology, B: Biology

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

In vitro fabrication of dental filling nanopowder by green route and its antibacterial activity against dental pathogens

Jeong-Ho Lee [a], Palanivel Velmurugan [b], Jung-Hee Park [b], Kui-Jae Lee [b], Jong-Sik Jin [c], Yool-Jin Park [d], Keuk-Soo Bang [c,*], Byung-Taek Oh [b,*]

[a] Sunchang Research Institute of Health and Longevity, Sunchang, Jeonbuk 56015, South Korea
[b] Division of Biotechnology, Advanced Institute of Environment and Bioscience, College of Environmental and Bioresource Sciences, Chonbuk National University, Iksan, Jeonbuk 54596, South Korea
[c] Department of Oriental Medicine Resources, Advanced Institute of Environment and Bioscience, College of Environmental and Bioresource Sciences, Chonbuk National University, Iksan, Jeonbuk 54596, South Korea
[d] Department of Ecology Landscape Architecture — Design, College of Environmental and Bioresource Sciences, Chonbuk National University, Iksan, Jeonbuk 54596, South Korea

ARTICLE INFO

Article history:
Received 1 February 2016
Received in revised form 21 March 2016
Accepted 21 March 2016
Available online 23 March 2016

Keywords:
Metal ions
Nanopowder
Characterization
Salvia miltiorrhiza
Dental
Antibacterial

ABSTRACT

The aim of this study was to introduce novel Sn, Cu, Hg, and Ag nanopowders (NPs) and a composite nanopowder (NP) synthesized using *Salvia miltiorrhiza* Bunge (SM) root extract as a reducing and capping agent to improve the antibacterial property of dental filling materials. All of the NPs obtained were characterized using a scanning transmission electron microscope (STEM), and energy dispersive X-ray (EDX) spectrum imaging was performed to map the elemental distributions of the NP composite. Fourier transform infrared (FTIR) spectroscopy was performed to identify the role of various functional groups in all of the obtained NPs and the phyto-compound responsible for the reduction of various metal ions. The X-ray diffraction (XRD) patterns clearly illustrated the crystalline phase of the synthesized NP. The antibacterial properties of the synthesized Sn, Cu, Hg, Ag, composite NP, SM root extract, and commercial amalgam powder were evaluated. The Cu, composite NP, SM root extract and Ag NP displayed excellent antibacterial activity against dental bacteria *Streptococcus mutans* and *Lactobacillus acidophilus*. The results of this study require further evaluation for signs of metal toxicity in appropriate animal models. However, the results are encouraging for the application of metal NPs as suitable alternatives for antibiotics and disinfectants, especially in dental filling materials.

1. Introduction

Metallic nanoparticles and their composites are extensively used in biomedical applications such as drug delivery, antimicrobial agents, and medical devices due to their small size, high surface area-to-volume ratio, inert nature, stability, high dispersity, non-cytotoxicity, and biocompatibility [1,2]. Due to the emergence of new bacterial pathogens, as well as microbes developing resistance against existing antibiotics, biomedical researchers and pharmaceutical companies are extensively studying the antibacterial properties of metallic nanoparticles against various microbial pathogens [3,4]. Elemental nanoparticles are used as an active antibacterial ingredient for dental materials [3,5]. In dentistry, failures in restoration are often attributed to the restorative materials themselves or to oral bacteria [6]. Researchers are in search of long-lasting antibacterial materials for dental applications, with a special emphasis on the metal used in teeth fillings, to control the formation of biofilms within the oral cavity. Dental amalgams are a combination of mercury (50%), silver (~22–32%), tin (~14%), and copper (~8%), with few other trace metals [7]. Dental filling amalgam made up of several metal compounds which frequently leach out from the tooth filling during mastication of food materials and it can cause several health hazards both in adults and children. To overcome this problem, it is necessary to fabricate dental filling amalgam using organic source as an alternative.

The development of a reliable and green chemical process for the biogenic synthesis of nanomaterials is important for their clinical application as antibacterial agents [8]. One potential solution to the development of green nanomaterials with high medicinal value is the use of medicinal plant extracts as a reducing and capping agent. *Salvia miltiorrhiza* Bunge (danshen in Chinese) is a well-known and important traditional Chinese herbal medicine [9]. *S. miltiorrhiza* roots (SMR) or rhizomes are widely used for the clinical treatment of cardiovascular and cerebrovascular diseases such as angina pectoris, myocardial infarction, and stroke in China, Japan, the United States, and some European countries [10,11]. The hydro-soluble extract of SM is rich in phenolic acids such as danshensu (3,4-dihydroxyphenyllactic acid), rosmarinic acid, and salvianolic acids A & B, which are excellent antioxidants [11]. The extract has been shown to improve blood rheology and protect

* Corresponding authors.
E-mail addresses: ksbang@jbnu.ac.kr (K.-S. Bang), btoh@jbnu.ac.kr (B.-T. Oh).

http://dx.doi.org/10.1016/j.jphotobiol.2016.03.028
1011-1344/

against myocardial damage, and is used in the treatment of cardiovascular and cerebrovascular diseases, hepatic fibrosis, liver fibrosis, and nephritis [11–15].

Streptococcus mutans and *Lactobacilli* sp. change the environment of the oral flora, enabling fastidious organisms to colonize and cause the formation of dental plaques [16]. These two bacteria are normally present in low numbers in the plaque of affected individuals. When salivary flow decreases, the pH of the plaque drops, leading to a selection for aciduric (acid-tolerant) bacteria such as *S. mutans* and *Lactobacilli* sp. [17]. For patients undergoing radiotherapy, new decayed lesions become obvious within 3 months of treatment, and the patient may average one or more new decayed surfaces per post-radiation month. During the development of this decay, the proportions of *S. mutans* and *Lactobacilli* sp. increase significantly and sequentially [16]. This sequence of events indicates that *S. mutans* is involved in the initiation of decay, whereas the *Lactobacilli* sp. is associated with the progression of the lesion [16,18].

In restorative materials, nanocomposites with nano fillers are developed in combination with advanced resins and curing technologies [19, 20]. Nano dental filling materials typically consist of silver, silver nanopowder, and combinations of various materials with silver [21]. However, there has not been a meticulous study on the green synthesis of dental filling materials such as Sn, Cu, Hg, and Ag nanopowders (NPs), or of composite nanopowder (NP). Thus, in this study we evaluated the potential of the green syntheses of various metal NPs and composite NP for dental fillings and then characterized their antibacterial activity against dental bacteria *S. mutans* and *L. acidophilus* using the Korean medical plant SM.

2. Materials and methods

2.1. Chemicals, medicinal plant, and biological materials

The reactants $SnCl_2$ (99.9%), $Cu(NO_3)_2$ (99.9%), $HgSO_4$ (99.9%), and $AgNO_3$ (99.9%) were acquired from DaeJung Chemicals, Seoul, South Korea, in stoichiometric amounts and were dissolved in aqueous solution as the metal precursors. Dried SMR (Fig. 1) was obtained from the Department of Oriental Medicine, Chonbuk National University, Iksan, South Korea, and the extract was prepared by grinding the dried root into a fine powder. 100 g of this powder was added to 500 mL of deionized water in a 1000-mL flask, mixed well, and maintained on a magnetic heating stirrer at 90 °C for 30 min. The extract yield was filtered using Whatman No.1 filter paper and stored at 4 °C for further use. Virulent strains of the skin pathogen *Streptococcus mutans* (KACC 16833) and *Lactobacillus acidophilus* (KACC 12419) were procured from the Korean Agriculture Culture Collection (KACC) in Suwon, South Korea. Each strain was cultivated initially using nutrient agar followed by incubation and stored at 4 °C for further use.

2.2. Sn, Cu, Hg, Ag, and composite NP preparation

The Sn, Cu, Hg, Ag NPs, and composite NP (a blend of Sn, Cu, Hg, and Ag ions) were synthesized using *S. miltiorrhiza* root extract (SMRE) in aqueous solutions at room temperature. Fifty mL of each metal solution of Sn (2 mM), Cu (1 mM), Hg (5 mM), and Ag (2 mM), and mixing of all the metal solutions (composite NP) were added dropwise over 2 h using a Gilson Minipuls 3 peristaltic pump (Villiers Le Bel, France) at flow rate of 1 μL/min in to a 250 mL-Erlenmeyer flask containing 50 mL of SMRE under rapid stirring. During stirring, different colors were formed in each reaction solution. After 1 day of incubation, each reaction solution was centrifuged at 12,000 rpm for 20 min to separate the fabricated NP and was then washed with deionized water several times in order to remove any uncoordinated particles. The obtained NPs were dried in a vacuum freeze dryer for 72 h.

2.3. Characterization of the NP synthesized with SMR extract

The size and morphology of the nanoparticles synthesized with SMRE were analyzed using TEM/STEM (TEM, JEM-2200FS, JEOL, Japan) operated at 200 kV, equipped with an energy dispersive X-ray spectroscopy (EDS). High angle annular dark field-scanning TEM (HAADF-STEM) images were collected over scattering angles from −30 to 30 mrad (*X* and *Y* axes). In addition, elemental mapping was used to study the microstructure and composite elemental distribution. Powder X-ray powder diffraction (XRD) of the samples was performed using a Rigaku X-ray diffractometer (Rigaku, Japan) with scanning performed from $2\theta = 20$ to 80° at 0.04°/min with a time constant of 2 s. The Fourier transform infrared spectroscopy (FTIR) spectra of the NPs were obtained with a Perkin-Elmer FTIR spectrophotometer (Norwalk, CT,

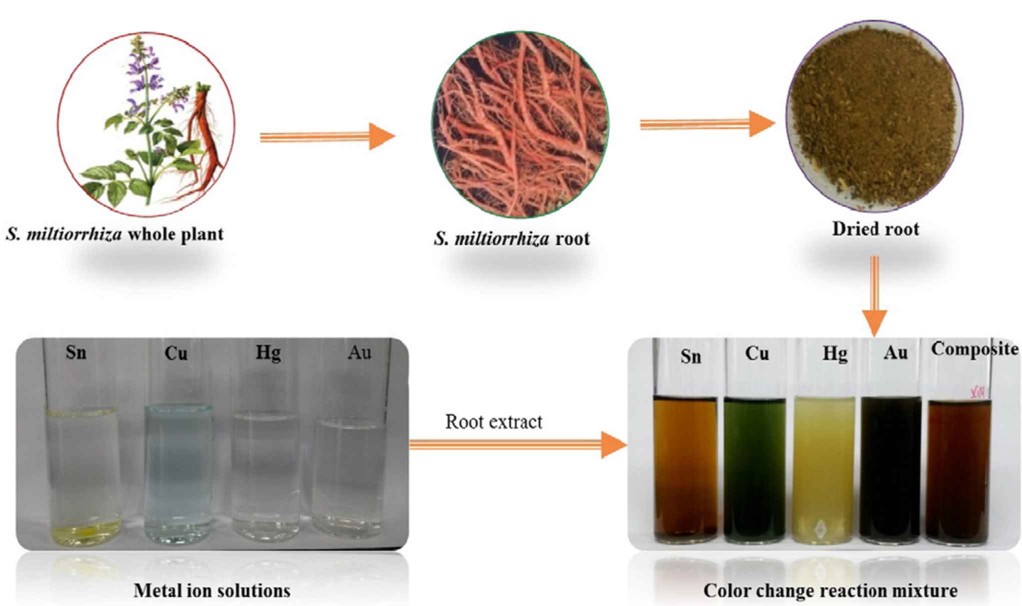

Fig. 1. *S. miltiorrhiza* whole plant, *S. miltiorrhiza* root, dried root material, metal ion solutions, and reaction mixtures with Sn, Cu, Hg, Au, and composite nanoparticles (color change).