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Full Length Article

Preparation and characterization of antibacterial orthodontic resin containing silver nanoparticles

Sang Jin Lee[a,1], Min Heo[a,1], Donghyun Lee[a], Seungheui Han[b], Ji-Hoi Moon[c], Ho-Nam Lim[d], Il Keun Kwon[d,*]

[a] Department of Dentistry, Graduate School, Kyung Hee University, 26 Kyungheedae-ro, Dongdaemun-gu, Seoul 02447, Republic of Korea
[b] Department of Orthodontics, School of Dentistry, Kyung Hee University, 26 Kyungheedae-ro, Dongdaemun-gu, Seoul 02447, Republic of Korea
[c] Department of Maxillofacial Biomedical Engineering and Institute of Oral Biology, School of Dentistry, Kyung Hee University, 26 Kyungheedae-ro, Dongdaemun-gu, Seoul 02447, Republic of Korea
[d] Department of Dental Materials, School of Dentistry, Kyung Hee University, 26 Kyungheedae-ro, Dongdaemun-gu, Seoul 02447, Republic of Korea

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ABSTRACT

In this study, we developed a hybrid dental resin containing silver nanoparticle (AgNPs) to eliminate periodontal disease causing bacteria such as *streptococcus mutans* (*S. mutans*) and *streptococcus sobrinus* (*S. sobrinus*). The silver nanoparticles enables the resin to prevent oral pathogen growth during orthodontic therapy. First, AgNPs were directly synthesized in dimethylformamide (DMF) solvent with a capping agent. Second, pure orthodontic primer was mixed with the synthesized AgNPs solvent-slurry followed by photocuring. The resultant material was characterized by physicochemical characterization. Finally, an *in vitro* antimicrobial test was carried out. The results showed that the AgNPs were fully synthesized and clearly embedded in dental resin. In the bacterial test, the dental resin containing AgNPs showed potent antimicrobial activity against two kinds of bacteria. In conclusion, our methodology may allow for the generation of a wide range of dental resin and composite products which inhibit periodontitis causing bacteria.

1. Introduction

In the orthodontic clinical area, malocclusion (MC) is a well-known, and potentially fatal, oral disease. Remarkably, about 30 percent of children and teenagers suffer from MC [1]. In order to cure MC, many patients are treated using an orthodontic appliance (OA). The appliance is emplaced over a long period of time to hold the jaw and teeth in proper alignment. The OA also acts to improve the patient's cosmetic outcome and quality of life [2]. During orthodontic treatment, patients are instructed to brush their teeth in order to prevent plaque and provide for good oral hygiene. Nevertheless, various oral bacteria still remain on the OA in the oral cavity. These residual microorganisms may cause the orthodontic treatment to fail [3,4]. Additionally, infectious bacteria are implicated in caries and periodontitis [5]. They may also result in cancer causation and progression [6,7]. Thus, inhibition and extermination of bacteria is required to prevent oral pathogen growth during orthodontic therapy.

In general, the OA is comprised of a metal base wire embedded in orthodontic composite resin [8,9]. One of the most commonly used orthodontic composite resins is dental adhesive, which is widely used as an orthodontic bracket bonding agent [10,11]. In order to functionalize the orthodontic adhesive, some groups have developed silver nanoparticles (AgNPs) loaded antimicrobial cement which acts to prevent dental caries. Akhavan et al., investigated the effect of incorporating silver (Ag) and hydroxyapatite nanoparticles on the shear bond strength of an orthodontic adhesive [12]. They confirmed that Ag/hydroxyapatite particles can increase the shear bond strength of orthodontic adhesive. Blöcher et al., established that the addition of AgNPs to an orthodontic primer affects shear bond strength and bracket strength, which reduces adhesive failure [13]. Other researchers have described that the addition of AgNPs exhibited neither resistance to adhesive failure nor shear bond strength. Although these researchers have characterized the physical behavior due to AgNP incorporation, the use of a facile AgNP manufacturing process and antimicrobial assessment remains to be accomplished. In the field of dentistry, one innovative strategy for promoting antimicrobial activity of dental materials is to incorpo-

* Corresponding author.
E-mail address: kwoni@khu.ac.kr (I.K. Kwon).
[1] These co-authors have made equal contributions to this work.

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rate AgNPs directly in the dental composite. This approach prevents dental caries and other diseases [14–17].

In this study, we designed and prepared an antimicrobial dental adhesive by directly incorporating the AgNPs, generated by a facile synthesis procedure, into the resin followed by curing from ultraviolet–visible light. The major purpose is to provide a novel antimicrobial dental resin for clean orthodontic treatment with reduced periodontal disease. The detailed process is shown in Fig. 1. The resultant products were analyzed by ultraviolet–visible absorption spectra (UV), transmission electron microscopy (TEM), scanning electron microscopy (SEM), X-ray photoelectron spectroscopy (XPS), thermogravimetric analysis (TGA), and inductively coupled plasma-mass spectrometer (ICP-MS). Finally, *in vitro* antibacterial tests were performed against oral disease related-pathogenic bacterium including *Streptococcus mutans* (*S. mutans*) and *Streptococcus sobrinus* (*S. sobrinus*) [18,19].

2. Materials and methods

2.1. Materials

Transbond™ XT primer adhesive was purchased from 3 M Unitek Orthodontic Products (Monrovia, CA, USA). Silver nitrate (ACS reagent, ≥99.0%), *N,N*-Dimethylformamide (DMF, anhydrous, 99.8%), and (3-Aminopropyl) trimethoxysilane (APS, 97%) were purchased from Sigma-Aldrich (St. Louis, MO). Deionized distilled water (DDW) was produced by an ultra-pure water system (Puris-Ro800, Bio Lab Tech., Korea). All other reagents and solvents were used as received without further purification.

2.2. Synthesis of AgNPs in dimethylformamide (DMF)

To prepare AgNPs, the particles were directly synthesized in DMF using (3-Aminopropyl) trimethoxysilane (APS) as a capping agent. The formation process was as previously described with some modifications [20,21]. Briefly, 100 mM of silver nitrate was dissolved in 100 ml of DMF under stirring at 100 °C. After complete dissolution of silver nitrate, 1.75 ml of 100 mM APS was poured into the silver nitrate dissolved DMF solution and the conditions were maintained for 24 h. After synthesis, AgNPs solutions were cooled down to room temperature and filtered to remove any impurities. These solutions were stored at room temperature with protection from light. In order to avoid aggregation of these nanoparticles, the slurries were used immediately.

2.3. Preparation of AgNPs incorporated dental resin

To prepare the antimicrobial resin, Transbond™ XT primer adhesive (100 λ) and AgNPs dissolved in DMF (10 λ) were thoroughly mixed followed by polymerization for 40 s using a LED light-curing unit (Elipar S 10, 3 M ESPE, Seefeld, Germany). After that, the manufactured products were completely washed with fresh DDW several times and dried under nitrogen gas. A control group was prepared using the same process without including AgNPs dissolved in DMF.

2.4. Ag ion release kinetic of AgNPs loaded dental resin

For confirmation of Ag ion release, ICP-MS was performed. The AgNPs loaded dental resin samples were incubated in 1 ml of phosphate buffered saline (PBS) at 37 °C under orbital shaking with 100 rpm for up to 1 week. The release was determined at predetermined time intervals of 3 and 7 days. At each time point, the entire supernatant was removed from the vial and refreshed with fresh PBS. The quantity of released Ag was then determined by ICP-MS.

2.5. In vitro *antimicrobial test*

Streptococcus mutans GS5 and *Strepcococcus sobrinus* 6715 were grown either in Trypticase soy agar (Difco Laboratories, Detroit, MI) or in Brain Heart Infusion (BHI, Difco) broth at 37 °C. An antibacterial assay was performed as described previously with some modifications [22–24]. Briefly, each bacterial strain was grown for 24 h and the density was adjusted in sterile phosphate buffered saline (PBS, pH 7.4) to give a final concentration of 5×10^7 colony forming units per milliliter (CFU/mL). Then, 100 μl of the diluted bacterial suspension was dropped on the surface of each sample. After a predetermined time interval, aliquots (30 μl) were removed from the suspension and the viable cells were enumerated by ATP-bioluminescence quantification using a BacTiter-GloTM Microbial Cell Viability Assay Kit (Promega, Madison, WI) according to the manufacturer's instructions.

2.6. Analytical equipment

AgNPs observations were performed by TEM using a model H-7100 (Hitachi, Japan) at an accelerating voltage of 100 kV at room temperature. Ultraviolet–visible spectrophotometry was carried out using an UV1650PC (Shimadzu, Japan). Manufactured dental resin observations were observed using a scanning electron microscope (SEM, Hitachi S-4700, Japan) at an acceleration voltage of 15 kV. X-ray photoelectron spectroscopy (XPS) was performed using a K-Alpha 89 (Thermo Electron, UK). In order to investigate the thermal decomposition properties of the dental resins, TGA was carried out using a TGA Q5000IR (TA Instruments, USA). The release profile of Ag ions was evaluated by inductively coupled plasma mass spectrometry (ICP-MS, Agilent 7500cx, USA).

2.7. Statistical analysis

Statistical analysis was performed using PASW Statistics 18 software (SPSS, Inc.). All values were expressed as means ± standard deviations, and differences with p-values ($^*P < 0.05$) were considered statistically significant [25,26].

3. Results

3.1. Characterization of synthesized AgNPs within DMF (TEM, UV)

In order to manufacture the AgNPs loaded dental resin, we prepared the AgNPs solution by directly synthesizing the AgNPs in the DMF solution using a capping agent. The presence of AgNPs in the synthesized DMF solution was examined by TEM and UV–vis analysis. Fig. 2a shows a TEM image of the AgNPs incorporated DMF solution after drying at room temperature. The TEM image shows the synthesized AgNPs in nano-sized dimensions and with a mostly spherical shape. The nanoparticles were also observed to be well dispersed without aggregation. The results of this TEM analysis agree with UV–vis absorption (Fig. 2b). The absorbance spectrum of AgNPs was investigated by UV–vis spectroscopy. Fig. 2b shows that pure DMF solution displays no substantial absorption. However, AgNPs in DMF solution display a broad surface plasmon resonance (SPR) peak at around 420 nm. This corresponds to the SPR of AgNPs. This result correlates with the results obtained in our previous report [23,27]. The TEM and UV–vis analysis revealed that AgNPs formation was completed in DMF solvent.

3.2. Surface characterization of manufactured dental resin and AgNPs incorporated hybrid dental resin (SEM, XPS)

SEM was carried out to study the morphology of resin surfaces after curing at room temperature. Fig. 3 shows that bare dental resin

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