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Effects on cytotoxicity and antibacterial properties of the incorporations of silver nanoparticles into the surface coating of dental alloys*

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Abstract: The aim of this study was to research the changes in cytotoxicity and antibacterial properties after silver nanoparticles (AgNPs) were incorporated into the surface coating of dental alloys. AgNPs were attached to cobalt chromium alloys and pure titanium using a hydrothermal method, according to the reaction: $AgNO_3+NaBH_4 \rightarrow Ag+1/2H_2+1/2B_2H_6+NaNO_3$. A 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay was used to evaluate the cytotoxicity of the alloys when in contact with osteogenic precursor cells (MC3T3-E1) from mice and mesenchymal stem cells (BMSC) from rats. The antibacterial properties of dental alloys incorporating three different concentrations (10, 4, and 2 μmol/L) of AgNPs were tested on *Staphylococcus aureus* (SA) and *Streptococcus mutans* (MS). High cytotoxicity values were observed for all dental alloys that contained 0% of AgNPs (the control groups). The incorporation of AgNPs reduced cytotoxicity values. No significant difference was observed for antibacterial performance when comparing dental alloys containing AgNPs to the respective control groups. The results demonstrated that the cobalt chromium alloys and pure titanium all had cytotoxicity to MC3T3-E1 and BMSC and that the incorporation of AgNPs could reduce this cytotoxicity. The concentrations of AgNPs adopted in this study were found to have no antibacterial action against SA or MS.

Key words: Silver nanoparticles (AgNPs); Dental casting; Cytotoxicity; Antibacterial; MC3T3-E1; BMSC
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1 Introduction

Implants and removable dentures were widely used in clinical practice to improve patients' quality of life, but they also resulted in the occurrence of peri-implantitis (Mombelli *et al.*, 2012; Derks and Tomasi, 2015) and denture stomatitis (Ramage *et al.*, 2004; Shulman *et al.*, 2005). Research on surface modifications of dental materials had been done to reduce the failure rate of implants (Morra, 2007; Stanford, 2008). Studies demonstrated that surface modifications using plasma treatments could reduce the adherence of *Candida albicans* to denture base resins, which reduced the incidence rate of denture stomatitis (Zamperini *et al.*, 2010; Wen *et al.*, 2016).

The antibacterial properties of silver were discovered as early as ancient Greece. With more recent in-depth research, nanosilver was found to have a better antibacterial effect when the particle size was less than 10 nm (Gliga *et al.*, 2014). Methods for

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preparing silver nanoparticles (AgNPs) are divided into two main categories according to the reaction mechanism: physical synthesis and chemical synthesis. The physical method uses techniques such as mechanical polishing and radiation to generate nanosilver directly from silver (Baker *et al.*, 2005; Gromov *et al.*, 2015). Chemical synthesis uses an oxidation-reduction reaction to reduce silver ions into nanosilver particles, and due to its easy operation and good controllability, this method has been widely used in various fields (Fahmy *et al.*, 2016; Samari and Dorostkar, 2016).

AgNPs are known for demonstrating high antibacterial properties, effectively inhibiting the growth of bacteria, eucaryotic microbes, and viruses (Jung *et al.*, 2009; Wani *et al.*, 2013; Chowdhury *et al.*, 2016). The antibacterial effects of AgNPs are superior to that of other antibacterial materials (Anisha *et al.*, 2013). Due to these properties, medical products containing AgNPs have been widely used, such as in wound dressings, the surface coating of medical devices, surgical sutures, antibacterial excipients, cardiac valves, and target agents (Wright *et al.*, 2002; Huh and Kwon, 2011).

Recently, AgNPs have been used to enhance the antibacterial performance of dental materials (Qin *et al.*, 2015; Vogel *et al.*, 2015). AgNPs were added to composite resins and results demonstrated that, in sufficient concentrations, the presence of nanosilver significantly increased the resins' antibacterial performance (Fan *et al.*, 2011; Ai *et al.*, 2017). Microwave solidification was applied to the denture base preparation after mixing the AgNPs with polymethyl methacrylate (PMMA) powder, and the results showed that the amount of *C. albicans* attached to the base surface was significantly reduced (Acosta-Torres *et al.*, 2012). AgNPs have also been added to the surface of titanium implants (Massa *et al.*, 2014; Matsubara *et al.*, 2015; Mishra *et al.*, 2015). This incorporation of AgNPs not only resulted in a significant increase in antibacterial properties (*Streptococcus mutans* (MS), *Porphyromonas gingivalis*, and *C. albicans*), but also significantly increased the expression of osteoblast phenotype genes (*Alp*, *Ocn*, *RunX2*) in cell growth on the Ag-implanted titanium surface (Zheng *et al.*, 2012).

However, safety issues concerning AgNPs have attracted attention. Numerous studies have shown that AgNPs can affect DNA replication and the mitochondrial functions of cells, producing cytotoxicity (Park *et al.*, 2009; Li *et al.*, 2010; You *et al.*, 2011; Chen and Zhang, 2012). AgNPs (15 and 100 nm) can damage the fetal liver cells of mice, thought likely to be mediated by oxidative stress (Hussain *et al.*, 2005), and can also result in long-term inhibition of the cell proliferation (after short-term exposure) of human-derived keratinocytes (Zanette *et al.*, 2011). Many factors are directly related to the biosafety of AgNPs, including size, shape, chemical composition, surface charge, solubility, biological binding site, and metabolic and excretion pathways (Park *et al.*, 2010; Philbrook *et al.*, 2011; Beer *et al.*, 2012). Protective effects have been reported for different compositions (vitamin E, inducible nitric oxide synthase (iNOS), thiolated-2-methacryloyloxyethyl phosphorylcholine (MPC-SH)) regarding AgNPs cytotoxicity (Zhang *et al.*, 2006; Sangsuwan *et al.*, 2016; Zielinska *et al.*, 2016). The method of green synthesis and the characterization of AgNPs have been reported, such as *Nigella sativa* leaf extract (Gogoi *et al.*, 2015) and the alcoholic flower extract of *Nyctanthes arbortristis* (Amooaghaie *et al.*, 2015). Until the publication of this article, there has been no complete evaluation method and/or indicator system devised that can determine the biosafety of AgNPs, so great caution should be taken with their use (and indication). The main concern for future research on nanosilver is to figure out how to make AgNPs antibacterial while reducing cytotoxicity to a level acceptable for the human body.

The aim of this study was to investigate whether incorporating AgNPs into the surface coating of different dental-relevant alloys has an effect on the cytotoxicity and antibacterial properties of the alloy. We hoped to find an effective antibacterial concentration of AgNPs that also had a low biological toxicity.

2 Materials and methods

2.1 Sample preparation

Wax discs (Dental Materials Factory of Shanghai Medical Instruments Co., Ltd., Shanghai, China) patterns were prepared with dimensions of 10 mm×10 mm×2 mm (n=96). The lost-wax technique (taking a wax pattern of a dental restoration and

converting it into a dental casting alloy) was used in order to obtain six groups of dental castings (Cobalt-chrome: Wirobond C (WC), BEGO, Germany; Keragen (KN), Eisenbacher Dentalwaren ED GmbH, Germany; Ceramill Sintron (CS), Amanngirrbach, Germany; Solibond C plus (SCP), YETI Dentalprodukte GmbH, Germany; QLD-Co, Qianluda Medical Instrument Co., Ltd., China. Pure titanium: QLD-Ti, Qianluda Medical Instrument Co., Ltd., China). For standardization of surface roughness, abrasive SiC (Shanghai Grinding Wheels Plant, Shanghai, China) papers were gradually used (grits 100#, 200#, 400#, and 800#) under water refrigeration. The samples were ultrasonically washed using absolute ethyl alcohol for 15 min, allowed to air-dry for 30 min, then stored in sterile containers at room temperature.

2.2 AgNPs synthesis/incorporation method

AgNPs were prepared using hydrothermal methods according to the reaction: $AgNO_3+NaBH_4\rightarrow Ag+1/2H_2+1/2B_2H_6+NaNO_3$. Twenty milliliters of $NaBH_4$ (Aladdin Reagent, Shanghai, China) at a concentration of 0.004 mol/L was diluted to 500 ml. We added 0.004 mol/L of $AgNO_3$ (Aladdin Reagent, Shanghai, China) dropwise to $NaBH_4$ solution in three separate volumes (2.5, 1.0, and 0.5 ml), with magnetic stirring for 5 min, after which three different concentrations of solutions (with disperse AgNPs) were prepared. Twelve milliliters of each concentration of AgNPs solutions were separately added to a hydrothermal inner tank with the dental casting samples (groups=3×6). The hydrothermal inner tanks were placed into the hydrothermal reactor for 2 h at 130 °C and were annealing for 2 h at 450 °C. The three concentrations of AgNPs were then added to the six groups of dental castings. They were then ultrasonically washed using absolute ethyl alcohol for 15 min, and allowed to air-dry for 30 min. The surface morphology was observed using scanning electron microscopy (SEM) (S-4800, Hitachi, Japan). The elemental composition of the samples was analyzed by energy dispersive spectrometer (EDS) (Oxford Instruments, UK; attached to the field emission scanning electron microscopy (FESEM)).

2.3 Preparation of "leach liquors" from the dental alloy samples

Samples were first marked and ultrasonically washed using absolute ethyl alcohol for 15 min, sterile water-rinsed three times, allowed to air-dry for 30 min and autoclaved at 121 °C for 30 min. Each sample was immersed individually in 2 ml Dulbecco's modified Eagle's medium (DMEM, HyClone, LA, USA) containing fetal bovine serum (FBS, HyClone, LA, USA), and allowed to leach at 37 °C (with 5% CO_2 concentration), based on the ISO requirement (0.5–0.6 cm^2/ml), for 7 d prior to cytotoxicity analysis. Then, the leached liquors went through filtration by using 0.22-μm micro porous membranes and were stored in a refrigerator at 4 °C.

2.4 Cytotoxicity test

Osteogenic precursor cells (MC3T3-E1) from mice (Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, China) and mesenchymal stem cells (BMSC) from rats (Shanghai SLAC Laboratory Animal Co., Ltd., Shanghai, China) were cultured in a DMEM culture medium containing two antibiotics (100 U/ml each of penicillin and streptomycin) and 10% FBS. After the cells grew up to the third generation, or the cell state tended to be stable, the number was counted. Cells were transferred to a 96-hole board according to specific cell density to incubate in the cell incubator (MCO-17AC, SANYO, Japan) for 24 h. The supernatant was removed after the cells had been adherent for 24 h. For positive control, 10 μl of DMEM solution was added to the cell culture, while for negative control, culture solution was added instead of cells, and different leach liquors were used to culture cells separately for Groups 1 to 6. Twenty microliters of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) (5 g/L) (Sigma-Aldrich, St. Louis, MO, USA) was added to each hole, after the cells reacted with the leach liquor for 1, 3, and 5 d. The leach liquors were removed after incubating for 4 h, and 150 μl of DMSO was added to each hole and vibrated with the oscillator (Shanghai Precision Instrument Co., Ltd., Shanghai, China) for 10 min. The enzyme-linked immunoassay instrument (SpectraMax i3x, Molecular Devices, SF, USA) was used to determine the absorbance value (A) of 570 nm immediately after the purple crystal had completely dissolved. The relative growth rate (RGR) of cells was calculated according to the formula: $RGR=A_{exp}/A_{neg}\times 100\%$, where A_{exp} and A_{neg} are the absorbance values of experimental and negative control groups, respectively.

The degree of cytotoxicity of the materials was evaluated based on the RGR value according to the corresponding relationship between cell growth rate and material toxicity level (Table 1).

Table 1 Cell growth rate and material toxicity level standards

Level	Growth rate (%)
0	≥100
1	75–99
2	50–74
3	25–49
4	1–24
5	0

2.5 Antibacterial test

Samples of QLD-Ti and QLD-Co (at three AgNPs concentrations) were distilled, water-rinsed, autoclaved at 121 °C for 30 min, and dried on a laminar flow bench (Suzhou Purification Equipment Company, Suzhou, China). Samples were then immersed in the test tube containing 2 ml of brain heart infusion (BHI) culture medium (Becton Dickenson, Franklin Lakes, NJ, USA) in order to leach (under vibration) for 24 h. The leached liquors were collected into centrifugal tubes. A *Staphylococcus aureus* (SA) bacterium solution was uniformly coated over a panel containing BHI agar medium. After 5 min, the BHI agar medium was punched by a sterilized puncher and 150 μl of leach liquor was added to the hole. Penicillin was considered a positive control, and the panel was placed in the incubator for 24 h, in order to observe the presence (or not) of an inhibition zone.

Samples were placed in the test tube containing 2 ml of culture medium. Penicillin was considered a positive control. The same amount of bacterial solution was inoculated. Test tubes were placed on a shaking table (HS-111B, Shanghai Hasuc Instrument Manufacture Co., Ltd., Shanghai, China) for 3 h (for SA) or 24 h (for MS). The OD_{600} (the absorbance of a sample measured at a wavelength of 600 nm) of the bacterial solutions was measured by spectrophotometer (ND-2000, Thermo Scientific, MA, USA) in order to determine the antibacterial effect. Samples were taken from the bacteria solution, sterile water-rinsed and air-dried on a laminar flow bench before being immersed in the test tube containing 5 ml of culture medium, and placed on the shaking table for 24 h. The OD_{600} was evaluated to observe the number of bacteria adhered to the samples.

2.6 Statistical analysis

Data were expressed as mean±standard deviation (SD) from at least two independent experiments. One-way analysis of variance and Tukey's multiple-comparison test were used for statistical analysis at a pre-set alpha of 0.05.

3 Results

3.1 Distribution of AgNPs on the surface of samples

SEM images were observed to ensure that the nanoparticles had successfully adhered to the dental castings. As shown in Fig. 1, uniform-grain-size AgNPs were evenly distributed on the surface of dental castings. Spectrum analysis was conducted on a sample of Keragen coated with a low concentration of AgNPs to analyze the metal compositions. Within the detected area as shown in Fig. 1e, four elements (Cr, Co, Ag, and W) were confirmed. We further confirmed that the ions attached to the dental castings surfaces were silver, in a concentration of 1.06% (Table 2).

Table 2 Results of the spectrum analysis

Element	Weight (%)	Atom (%)
Cr	26.32	31.88
Co	58.69	62.73
Ag	1.06	0.62
W	13.94	4.78

3.2 Cytotoxicity test

The number of MC3T3-E1 cells, cultured with the negative control group (Fig. 2a) leach liquors of dental castings, decreased gradually with time. The cell growth rate was also negative, which indicated strong cytotoxicity of the dental castings. The cytotoxicity in the group with a concentration of 10 μmol/L (Fig. 2b) was reduced after AgNPs were incorporated, but still strong. The cytotoxicity in the groups at the concentrations of 4 μmol/L (Fig. 2c) and 2 μmol/L (Fig. 2d) was strong, and the cells were almost disappeared. No significant differences were

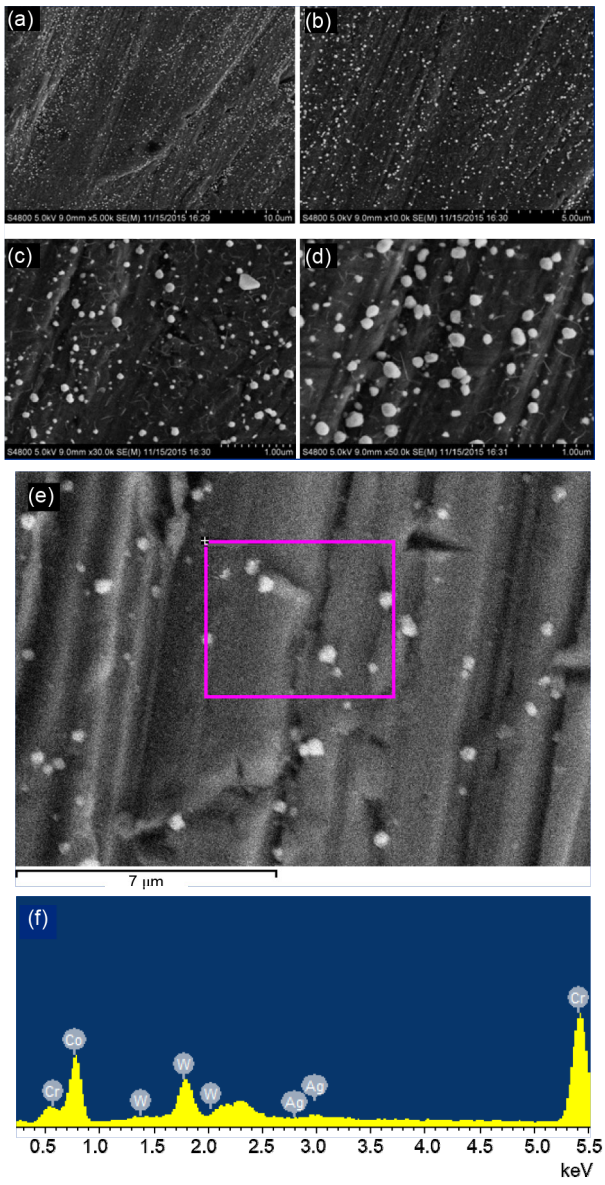

Fig. 1 Scanning electron microscopy (SEM) images and spectrum analysis of dental castings (KN) coated with a low concentration of silver nanoparticles (AgNPs)

Image (a) demonstrates a 5000× magnification of the sample surfaces. Images (b), (c), and (d) display details of the nanoparticles at 10000×, 30000×, and 50000×, respectively. Image (e) exhibits the detected area of spectrum analysis. Image (f) demonstrates the metal compositions

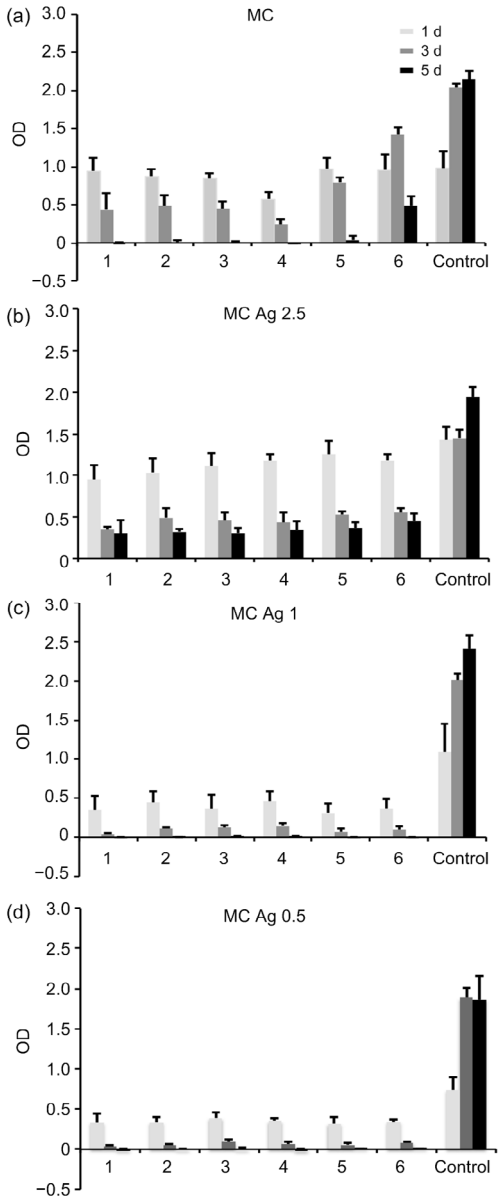

Fig. 2 Effects of silver nanoparticles at three concentrations on cytotoxicity of MC3T3-E1 cells

(a) The negative control group; (b) The AgNPs concentration of 10 μmol/L; (c) The AgNPs concentration of 4 μmol/L; (d) the AgNPs concentration of 2 μmol/L. 1, WC; 2, KN; 3, CS; 4, SCP; 5, QLD-Co; 6, QLD-Ti. Data are expressed as mean±SD (n=4)

observed between the five kinds of cobalt-chrome alloy in the experimental group and the control group, while significant differences were found between the Ti and cobalt-chrome alloys in the control group (P<0.05). Significant differences were observed between the group at the concentration of 10 μmol/L and the control group after the MC3T3-E1 cells had reacted with the leach liquor for 5 d.

Fig. 3a shows the number of BMSC cells cultured with the negative control group leach liquors. The number gradually decreased with time and the cell growth rate was negative, which indicates strong cytotoxicity of the dental castings. The cytotoxicity was reduced after the AgNPs incorporation but still high. No significant differences were found in the cytotoxicity among the three AgNPs concentrations (Figs. 3b–3d).

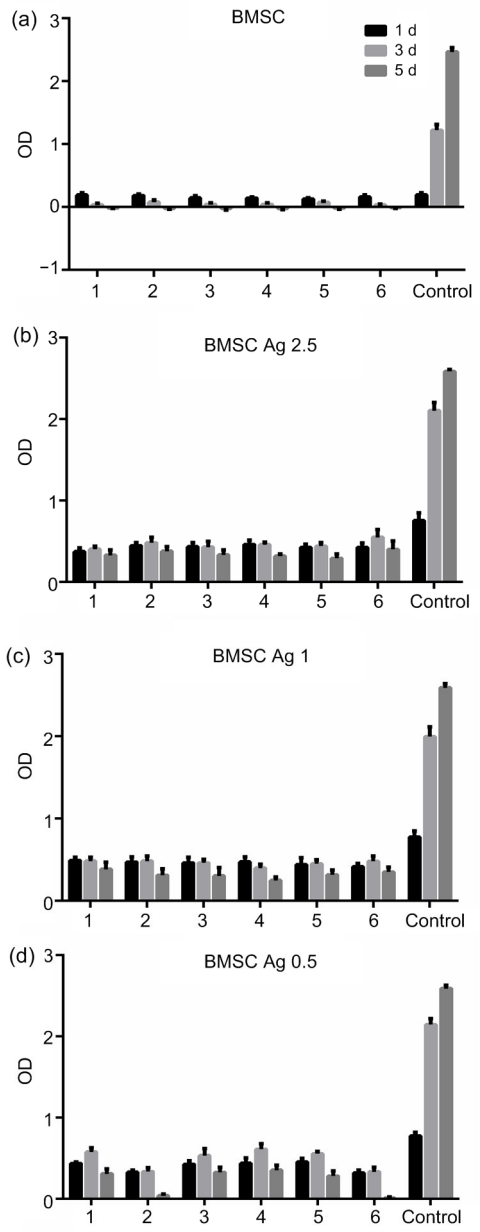

Fig. 3 Effects of three concentrations of silver nanoparticles (AgNPs) on the cytotoxicity of BMSC cells

(a) The negative control group; (b) The AgNPs concentration of 10 μmol/L; (c) The AgNPs concentration of 4 μmol/L; (d) The AgNPs concentration of 2 μmol/L. 1, WC; 2, KN; 3, CS; 4, SCP; 5, QLD-Co; 6, QLD-Ti. Data are expressed as mean±SD (n=4)

3.3 Antibacterial test

Fig. 4 demonstrates the inhibition zone of the three different concentrations of AgNPs. The leach liquors had no significant antibacterial effect when compared to the positive control group of penicillin (Fig. 4c-1).

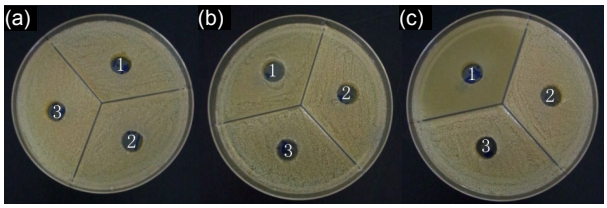

Fig. 4 SA inhibition zone of silver nanoparticles at three concentrations

(a) The inhibition zone of QLD-Ti: 1, AgNPs concentration of 10 μmol/L; 2, AgNPs concentration of 4 μmol/L; 3, AgNPs concentration of 2 μmol/L. (b) The inhibition zone of QLD-Co: 1, AgNPs concentration of 10 μmol/L; 2, AgNPs concentration of 4 μmol/L; 3, AgNPs concentration of 2 μmol/L. (c) Control group: 1, the positive control group of penicillin; 2, the negative control group of QLD-Ti; 3, the negative control group of QLD-Co

Fig. 5 demonstrates that the inhibition rate of AgNPs samples at different concentrations compared to SA and MS resulted in no significant increase when compared to the negative control group. The amount of bacteria attached to the surface of samples was not statistically reduced when compared to the negative control group.

4 Discussion

AgNPs have generated a great deal of interest in the field of research on dental materials. Fifty percent of patients who wear complete or partial dentures have problems with stomatitis (Budtz-Jorgensen, 1981). AgNPs were added to composite resins and the antibacterial performance of these resins would significantly increase at appropriate AgNPs concentrations (Fan *et al.*, 2011; Kasraei *et al.*, 2014; Ai *et al.*, 2017). Because of their antimicrobial properties, AgNPs have been added to poly(methyl methacrylate) (PMMA). The results of this study found that PMMA-AgNPs significantly reduced the adherence of *C. albicans*, while flexural strength of PMMA was decreased (Acosta-Torres *et al.*, 2012; Sodagar *et al.*, 2012). The colonization of the soft lining of denture material by oral fungi could result in infections and stomatitis, and therefore AgNPs were incorporated into composites as antimicrobial agents in order to reduce the microbial colonization of lining materials (Grzegorz *et al.*, 2011; Chladek *et al.*, 2013). Bacterial adhesion to the surface of implants may result in peri-implant diseases and cannot always be avoided

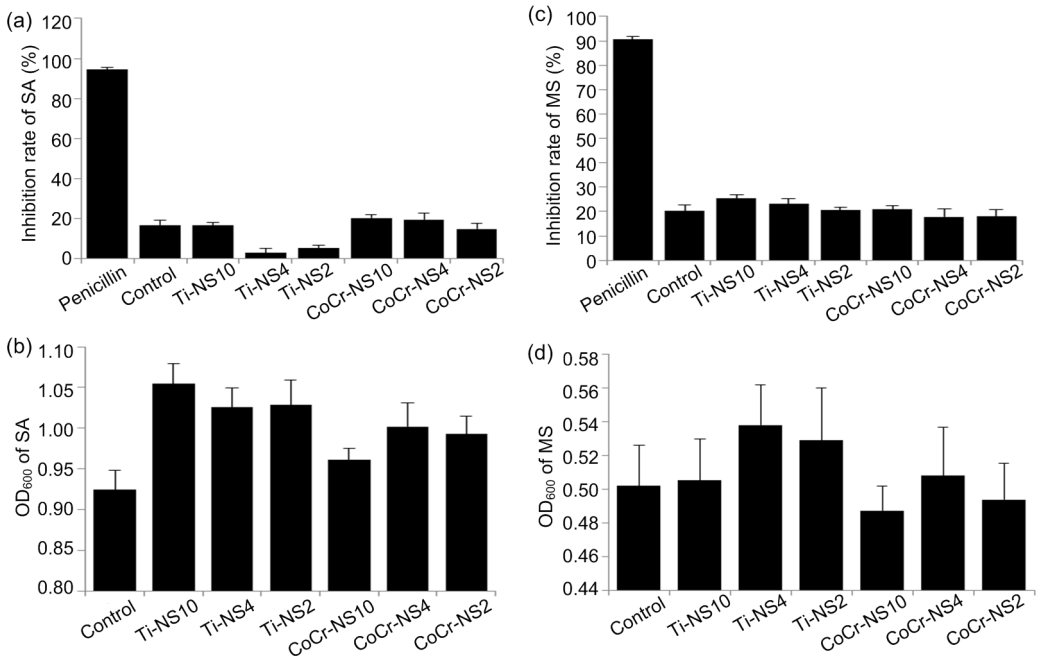

Fig. 5 Antibacterial effects of AgNPs samples at three concentrations compared to SA and MS

(a) Inhibition rate of SA; (b) OD_{600} of SA adhered to the samples; (c) Inhibition rate of MS; (d) OD_{600} of MS adhered to the samples. Penicillin: the positive control group; Control: the negative control group; Ti-NS10: QLD-Ti with 10 μmol/L of AgNPs; Ti-NS4: QLD-Ti with 4 μmol/L of AgNPs; Ti-NS2: QLD-Ti with 2 μmol/L of AgNPs; CoCr-NS10: QLD-Co with 10 μmol/L of AgNPs; CoCr-NS4: QLD-Co with 4 μmol/L of AgNPs; CoCr-NS2: QLD-Co with 2 μmol/L of AgNPs. Data are expressed as mean±SD (n=4)

by sterilization before implantation. AgNPs coating produced a strong antibacterial effect on the titanium surface by inhibiting the adhesion of bacteria (Massa *et al.*, 2014; Matsubara *et al.*, 2015; Mishra *et al.*, 2015; Qin *et al.*, 2015; Vogel *et al.*, 2015). In our study, AgNPs were incorporated to the surface of dental alloys to improve their antibacterial properties, in order to further reduce the incidence of denture stomatitis and peri-implantitis.

When AgNPs were reduced to sizes in the nanometer range (<100 nm), their biological activity could be changed and their antibacterial properties materialized. Due to their size and surface chemistry, AgNPs could be internalized by endosomal or lysosomal endocytosis (Asharani *et al.*, 2009a; Luther *et al.*, 2011) and then translocate to target organelles such as the nucleus and mitochondria, eliciting a series of biological effects including altered cell morphology, mitochondrial dysfunction, DNA damage, oxidative stress, inflammation, and genotoxicity, and result in cell death by apoptosis or necrosis (Asharani *et al.*, 2012; Yang *et al.*, 2012; Zhang *et al.*, 2014). Oxidative stress and the release of Ag^+ ions from AgNPs are two primary mechanisms that mediate AgNPs-associated cytotoxicity (Volker *et al.*, 2013). Intracellularly released Ag^+ ions interact with thiol groups of antioxidants such as glutathione, superoxide dismutase, and thioredoxin, leading to increased lipid peroxidation, DNA damage, oxidative stress, and subsequent apoptotic cell death (Arora *et al.*, 2008).

AgNPs toxicity may be attributed to either non-specific interactions, such as size and shape, or specific interactions with cells, such as surface coating, chemical composition and the release of Ag^+ ions (Riaz Ahmed *et al.*, 2017). The size of the AgNPs is a main factor in mediating various biological effects such as oxidative stress, DNA damage, cellular uptake, mitochondrial dysfunction, and permeabilization across biological barriers (Gliga *et al.*, 2014; Wang *et al.*, 2014). A study on the effects of three different AgNPs sizes (20, 80, and 110 nm) on RAW 264.7 macrophage viability demonstrated that 20 and 80 nm AgNPs inhibited cell metabolic activity at 7 and 38 μg/ml, respectively. However, no changes in viability were observed in the group of 110 nm AgNPs, which indicates that the smaller-sized AgNPs

were more cytotoxic (Park *et al.*, 2011). Another study, however, demonstrated that 100 nm AgNPs caused a higher level of cytotoxicity, while similarly at the same concentrations for both sizes (Hussain *et al.*, 2005). As a result, it was difficult to prove the correlation between cytotoxicity and the size of AgNPs. The shape of AgNPs can also influence the degree of particle toxicity and immunological effects in cells. The effects of silver wires, spherical AgNPs, and silver microparticles on lung epithelial A549 cells were investigated and it was found that silver nanowires had the highest levels of cytotoxicity, while silver nanospheres had minimal cellular effects (Stoehr *et al.*, 2011). As shown in the SEM images of Fig. 1, silver nanowires and spherical AgNPs (<100 nm) were observed, which met the requirements for the size and shape of nanosilver with antibacterial properties.

Studies conducted in vivo have demonstrated that different cell types display different sensitivities to AgNPs (Asharani *et al.*, 2009b). Differential sensitivity to AgNPs has also been discovered among cell lines of the same cell type (Singh and Ramarao, 2012). Fibroblasts are commonly used in toxicological studies as generalized representative cell types since they exist in every organ/tissue. NIH3T3 fibroblast cells (Hsin *et al.*, 2008), normal human lung fibroblasts (IMR-90) (Asharani *et al.*, 2009b), and L929 murine fibroblasts (Park *et al.*, 2011) are often used in in vitro studies. The results of these experiments were directly determined by the sensitivity of cell types, the more sensitive cell lines were more able to reflect the toxicity of materials (Hensten-Pettersen and Helgeland, 1981). A longer period of contact with these dental alloys in the oral environment could lead to higher cytotoxicity to oral fibroblast cells or osteoblasts. In this study, MC3T3-E1 and BMSC cells were selected to replace osteoblasts in order to evaluate the cytotoxicity of dental alloys (Tian *et al.*, 2016).

The results of this study, using the MTT method, determined that the cytotoxicity of dental alloys to MC3T3-E1 and BMSC cells was high, but this same cytotoxicity was reduced (to some extent) when AgNPs, at a certain concentration, were incorporated into the surface coating. The cytotoxicity of AgNPs has been confirmed by numerous in vitro studies (Park *et al.*, 2009; Li *et al.*, 2010; You *et al.*, 2011; Chen and Zhang, 2012), but cytotoxicity has rarely been reported in dental materials. It has been determined that chrome-cobalt alloy has a cytotoxic effect on fibroblast cells (Sabaliauskas *et al.*, 2011). The cytotoxic effects of cobalt-chromium, commercially pure titanium, and palladium-based alloys on L929 cells were evaluated by flow cytometry (FCM) and a real-time quantitative polymerase chain reaction (PCR) assay. The results determined that dental alloys might cause time-dependent early apoptosis via the intrinsic pathway (Pan *et al.*, 2017). It was reported that human cells in culture were less sensitive to coated-AgNPs than mouse cells irrespective of the type of coating used (de Lima *et al.*, 2012). Therefore, the cytotoxicity of dental alloys may be related to the culture cells.

Results from inhibition zone tests showed that AgNPs were ineffective in inhibiting SA and were significantly less effective than the positive control group (penicillin), which was consistent with other findings in the literature (Park *et al.*, 2010). There was no significant difference in the inhibitory rate of SA or MS between the three concentrations of AgNPs samples. The concentrations of AgNPs evaluated for this study had no significant inhibitory effect on SA or MS. The results of in vitro research have demonstrated that AgNPs, though cytotoxic to mammalian cell lines, exhibit no antimicrobial efficacy against bacterial cells such as *Escherichia coli* and *Bacillus subtilis* (Suresh *et al.*, 2010). Studies should be conducted in order to determine an effective preparation method which could lead to increased antibacterial properties and reduced cytotoxicity in AgNPs.

5 Conclusions

The dental alloys evaluated by this study demonstrated high cytotoxicity on MC3T3-E1 and BMSC cells. Cytotoxicity was reduced after AgNPs incorporation. No significant difference was observed for cytotoxicity or antibacterial properties among the three AgNPs concentrations. However, the mechanism, by which cytotoxicity was reduced after the incorporation of AgNPs, is still unknown, and studies should be conducted to determine this mechanism.

Compliance with ethics guidelines

Xiao-ting SHEN, Yan-zhen ZHANG, Fang XIAO, Jing ZHU, and Xiao-dong ZHENG declare that they have no conflict of interest.

This article does not contain any studies with human or animal subjects performed by any of the authors.

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中文概要

题　目：纳米银颗粒粘附对牙科合金细胞毒性和抗菌性的影响

目　的：评估纳米银颗粒的粘附对牙科合金的细胞毒性和抗菌性的影响，并初步探讨其作用机制。

创新点：运用 MTT 法证实钴铬合金和纯钛对小鼠成骨前体细胞（MC3T3-E1）及大鼠骨髓间充质干细胞（BMSC）产生细胞毒性，粘附纳米银颗粒后细胞毒性有所降低。

方　法：将化学法制得的 3 种浓度的纳米银颗粒分别粘附于 6 种牙科合金表面，扫描电镜观察并确认纳米银的粘附情况。采用 MTT 法检测不同浓度纳米银颗粒的牙科合金对 MC3T3-E1 及 BMSC 的细胞毒性。评价 3 种浓度纳米银颗粒的钴铬合金和纯钛试件浸提液对金黄色葡萄球菌和变形链球菌的抗菌性。

结　论：牙科合金对 MC3T3-E1 和 BMSC 细胞具有较强的毒性，粘附纳米银颗粒后细胞毒性有所降低。3 种浓度的纳米银颗粒细胞毒性之间无显著性差异，且这 3 种浓度纳米银颗粒粘附后对牙科合金的抗菌性无明显影响。

关键词：纳米银颗粒；牙科合金；细胞毒性；抗菌性能；MC3T3-E1细胞；BMSC细胞