Characterization and evaluation of a novel silver nanoparticles-loaded polymethyl methacrylate denture base: In vitro and in vivo animal study

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The aim of this study was to optimize the preparation method of polymethyl methacrylate (PMMA) denture base loaded with nano silver (NAg), to more effectively and safely impart sustainable antibacterial functions. NAg solution was synthesized and mixed with acrylic acid and methyl methacrylate (MMA) monomer in order to prepare a new type of NAg solution (NS)/polymer methyl methacrylate denture base specimens (NS/PMMA). The surface morphology, mechanical strength, antimicrobial activity, anti-aging performance, cytotoxicity and biocompatibility of NS/PMMA denture base were evaluated in comparison with specimens fabricated using traditional NAg adding methods and NAg-free denture base. The aesthetic characteristics and mechanical strength of NS/PMMA denture base met the clinical application requirements. Meanwhile, NS/PMMA denture base showed better antibacterial activity, anti-aging properties, no cytotoxicity and displayed exceptional biocompatibility. NS/PMMA denture base thus has great potential for clinical application.

Keywords: Denture base, Nano silver, Antibacterial properties, Polymethyl methacrylate

INTRODUCTION

Polymethyl methacrylate (PMMA) polymer is commonly used for denture base fabrication due to its easy handling, excellent performance, and reasonable cost. Most of these materials are susceptible to fungal and bacterial adhesion1), which may cause denture stomatitis2,3). Mechanical-based cleaning systems and chemical immersion methodologies can effectively remove bacteria and fungus adhering to the surface of the denture base4-7). However, these methods can also induce irreversible damage to denture base surfaces.

Alternatively, customized coatings and/or chemical modifications of surfaces or near-surface layers of the denture base have been tested8). However, imparted functions into the surfaces and surface layers were easily lost due to physical, chemical, or mechanical related influences and damages9). For such reasons, researchers recently have begun to consider new ways to manually induce increased functionality and long-term antimicrobial activity in the materials by increasingly uniformly mixing antibacterial agents and denture base materials10-12). In such approaches, even if aspects of functionalized surfaces are damaged, new surfaces with the desired and effective types of antibacterial properties will appear subsequently and establish in their place13-15). Furthermore, the addition of antibiotics to PMMA has been examined, and was found to provide additional antimicrobial properties, however the mechanical properties were negatively affected over time. To overcome these problems, some studies have proposed that silver-based materials including oxides16), ions17), and commonly used nano silver (NAg)18) can be used effectively as antimicrobial agents19,20). Previous studies have concluded that silver-loaded PMMA denture bases significantly reduced bacterial growth and prevented the formation of biofilms compared to non-silver loaded PMMA denture bases16-21).

NAg is a leading type of antimicrobial nanomaterial presently in use, is readily available, and has exhibited a broad-spectrum, a high-efficiency, high antimicrobial activity, and relatively high biocompatibility22). Compared to other nanoparticle types, NAg have been deemed is more sensitive to oxygen levels and their catalytic action facilitates their role in the conversion of oxygen in to active oxygen. The resultant active oxygen subsequently can induce structural damage of microorganisms, which is a process specific to silver and is known as “oligodynamic action”23-25). Because of such properties, silver has become increasingly and widely used in antimicrobial gels26), dressings for burns27), and cosmetics with characteristically antiseptic properties28).
Previously NAg-reinforced denture bases were prepared by blending NAg powders (NP) with PMMA powder using mechanical ball-based milling to form NAg powders/PMMA denture base (NP/PMMA)\(^9\). Although the resulting denture bases had some antimicrobial properties, the distributions of NAg particles in the respective denture bases was uneven\(^30\), and the denture bases color was poor\(^31-34\). In addition, increased relative proportions of NAg in the mixture adversely affected mechanical properties of the denture base\(^30\).

Acrylic acid is the simplest type of unsaturated carboxylic acids, and consists of a vinyl group connected directly to a carboxylic acid terminus. Acrylic acid is miscible with water, alcohols, ethers, and chloroform. With water in any proportion, acrylic acid can be miscible by emulsion polymerization induced reactions with MMA liquid\(^36,37\).

Therefore, the aim of our study was to use acrylic acid, which is an important raw material for synthetic resin monomers\(^8\), as an amphiphilic intermediate medium to facilitate equal distributions of introduced NAg solutions into denture bases for preparing a new type of NAg solution (NS)/polymer Methyl methacrylate denture base (NS/PMMA) and comparing it with the traditional denture base and with the denture base fabricated by adding NAg powder with the monomer powder in terms of surface morphology, mechanical strength, antimicrobial activity, anti-aging performance, cytotoxicity and biocompatibility.

We hypothesize that using acrylic acid as intermediate medium will facilitate increasingly uniform dispersal of NAg particles into denture bases, thereby increasing NAg particle surface areas such that antibacterial effects would be expected to be greater versus NP/PMMA denture bases lacking acylic acid as a medium.

**MATERIALS AND METHODS**

**Specimens**

Three groups were designed for this study as follows: (1): the PMMA group (NAg-free); (2) the NP/PMMA group (NAg powders (NP) mixed with PMMA powder then with MMA liquid); and (3) the NS/PMMA group (NAg solution was mixed with the acrylic acid and then with MMA liquid and PMMA powder). Fifty-four disk-shaped specimens (10 mm in diameter and 2 mm thick) and twelve rectangular specimens (length×width×thickness: 20×10×2 mm) were fabricated using teflon molds, and then mixed with MMA liquid at a powder to liquid ratio of 3:1 (w:v) to prepare the test specimens. The aesthetics of the three denture base test specimens were observed and photos were taken.

**Mechanical properties**

Twelve rectangular samples (length×width×thickness: 20×10×2 mm) were fabricated using teflon molds, 6 specimens from the NS/PMMA group, and the other 6 specimens were constructed from NP/PMMA group, and subsequently examined for the dispersion degree of NAg and the smoothness of the surface with the scanning electron microscopy (SEM; S4800, Hitachi). The mechanics of the three denture base test specimens were observed and photos were taken.

**Materials and methods**

**Materials**

| Component | Quantity |
|-----------|----------|
| PMMA      | 2.5 g    |
| Solid silver nitrate | 0.5 g |
| Deionized water | 50 mL |
| Isopropanol | 4 mL |
| Glucose powder | 4 g |

**Methods**

1. For preparing the NP/PMMA group the NAg solution prepared as mentioned before was centrifuged at 3,000 rpm for 20 min. The supernatant was gently discarded leaving the NAg precipitate. Isopropanol (10 mL) was added dropwise, the precipitate was resuspended, and the solution was then centrifuged at 3,000 rpm for 15 min. This was repeated three times to remove excess water. Finally, the precipitate was collected in a glass dish and air-dried to obtain NAg particles. The NAg particles and PMMA powder were uniformly mixed at mass ratio 1:20 (w:w) by ball milling, and then mixed with MMA liquid at a powder to liquid ratio of 3:1 (w:v) to prepare the denture base specimens.

**Aesthetic of denture base**

The mixed NAg/acrylic acid/MMA liquid was gently dropped onto a copper mesh and dried overnight. By transmission electron microscopy (TEM; H-7650, Hitachi, Tokyo, Japan), the size and dispersion degree of the NAg in the solution was examined. Twelve disk specimens (10×2 mm) were prepared using teflon molds, 6 specimens from the NS/PMMA group, and the other 6 specimens were constructed from NP/PMMA group and subsequently examined for the dispersion degree of NAg and the smoothness of the surface with the scanning electron microscopy (SEM; S4800, Hitachi). The aesthetics of the three denture base test specimens were observed and photos were taken.

**Elastic modulus**

Twelve rectangular samples (length×width×thickness: 20×10×2 mm) were fabricated for measuring the elastic modulus (E) and bending strength (σ). They were determined in accordance with ISO 178 (Plastics—Determination of flexural properties) through a three-point bending tests by a universal testing machine (Instron 5967, Norwood, MA, USA) at a crosshead speed of 2 mm/min. E and σ were calculated according to the following equations:

\[
E (\text{MPa}) = \frac{Pl^3}{4bd^3} \quad (1)
\]

\[
\sigma (\text{MPa}) = \frac{3Pl}{2bd^2} \quad (2)
\]
where, $P$ is the maximum load, $P_1$ is the load at a selected point of the elastic region of the stress–strain plot, $l$ is the distance between supports, $b$ is the specimen width, $d$ is the specimen thickness, and $f$ is the deflection of the specimen at $P$.

The impact strength ($a_n$) was determined in accordance with DIN 53435 (Testing of plastics. Bending test and impact test on Dynstat test pieces) using VEB Werkstoffprüfmaschinen Dynstat apparatus on rectangular unnotched specimens (length×width×thickness: 20×10×2 mm). The following formula was applied to calculate $a_n$:

$$a_n \left( \frac{kj}{m^2} \right) = \frac{A_n}{bd}$$

where, $A_n$ is the impact energy required to cause a material to fracture, and $b$ and $d$ are the width and thickness of the specimen, respectively.

**MTT cytotoxicity assay**

The 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H tetrazolium bromide assay (MTT; Sigma-Aldrich, St. Louis, MO, USA) was used to evaluate the cytotoxicity of denture base specimens. The following groups were evaluated: buffered Dulbecco's modified Eagle's essential medium (H-DMEM; Gibco Life Technologies, Carlsbad, CA, USA), PMMA, NP/PMMA, and NS/PMMA groups. A 20 mL of N-2-Hydroxyethylpiperazine-N-2-ethane sulfonic acid (HEPES) -H-DMEM were supplemented with 10% heat-inactivated fetal bovine serum (FBS; Gibco Life Technologies), and added to each group at a ratio of test specimens to medium of 1:3 (v:v). Test specimens should be completely immersed in the medium and allowed to extract at 37°C for 72 h. The extract was collected for all the groups and stored at 4°C refrigerator for future use. L929 cells (preserved by the National Key Laboratory of Stem Cells of the Academy of Military Medical Sciences, Beijing, China) were used for this study to detect the cytotoxicity by MTT method. L929 cells were uniformly inoculated into a 96-well plate at a density of $1\times10^4$ cells/well, divided into four groups, and the denture base extracts of PMMA, NP/PMMA and NS/PMMA were added respectively, and H-DMEM as the control group and cultured overnight at 37°C in an atmosphere containing 5% CO2. The cells were first inoculated for 24 h, which was counted as the 0th day. From the first experimental day, cells from each group were selected at a fixed time every day. Twenty microliters of 5 mg/mL MTT solution was added to each test well, and the mixture was shaken and incubated for 4 h. The supernatant was discarded, then, 100 µL of Dimethylsulfoxide (DMSO; Sinopharm Chemical Reagent) was added to each test well, and the mixture was shaken for 30 s. The absorbance value of the wells was measured at 492 nm using a microplate reader. Measurement was continued for 7 days.

**Intradermal injection**

Twenty male BALB/c mice (18–21 g) and three New Zealand Big Eared White rabbits (2.5–3.0 kg) were purchased from SPF (Beijing) Biotechnology. The animals were maintained at room temperature in a 12 h light/dark cycle with free access to water and chow in the SPF environment. All the animal protocols in this study were conducted with the approval of Animal Study Committee of Chinese PLA General Hospital. The experimental process followed “Consensus Author Guidelines on Animal Ethics and Welfare” produced by the International Association for Veterinary Editors (IAVE). The experimental animals underwent all operations under sodium pentobarbital anesthesia, and every effort was made to minimize their pain, suffering and death.

The biocompatibility of the denture base was evaluated by an intradermal injection experiment using four groups: blank, PMMA, NP/PMMA, and NS/PMMA according to the international standard for the preparation of leaching injections. Eight sterile glass bottles were labeled from 1 to 8 in sequence. Five milliliters of sterile physiological saline were added to the glass bottles from 1 to 4 as a polar extraction medium. Another 5 mL of sterile vegetable oil were added to glass bottles 5 to 8 as a non-polar extraction medium. According to the extraction ratio of 3 cm²/mL, 3 test specimens from each group were put into glass bottles. Glass bottles No. 1 and No. 8 were considered as the control groups, where no test specimens were added. Glass bottles No.2 and No.7 contained specimens from the PMMA group and were considered as the negative control group. Glass bottles No.3 and No.6 contained samples from the control NP/PMMA group. Finally, specimens from the experimental NS/PMMA group were placed in glass bottles No.4 and No.5. The test piece specimens were completely immersed in the extraction solution at 37°C for 72 h, the extraction solution was temporarily stored at 4°C for future use.

Three healthy New Zealand Big Eared White rabbits were used. After anesthesia was given through the ear vein, the back was divided into left and right sides. Four points were selected and injected on each side. The space between each point was >2 cm. The first to fourth injections were of polar medium extracts from the blank, PMMA, NP/PMMA and NS/PMMA groups, respectively. The fifth to eighth injections were of non-polar medium extracts from the blank, PMMA, NP/PMMA, and NS/PMMA groups, respectively. After the injection, inflammatory sensitization reactions such as erythema, edema and ulcer were observed and recorded at 0, 24, 48 and 72 h.

**Subcutaneous transplantation**

The biocompatibility of the denture base was further evaluated by subcutaneous transplantation experiments for the three groups: PMMA, NP/PMMA, and NS/PMMA. Twenty male BALB/c mice were randomly divided into two groups. After abdominal anesthesia and longitudinal incision along the center of the back, three subcutaneous sacs were prepared around the incision by blunt dissection, and the three sacs of skin were connected.
into an equilateral triangle. Test specimens were placed in the subcutaneous sacs, and the back incisions were sutured and bandaged. The mental state and body weight of the mice were closely observed after operation. On the 7th and 14th days after surgery, respectively, 10 mice were sacrificed and the specimens were exposed. First, the tissue surrounding the test specimens was observed by the naked eye, and photographed. The specimens and their surrounding tissues were then cut out, fixed with 4% paraformaldehyde, dehydrated, embedded in paraffin, stained with hematoxylin and eosin (HE), and observed under a light microscope.

**Antimicrobial activity**

The antimicrobial activity of each group of specimens was detected by Bauer-Kirby agar disk diffusion method. This experiment was carried out for PMMA, NP/PMMA, and NS/PMMA groups. Six test specimens of each group were quickly placed in the center of the medium coated with 50 µL of activated *Escherichia coli* (ATCC25922), *Candida albicans* (ATCC90028), *Streptococcus mutans* (ATCC25715) (1×10⁵ colony-forming units/mL) (preserved by the National Key Laboratory of Stem Cells of the Academy of Military Medical Sciences, Beijing, China). One test piece was placed in each dish culture medium, and at least 3 parallel tests were set for each group. The culture dish was placed in an incubator at 37°C, and, after standing for 2 h, the medium was gently inverted and culture was continued for 18 h. The diameter of the inhibition zone was measured with a ruler and recorded in mm and photographed. PMMA did not inhibit the growth of microorganisms (tested piece diameter=10 mm). The clear halos with a diameter larger than 10 mm were considered positive results. Tests were performed in hexaplicate, and values are presented as the average value±standard deviation.

**Artificial material aging test**

The test was carried out for PMMA, NP/PMMA, and NS/PMMA groups. Each group of specimens was immersed in 10 mL of artificial saliva, and heated in a water bath at 57°C to accelerate the aging of the denture base for 7 and 14 days. The Bauer-Kirby agar disk diffusion method was used to determine the durability of the antimicrobial properties of the test specimens from each group.

**Statistical analysis**

Mechanical testing data, antimicrobial testing data and accelerated aging testing data were analyzed using SPSS 17.0 statistical software (SPSS software, Munich, Germany). One-way analysis of variance was used for comparison between groups. The SNK test was used for comparison between two groups. The test level was α=0.05. When p<0.05, a difference was considered statistically significant.

**RESULTS**

**Aesthetics of denture base test specimens**

When aqueous NAg solution was directly added to the MMA liquid, the solution was incompatible (Fig. 2A). However, when aqueous NAg solution mixed with acrylic acid was added to the MMA liquid, it was found that the two were compatible and they formed a uniform solution (Fig. 2B). The NAg solution-acrylic acid-MMA mixed solution observed by TEM showed that the NAg particles had a uniform diameter of 40–60 nm and uniformly dispersed in the solution (Fig. 2D).

Visual observation showed that PMMA test specimens were light pink with uniform color distribution and smooth surface, which were almost the same as the color of the oral mucosa (Fig. 2E1). Denture base test specimens of the NS/PMMA group were orange-yellow with uniform color distribution similar to that of the oral mucosa, which could meet the requirements of clinical applications (Fig. 2E3). The denture base test specimens of the NP/PMMA group were tan, the surface color distribution was uneven and does not meet the aesthetic requirements (Fig. 2E2). SEM observations of the NS/PMMA group (Fig. 2F) and the NP/PMMA group (Fig. 2C) test specimens showed that the surface of the
NS/PMMA group piece was smoother with no obvious agglomerated NAg particles.

**Mechanical properties**

Compared with the PMMA group material, the bending strength of NP/PMMA and NS/PMMA group material decreased significantly \((p<0.05)\), but they still conformed to the requirements of ISO Specification 20795-1: Dentistry-Base polymers-Part 1: Denture base polymers \((>65\text{ MPa})\). The impact strength and elastic modulus were similar to those in the control (PMMA) group (Table 1).

**Cytotoxicity by the MTT assay**

The proliferation curve of L929 cells in the H-DMEM group was “S” type: they were in the stationary phase on the 1st to 2nd day; from the 3rd to the 6th day, the cells entered the logarithmic growth phase; on the 7th day, cell proliferation entered the plateau phase. The proliferation in each test group was essentially the same as that in the H-DMEM group; there was no significant difference in the proliferation rate at any time point (Fig. 3). These data indicated that the extract from each group had no cytotoxicity toward L929 cells.

**Intradermal injection**

The injection area was observed immediately after injection, and after 24, 48, and 72 h. No sensitizations such as erythema, edema, ulcer or induration were observed (Fig. 4), indicating that the extract from each group had no primary stimulation effect in rabbits.

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### Table 1  Mechanical properties of each group of denture base test pieces \((n=6, \bar{x}\pm s)\)

| Group      | Flexural strength (MPa) | Impact strength (kJ/m²) | Modulus of elasticity (GPa) |
|------------|-------------------------|-------------------------|-----------------------------|
| PMMA       | 88.1±0.49               | 6.33±0.28               | 2.31±0.16                   |
| NS/PMMA    | 83.3±0.24*              | 6.47±0.36               | 2.19±0.32                   |
| NP/PMMA    | 82.2±0.25**             | 6.35±0.68               | 2.12±0.45                   |

* Indicates a statistically significant difference \((p<0.05)\) between the NS/PMMA group and the PMMA group.
** Indicates a statistically significant difference \((p<0.05)\) between the NP/PMMA group and the PMMA group.

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**Fig. 4** Intradermal injection experiments in rabbits.
1–4 are injection sites of polar medium and 5–8 are injection sites of non-polar medium.
1, Blank group, injected with sterile physiological saline; 8, blank group, injected with sterile vegetable oil; 2 and 7, PMMA group, injected with extract of PMMA-group denture base test piece; 3 and 6, NP/PMMA group, injected with extract of NP/PMMA-group denture base test piece; 4 and 5, NS/PMMA group, injected with extract of NS/PMMA-group denture base test piece.
**Subcutaneous transplantation**

During the intradermal transplantation experiment, no systemic poisoning reaction occurred in any mouse. The naked eye examination indicated that the back of the mouse healed completely 7 days after transplantation. The tissues around the implanted specimens were normal. HE stained sections were observed under a microscope; the skin structure around the specimens was normal, the muscle layers were intact, and there was no infiltration of inflammatory cells or cellular necrosis (Fig. 5).

**Antimicrobial tests**

Results obtained by measuring the diameters of growth inhibition zones of the denture base tested pieces are presented in Table 2. Based on the size of inhibition zones around test materials, the PMMA group had no antimicrobial ability toward *E. coli*, *C. albicans* or *S. mutans*. The NP/PMMA group denture base and NS/PMMA group denture base did show antimicrobial effects, and the antimicrobial effect of the latter was greater. Based upon the resistance to three different microbial species, the NP/PMMA and NS/PMMA denture bases performed differently. NP/PMMA and NS/PMMA denture bases against *E. coli* and *C. albicans* were similar, but the antimicrobial effect against *S. mutans* was greater (Fig. 6).

**Accelerated aging**

Results obtained by measuring the diameters of growth inhibition zones for each test piece after 7 days and 14 days of accelerated aging of the test material are presented in Table 3. Observing the size of the inhibition zones, the NP/PMMA and NS/PMMA groups showed better resistance to accelerated aging than the PMMA group (Fig. 7).

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**Table 2**  
Inhibition zone diameters on denture base tested pieces (*n*=6, x±s)

| Samples   | Diameter of the inhibition zone (mm) |
|-----------|--------------------------------------|
|           | PMMA | NP/PMMA | NS/PMMA               |
| *E. coli* | 10    | 14.88±0.26 | 20.28±0.36            |
| *C. albicans* | 10    | 15.08±0.26 | 20.47±0.44            |
| *S. mutans* | 10    | 24.26±0.54 | 25.4±0.26             |

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**Fig. 5**  
Hematoxylin and eosin staining of mouse tissues 7 and 14 days after subcutaneous transplantation of test materials.

**Fig. 6**  
Inhibition zones of microbes with different denture base test specimens. A, B, and C are tests against *E. coli* of the PMMA, NP/PMMA and NS/PMMA group denture base, respectively; D, E, F are tests against *C. albicans*; G, H, and I are tests against *S. mutans*.
zones, it was found that the PMMA group, as expected, had no antimicrobial ability. The NP/PMMA group showed a significant decrease in antimicrobial ability compared with unaged NP/PMMA group \((p<0.05)\). Compared with the aged NP/PMMA group denture base, the antimicrobial effect of the NS/PMMA group denture base toward the three strains were still obvious after accelerated aging (Fig. 7).

After 14 days of accelerated aging, the antimicrobial effect of the NP/PMMA group denture base essentially disappeared. The antimicrobial effect of the NS/PMMA group denture base toward the three strains also decreased, but there were still clear inhibition zones (Fig. 7).

**DISCUSSION**

Variety of methods have been proposed for the antimicrobial activity of denture surfaces, including the use of mechanical-based cleaning systems, chemical immersion methodologies and, recently, customized coatings and/or chemical modifications of surfaces or near-surface layers. However, studies have shown some disadvantages regarding the use of these methods\(^4\text{-}^9\).

The results of this study present a low-cost, easy-access procedure with high potential to become an antimicrobial protocol for removable partial or total dentures, which can be used for the aging patient population. Furthermore, there are no reports in the literature for this method. The null hypothesis of this study was partially accepted.

NAg has a broad-spectrum, high-efficiency antimicrobial ability, high biocompatibility, no drug resistance and “oligodynamic action”. The antimicrobial activity is maximal when the NAg particle size is around 50 nm\(^4\text{1}\). The greater the contact area between silver ions and bacteria is, the greater the antimicrobial effect can be achieved\(^4\text{2}\). In previous studies, the common method for loading NAg into the denture base was by drying the NAg solution in order to make NAg powder and mixing it with the PMMA powder, by using the mechanical ball milling\(^2\text{9}\). There are several major issues in this production process. First, in the process of making NAg powder, the NAg changed from a state of water solvent that was originally dispersed and dissolved to a relatively aggregated solid state, which reduced the degree of dispersion of the NAg itself. In addition, the ball milling method is purely mechanical mixing, which cannot achieve a more uniform mixing state of the two powders in space\(^2\text{9}\). This has caused the NAg particles to aggregate into the denture base and disperse unevenly, resulting in a significant decrease in the antibacterial efficacy of the modified base material. Therefore, the agglomeration of NAg has become a major problem in the modification of the base. Second, to ensure the good antibacterial effect of the modified base, a sufficient amount of NAg powder is added which is a black powder. After mixing it with the powder of base resin, the color of the resin itself is changed from pink that is very similar...
to oral mucosa to brown or dark gray, and the aesthetic properties of the denture base is lost. Furthermore, the NAg powder can pollute and harm the environment and users through powder scattering during the process of making and adding\textsuperscript{46}. Acrylic acid is an important raw material for synthetic resin monomers\textsuperscript{44}. It is a colorless, clear liquid at room temperature and miscible with water at any ratio. Acrylic acid is miscible with water at any proportion, and can be miscible with MMA liquid by way of emulsion polymerization reactions\textsuperscript{47,48}. In this study, acryllic acid was first mixed with the NAg solvent and then the NAg suspended in acrylic acid was added to the denture base, which did not only increase the dispersion of the NAg in the denture base but also reduced the generation of bubbles, ensuring appropriate mechanical properties of the resulting denture base.

The relevant mechanical properties of the material are mainly deformation and fracture characteristics under external force. The denture base prepared in this work showed a decrease in bending strength and elastic modulus compared with PMMA group denture base, which may be related to the addition of NAg. This result is consistent with the conclusions of other studies\textsuperscript{45,46}. However, although the physical properties of the denture base declined, they still met clinical requirements for denture base. Biocompatibility of the NS/PMMA denture base test specimens was confirmed at the cellular level by MTT assays and in vivo in experiments in rabbits and mice.

The NP/PMMA group specimens contained 5% NAg powder, which is the minimum inhibitory concentration (MIC)\textsuperscript{65}. The NS/PMMA group was diluted by acrylic acid, and the final NAg concentration was much less than 5%. However, because acrylic acid was used as the intermediate medium, the NAg can be better dispersed into the denture base, increasing the NAg surface area, so the antibacterial effect of NS/PMMA was significantly greater than that of NP/PMMA. In clinical practice, a long-lasting antimicrobial effect is required. Here, after accelerated aging experiments, the NS/PMMA test material retained higher antimicrobial activity, indicating that the antimicrobial effect of the material can be maintained for at least one year at 37°C. This can largely meet the requirements for the denture base in clinical application. Despite the favorable results, there are some limitations to the study, such as not including a positive control group, use of single cultures and without clinical studies.

CONCLUSIONS

The NS/PMMA denture base has high-efficiency, long-lasting antimicrobial activity; it can inhibit the proliferation of common oral microbiota such as \textit{C. albicans}, \textit{E. coli} and \textit{S. mutans}. Compared with denture base made by adding NAg powder, the NS/PMMA denture base had better aesthetic performance and antimicrobial properties. The material had high biocompatibility with no evident cytotoxicity, and its mechanical properties meet clinical requirements. NS/PMMA denture base thus has great potential for clinical application.

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