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In Vitro Antibacterial Effect of Nano-Silver Loaded Zirconium Phosphate-Modified PMMA Resin against *Streptococcus Salivarius* and *Lactobacillus Salivarius*

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Abstract: *Polymethyl methacrylate (PMMA) denture base resin lacks antibacterial properties, which can easily lead to microbial adhesion and oral microecological imbalance. In this study, nano-silver loaded zirconium phosphate was incorporated into PMMA at mass fractions ranging from 0% to 7% to evaluate the in vitro antibacterial efficacy, mechanical properties, silver ion release, and cytocompatibility of the modified material against Streptococcus salivarius and Lactobacillus salivarius. The 3% incorporation group showed the best overall mechanical properties with a cell proliferation rate > 80%, and silver ion release was dose- and time-dependent. The antibacterial rate increased with increasing proportion. At mass fractions of ≥ 1%, significant inhibition was observed against both bacterial species ($P<0.05$); the 7% group achieved antibacterial rates of 91% against S. salivarius and 88% against L. salivarius, while the 0.7% group showed no significant effect. In summary, the 3% mass fraction of nano-silver loaded zirconium phosphate provides reliable antibacterial activity while maintaining favorable mechanical properties and biosafety, and is therefore recommended as the optimal formulation for denture base resin modification.*

Keywords: *Nano-silver loaded zirconium phosphate, Surface modification, Bacterial adhesion, Oral microecology*

1. Introduction

Currently, due to numerous regional factors, the proportion of patients with missing teeth is relatively high. Removable partial dentures, complete dentures, and interim dentures remain among the preferred treatment options for dentition defects and edentulous arches [1]. Polymethyl methacrylate (PMMA) is the core material for denture bases and is widely used in removable partial dentures, complete dentures, interim dentures, occlusal rehabilitation, occlusal pads, retainers, orthodontic appliances, prefabricated resin teeth, provisional crowns and bridges, etc. [2–5].

The placement of PMMA resin-based dentures into the oral cavity can affect the pre-existing oral microecology. On one hand, the denture interferes with the self-cleaning action of the tongue and soft tissues on the teeth, and the reduced flushing effect facilitates biofilm formation and adhesion. On the other hand, microorganisms such as bacteria and fungi readily adhere to the porous and rough surface of PMMA, leading to massive colonization by dominant pathogenic bacteria, which significantly increases the incidence of caries in remaining teeth, periodontal disease, and denture stomatitis on the mucosa of denture-bearing areas [6,7].

With the development of dental materials science and nanotechnology, modified PMMA resins incorporating nano-antimicrobial agents have become a research hotspot. In recent years, a systematic review by Lima et al. [8] pointed out that incorporating inorganic antimicrobial substances into heat-cured PMMA denture base materials can endow the material with antibacterial properties, among which silver-based nanoparticles are one of the most commonly used antimicrobial agents. Silver nanoparticles (AgNPs) are a novel type of inorganic antimicrobial agent, characterized by small volume, large specific surface area, sustained and effective bactericidal activity, low propensity for inducing drug resistance,

ISSN 2706-6819 Vol.8, Issue 3: 7-20, DOI: 10.25236/IJFM.2026.080302

and good biosafety [9–11]. Chen et al. [12] demonstrated that incorporating nano-silver loaded zirconium phosphate (Ag-ZrP) into room-temperature-cured PMMA materials achieved optimal mechanical properties at addition levels of 1.0%–1.5%, with good biosafety and no significant cytotoxicity below 3.0 wt%. Our preliminary studies found that the addition of nano-silver loaded zirconium phosphate to PMMA conferred good antibacterial effects against six dominant oral pathogens, including Streptococcus mutans, Actinomyces viscosus, Streptococcus sanguinis, Actinomyces naeslundii, Porphyromonas gingivalis, and Candida albicans, with the antibacterial rate increasing with the addition ratio [13].

The oral cavity harbors a wide variety of microbial species, which maintain a dynamic balance through symbiosis and antagonism under healthy conditions [14,15]. After denture placement, the microbiota composition changes, with an increase in opportunistic pathogens and a greater tendency for plaque biofilm formation, which affects patients' oral health and the long-term outcomes of prosthodontic rehabilitation [16]. Currently, there are relatively few studies on the effects of novel antimicrobial PMMA restorative materials on non-dominant oral pathogens. Streptococcus salivarius is the most numerous bacterial group in the oral mucosal microbiota, accounting for approximately 60% of the total cultivable bacteria, and can produce bacteriocin-like inhibitory substances, which are important for maintaining oral health. Reichardt et al. [17] demonstrated that bacteriocins produced by Streptococcus salivarius K12 and M18 significantly inhibit the growth and biofilm formation of Streptococcus mutans. Lactobacillus salivarius (currently reclassified as Ligilactobacillus salivarius) is an important probiotic in the oral cavity and exerts inhibitory and bactericidal effects against Porphyromonas gingivalis, Fusobacterium nucleatum, Streptococcus mutans, and Aggregatibacter actinomycetemcomitans [18]. A systematic review by Hu et al. [19] summarized the mechanisms of Ligilactobacillus salivarius in the prevention and treatment of oral diseases, including inhibition of S. mutans growth and expression of cariogenic virulence factors, as well as antagonism against Candida albicans.

Nano-silver loaded zirconium phosphate Ag-ZrP has the advantages of good biocompatibility, low toxicity, low cost, and strong antibacterial activity [12]. In this study, we planned to add nano-silver loaded zirconium phosphate to PMMA resin at different proportions, according to the national standard GB/T 21510-2008, to evaluate its antibacterial effects against Streptococcus salivarius and Lactobacillus salivarius using the film adhesion method, and to analyze the silver ion release profile, to provide a theoretical basis for the application of nano-silver loaded zirconium phosphate in resin denture bases.

2. Materials and Methods

2.1 Main Experimental Materials and Instruments

Heat-cured denture base resin powder and liquid (Shanghai Huger Dental Medical Equipment Co., Ltd.); silver-loaded zirconium phosphate ($Ag_aNa_bZr_2(PO_4)_3$, a+b=1, Ag content > 3 wt%, Conval PAg-40, Xi'an Kangwang Antimicrobial Technology Co., Ltd.) [20]; BHI broth, MRS broth, PBS buffer, agar powder, Gram staining solution (Beijing Solarbio Science & Technology Co., Ltd.); anaerobic gas packs (Mitsubishi Gas Chemical Company, Japan); OLS4000 laser confocal microscope (Olympus, Japan); scanning electron microscope (Japan); universal testing machine (Instron 5967, USA); inductively coupled plasma mass spectrometer (ICP-MS, Agilent 7900); CCK-8 kit (Dojindo). Lactobacillus salivarius BNCC138618 and Streptococcus salivarius BNCC134928 were purchased from the microbial culture collection center of Beina Biotechnology Co., Ltd. L929 mouse fibroblasts (ATCC CCL-1) were obtained from the Cell Bank of the Chinese Academy of Sciences Typical Culture Collection Committee.

2.2 Experimental Methods

2.2.1 Preparation and Grouping of Test Pieces

At mass ratios of antimicrobial agent to base powder of 0:100, 0.7:100, 1:100, 3:100, 5:100, and 7:100, Conval PAg-40 nano-silver loaded zirconium phosphate was mixed with Meliodent denture base resin powder, and the mixture was blended at a powder-to-liquid ratio of 2:1, packed into 20 mm × 20 mm × 2 mm molds (mechanical property specimens were prepared according to ISO 20795-1:2013), and cured under pressure at 60 °C for 15 min. After grinding and polishing, specimens free of bubbles and with smooth edges were selected and divided into six groups: 0% (control), 0.7%, 1%, 3%, 5%, and 7%. The specimens were sequentially cleaned by ultrasonication in distilled water and acetone for 10 min each, wiped with anhydrous ethanol, rinsed with distilled water, dried at 37 °C for 2 h, exposed to UV irradiation on both sides for 1 h, and kept sterile until use. The grouping of experimental samples is shown in Table 1.

ISSN 2706-6819 Vol.8, Issue 3: 7-20, DOI: 10.25236/IJFM.2026.080302

Table 1: Grouping of test pieces.

Group	Composition
0%group(control)	PMMA resin specimens
0.7%group(experimental)	0.7% AgaNabZr2(PO4)3/PMMAresin specimens
1%group(experimental)	1% AgaNabZr2(PO4)3/PMMA resin specimens
3%group(experimental)	3% AgaNabZr2(PO4)3/PMMA resin specimens
5%group(experimental)	5% AgaNabZr2(PO4)3/PMMA resin specimens
7%group(experimental)	7% AgaNabZr2(PO4)3/PMMA resin specimens

2.2.2 Surface Roughness and Mechanical Property Testing

A laser confocal microscope (OLS4000) was used to observe the polished surface morphology and measure the arithmetic mean deviation of the profile (Ra). Three regions per test piece were averaged. In accordance with ISO 20795-1:2013, a universal testing machine (Instron 5967) tested the tensile strength, flexural strength, flexural modulus, elongation at break, compressive strength, and compressive modulus at a loading rate of 1 mm/min, with five test pieces per group.

2.2.3 Silver Ion Release Experiment

Test pieces from each group were immersed in artificial saliva at 37 °C. The leaching solution was collected at weeks 1, 2, 3, and 4, and the cumulative Ag^+ release ($\mu g \cdot cm^{-2}$) was determined by inductively coupled plasma mass spectrometry (ICP-MS).

2.2.4 Cytocompatibility Assay

According to ISO 10993-5, L929 mouse fibroblasts were used as the cell model. The cells were cultured for 24 h in the 4-week leaching solution (100% concentration) obtained from each group, and the relative cell proliferation rate was determined by the CCK-8 assay, with the 0% group as the control.

2.2.5 Scanning Electron Microscopy Observation

Specimens from the control group and the 3% group were fractured in liquid nitrogen and sputter-coated with gold for cross-sectional morphology observation by scanning electron microscopy (SEM).

2.2.6 Strain Activation and Preparation of Bacterial Suspension

Lyophilized powders of Streptococcus salivarius (BNCC134928) and Lactobacillus salivarius (BNCC138618) were reconstituted and then spread onto BHI agar plates (aerobic) and MRS agar plates (anaerobic, H_2:10%, N_2:80%, CO_2:10%), respectively, and incubated at (37 ± 1) °C for 48 h. After confirmation of purity by colony morphology and Gram staining, single colonies were picked and subcultured for three passages. Colonies in the logarithmic growth phase from the third passage were inoculated into the corresponding liquid media, adjusted to the desired OD_{600} value using a spectrophotometer, and diluted to 1×10^6 CFU/mL by the plate count method, then stored at 4 °C for no more than 4 h.

2.2.7 Adhesion Assay and Calculation of Antibacterial Rate

Sterile test pieces were placed in 50 mm Petri dishes with the polished surface facing up. After adding 10 μL of bacterial suspension onto each test piece, a PE membrane (20 mm × 20 mm, treated with 75% ethanol and sterile water) was placed on top, and the dishes were incubated at (37 ± 1) °C for 24 h (S. salivarius under aerobic conditions, L. salivarius under anaerobic conditions). The test pieces and PE membranes were transferred to 10 mL of sterile PBS and vortexed for 1 min. After 100-fold dilution, 100 μL aliquots were spread onto corresponding agar plates and incubated for 24 h for colony counting. Each group was tested in triplicate. According to GB/T 21510-2008 [22], the antibacterial rate R (%) was calculated as $R = (A - B) / A \times 100\%$, where A is the mean colony count of the control group, and B is the mean colony count of the test group.

2.2.8 Statistical Analysis

GraphPad software was used for one-way analysis of variance. Results are expressed as mean ± standard deviation. The significance level was set at $\alpha = 0.05$, and a P value of < 0.05 was considered statistically significant.

ISSN 2706-6819 Vol.8, Issue 3: 7-20, DOI: 10.25236/IJFM.2026.080302

3. Results

3.1 Surface Roughness of Test Pieces

Under laser confocal microscopy, the polished surfaces of all test pieces were generally smooth, with only a few shallow regular scratches (Fig. 1). The three-dimensional surface topography showed similar colors across the entire surface, indicating a highly flat surface (Fig. 2). Compared with the control group, no statistically significant differences in roughness were observed in any of the test groups ($P > 0.05$) (Fig. 3).

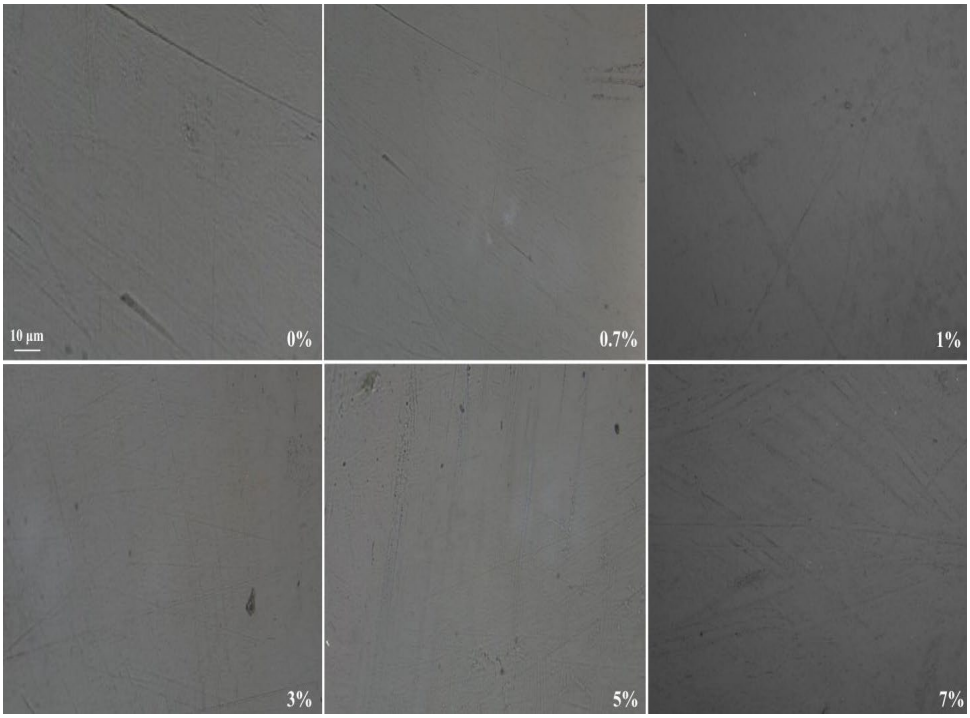


Figure 1: Observation of the roughness of each group of specimens under a laser confocal microscope (x500).

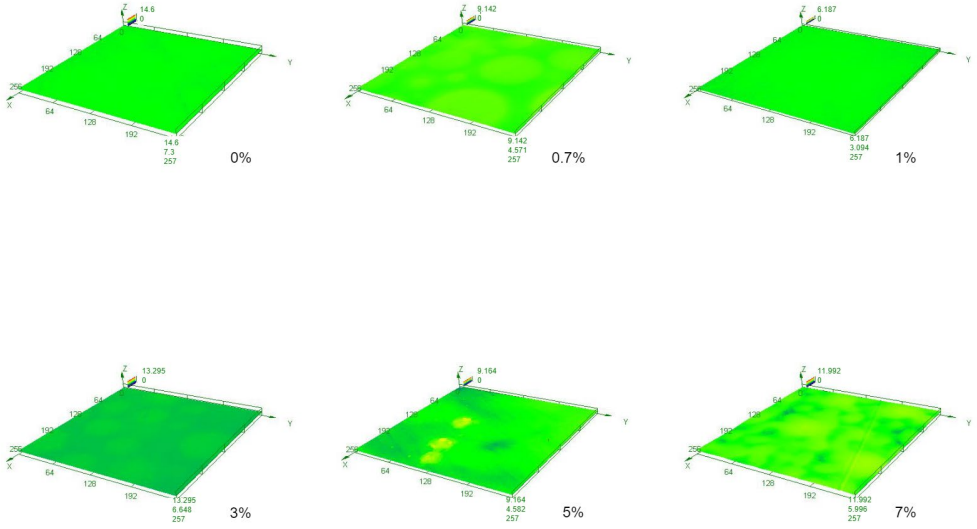


Figure 2: Three -dimensional images of the undulation of each group were drawn by a laser confocal microscope.

ISSN 2706-6819 Vol.8, Issue 3: 7-20, DOI: 10.25236/IJFM.2026.080302

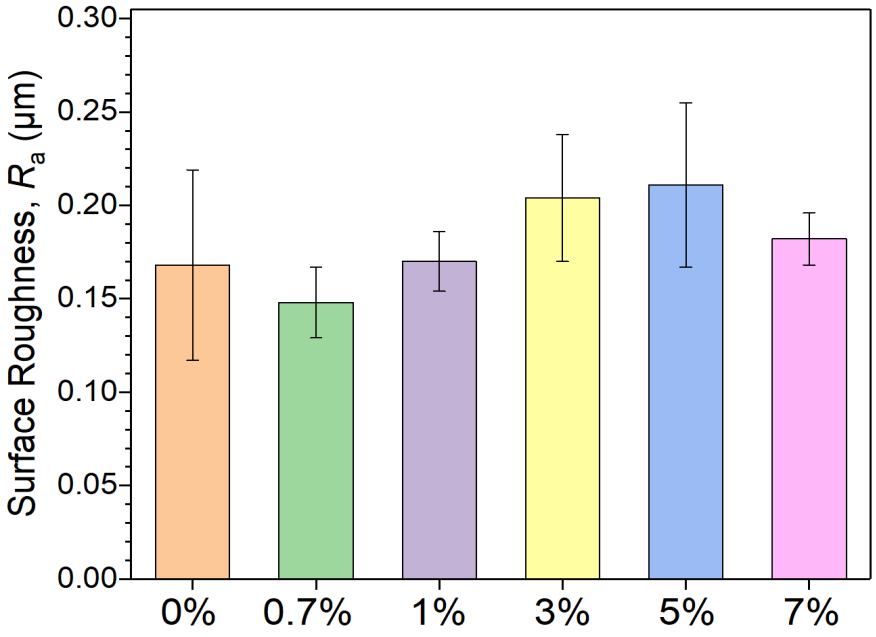
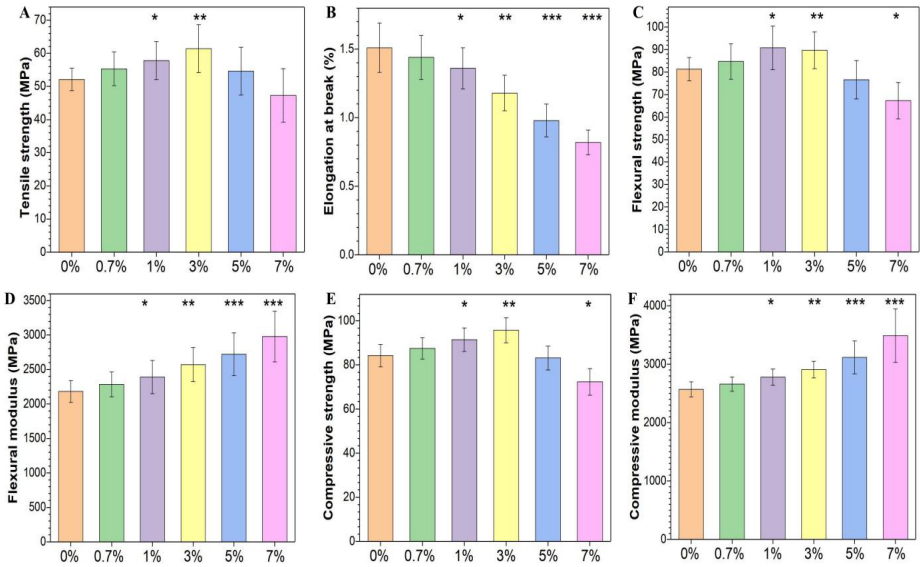


Figure 3: Ra values of specimens.

Note: Data are shown as mean ± SD. NS, not significant vs. control group (P > 0.05).

3.2 Mechanical Property Testing

With increasing addition ratio of nano-silver loaded zirconium phosphate, the compressive modulus showed a trend of first increasing and then decreasing, while the tensile strength, flexural strength, and flexural modulus exhibited similar trends, peaking at 3% and decreasing significantly at 7%. The elongation at break gradually decreased with increasing addition ratio, but the differences among groups were not statistically significant (P > 0.05). The compressive strength followed a similar pattern to the compressive modulus (Fig. 4). Overall, the 3% addition group exhibited the best mechanical properties.



(A) Tensile strength; (B) Elongation at break; (C) Flexural strength; (D) Flexural modulus; (E) Compressive strength; (F) Compressive modulus.

Figure 4: Mechanical properties of specimens with different mass fractions.

ISSN 2706-6819 Vol.8, Issue 3: 7-20, DOI: 10.25236/IJFM.2026.080302

3.3 Silver Ion Release Profile

The silver ion release from the test pieces showed that the 3% group exhibited a faster release in the initial stage, which then leveled off, while the 7% group consistently showed higher release than the other groups. The Ag^+ release increased with increasing addition ratio and time (Fig. 5), demonstrating that the incorporation of nano-silver loaded zirconium phosphate enhanced the silver ion release from the test pieces.

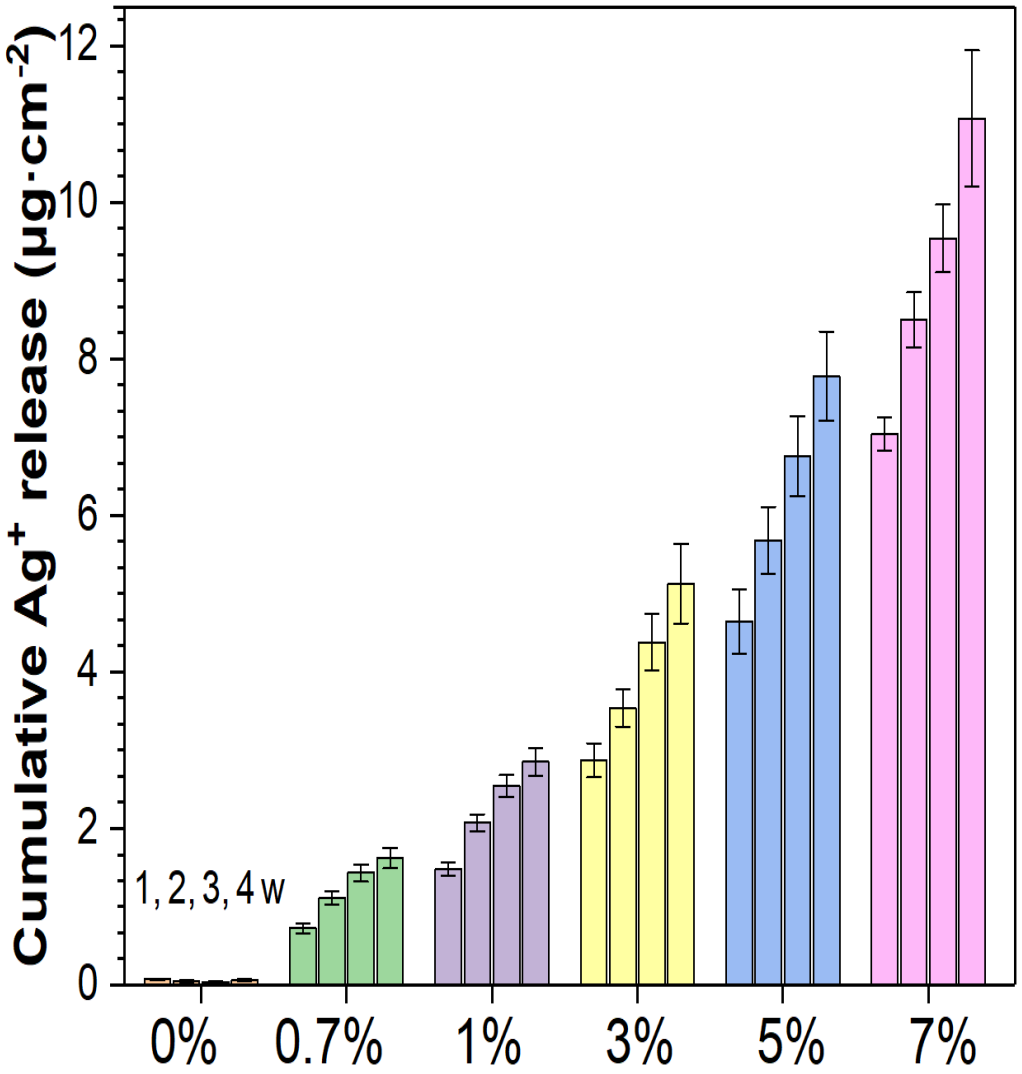


Figure 5: Ag^+ release detection of specimens with different mass fractions.

3.4 Cytocompatibility Evaluation

The cytocompatibility assay indicated that as the silver content in the test pieces increased, cell proliferation was inhibited to a certain extent. However, the 3% group maintained a cell proliferation rate above 80%, which is within the safe range (Fig. 6). In summary, the 3% nano-silver loaded zirconium phosphate group maintained good mechanical properties while balancing antibacterial silver ion release performance and cytocompatibility.

ISSN 2706-6819 Vol.8, Issue 3: 7-20, DOI: 10.25236/IJFM.2026.080302

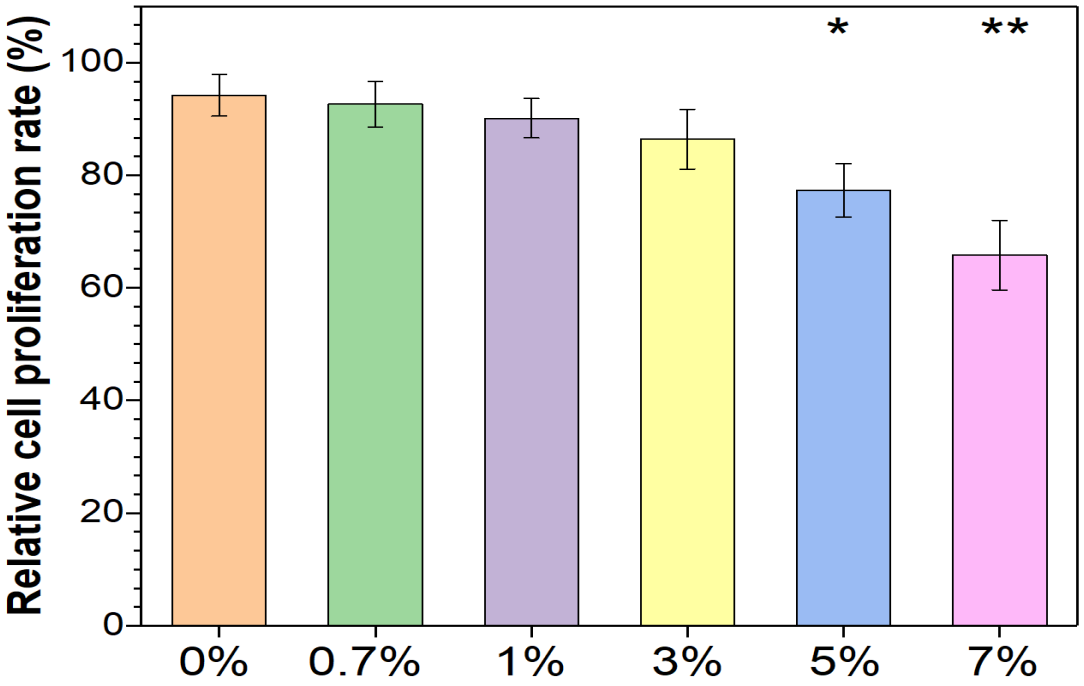


Figure 6: Relative proliferation rate of L929 cells.

3.5 Scanning Electron Microscopy Results

SEM revealed that nano-silver loaded zirconium phosphate particles were dispersed in the PMMA matrix in the cross-section of the 3% group specimens, while no such particles were observed in the control group (Fig. 7).

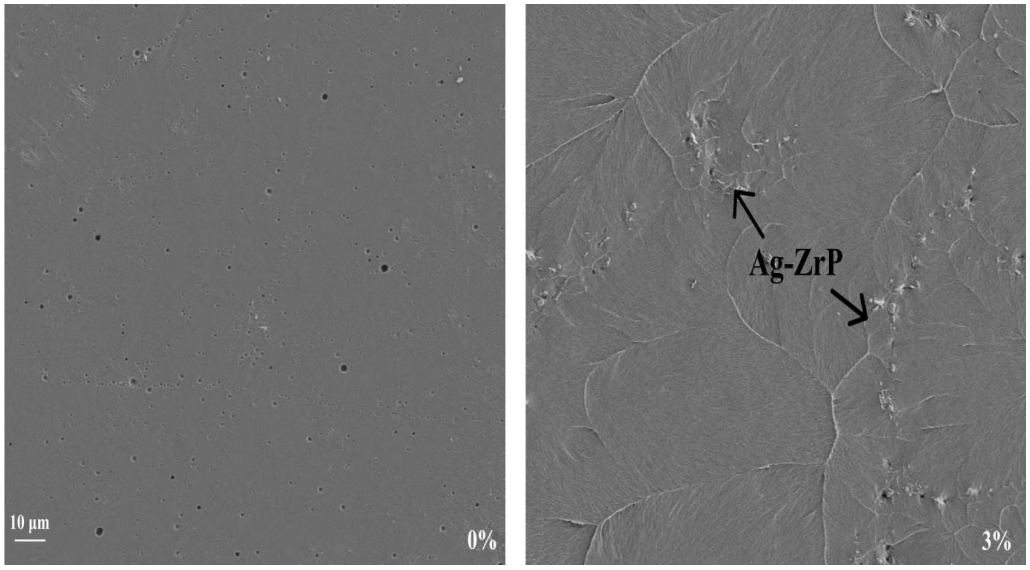
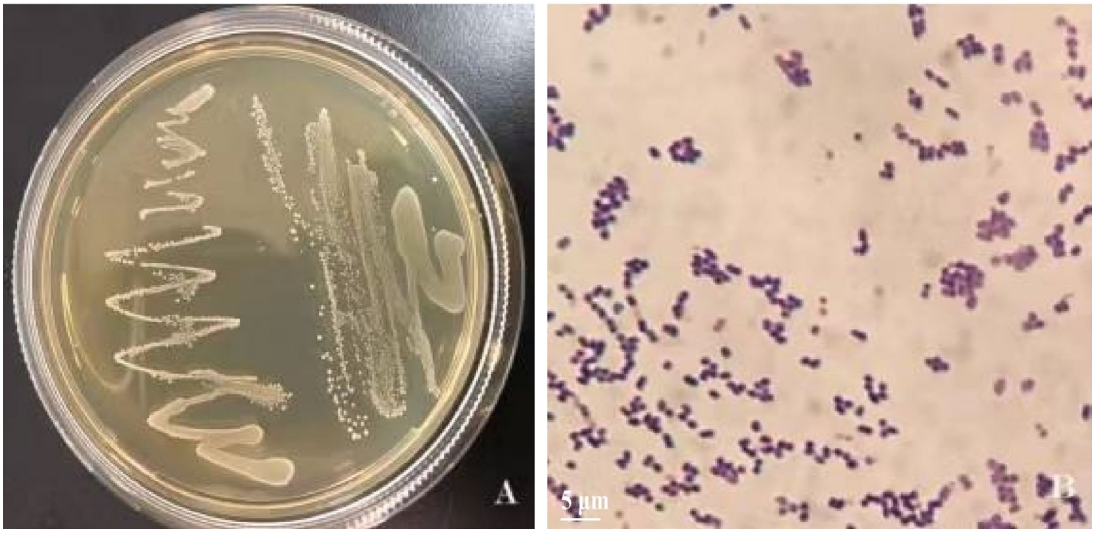


Figure 7: Scanning Electron Microscopy Images.

3.6 Strain Identification

Streptococcus salivarius appeared as white, smooth, circular, convex colonies with regular margins on BHI agar plates, measuring approximately 0.5–1 mm in diameter. Gram staining showed purple, spherical cells arranged in chains or pairs (Fig. 8).

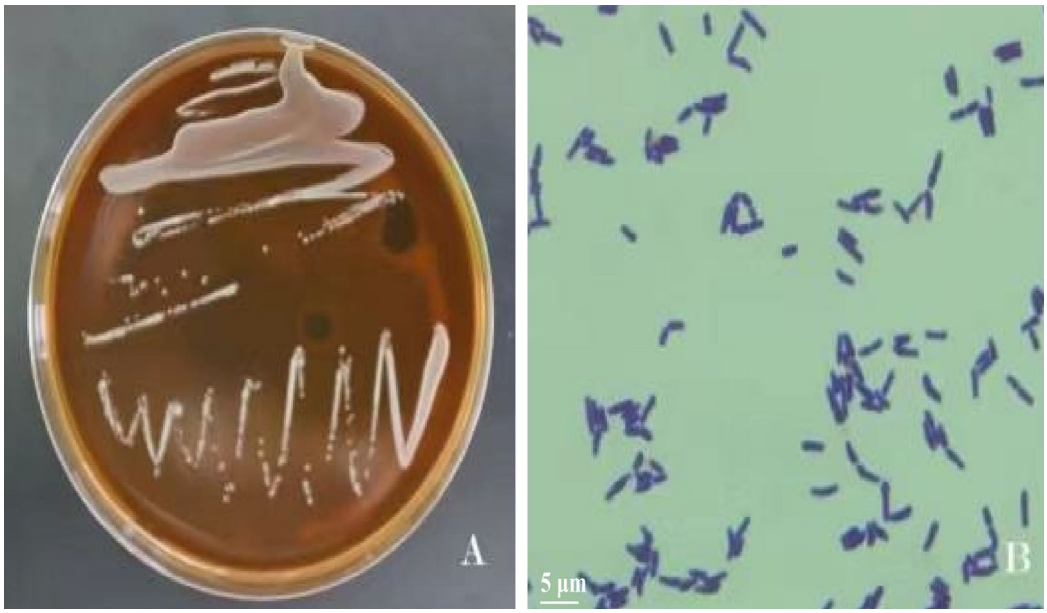
ISSN 2706-6819 Vol.8, Issue 3: 7-20, DOI: 10.25236/IJFM.2026.080302



A: Streaking *B: under oil immersion (x100)*

Figure 8: Streaking and Gram staining of Streptococcus salivarius.

Lactobacillus salivarius appeared as creamy white, circular, convex colonies with regular margins on MRS agar plates, measuring approximately 1.0–2.0 mm in diameter. Gram staining showed blue, straight rod-shaped cells arranged singly, in pairs, or in multiples (Fig. 9).



A: Streaking *B: under oil immersion (x100)*

Figure 9: Streaking and Gram-staining of Lactobacillus salivarius.

3.7 Determination Results of Antibacterial Rate

3.7.1 Antibacterial Effect against Streptococcus salivarius

Resin test pieces containing nano-silver loaded zirconium phosphate effectively inhibited the growth of Streptococcus salivarius (Fig. 10). Compared with the 0% group, the colony counts of the 1%, 3%, 5%, and 7% groups showed statistically significant differences ($P < 0.05$), with antibacterial rates of 26%, 42%, 78%, and 91%, respectively. The 0.7% group showed no statistically significant difference ($P > 0.05$) (Fig. 11).

ISSN 2706-6819 Vol.8, Issue 3: 7-20, DOI: 10.25236/IJFM.2026.080302

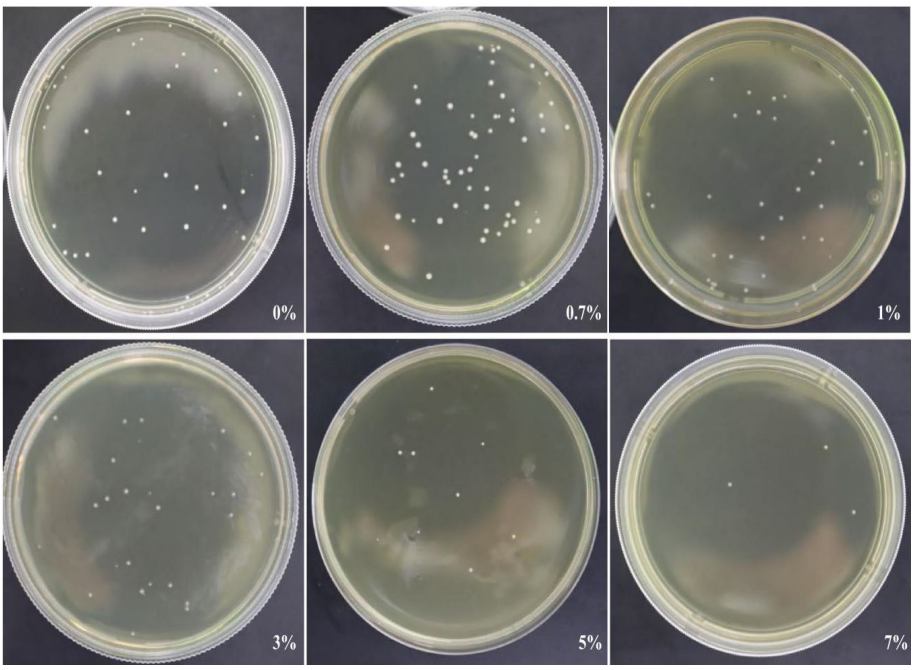


Figure10: Colony number of Streptococcus salivarius adhering to resin specimens with different mass fractions (1 × 104 CFU / ML)

61 in 0% control group; 58 in 0.7% group; 38 in 1% group; 29 in 3% group; 9 in 5% group; 3 in 7% group

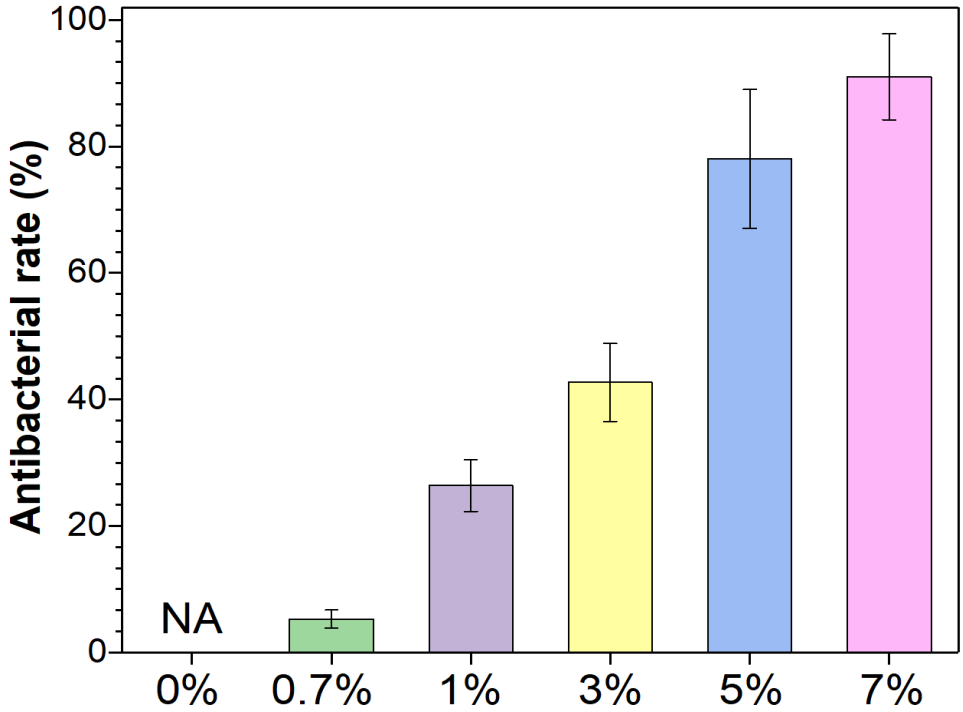


Figure 11: Antibacterial rate against Streptococcus salivarius of resin specimens with different mass fractions of the agents R(%).

3.7.2 Antibacterial Effect against Lactobacillus salivarius

Resin test pieces containing nano-silver loaded zirconium phosphate can effectively inhibit the growth of *Lactobacillus salivarius* (Fig. 12). Compared with the 0% group, the colony counts of the 1%, 3%, 5%, and 7% groups showed statistically significant differences ($P < 0.05$), with antibacterial rates of 22%, 49%, 68%, and 88%, respectively; the 0.7% group showed no statistically significant difference ($P > 0.05$) (Fig. 13).

ISSN 2706-6819 Vol.8, Issue 3: 7-20, DOI: 10.25236/IJFM.2026.080302

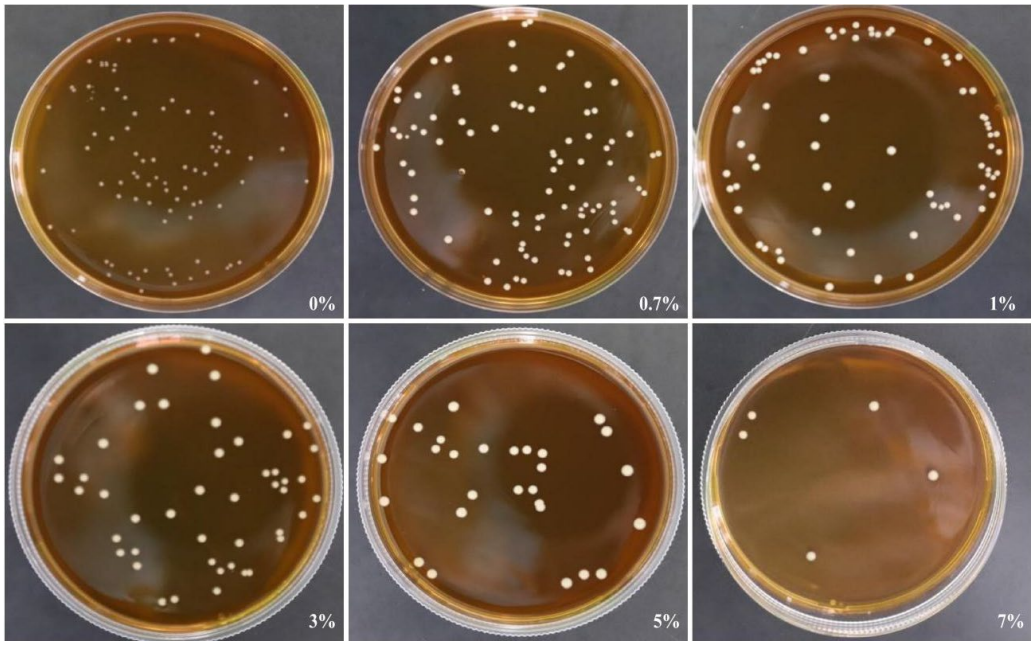


Figure 12: Colony number of Lactobacillus salivarius adhering to resin specimens with different mass fractions (1 × 104 CFU / ML) 86 in 0% control group;89 in 0.7% group;69 in 1% group;48 in 3% group;27 in 5% group;5 in 7% group.

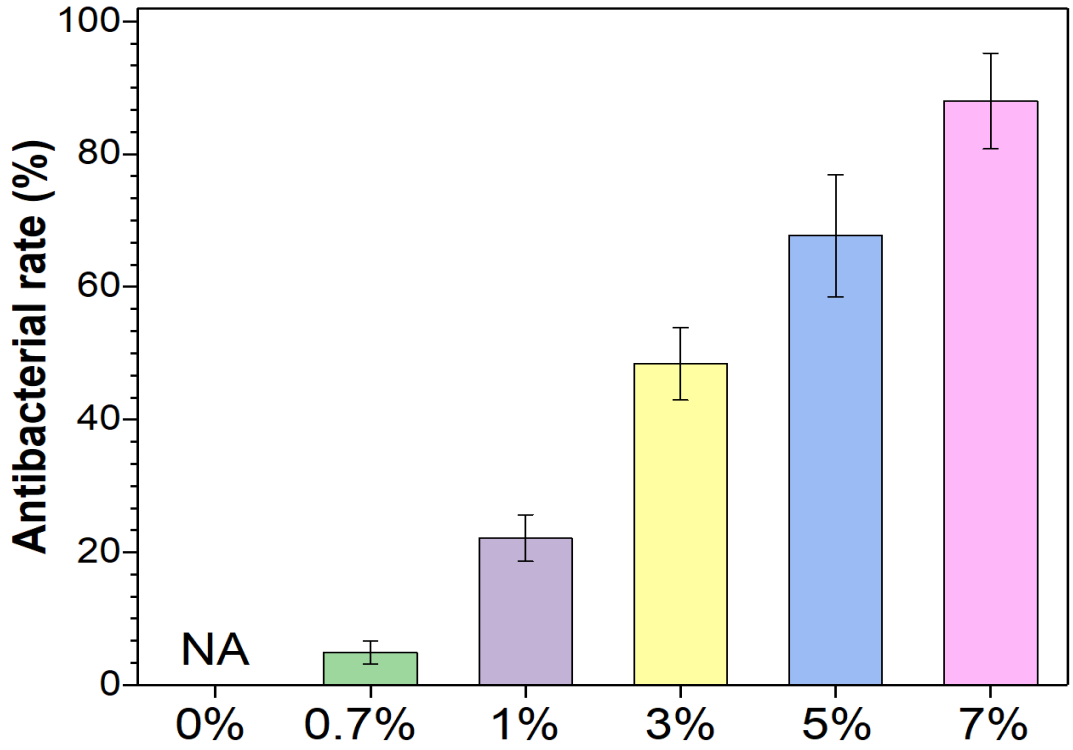


Figure 13: Antibacterial rate against Lactobacillus salivarius of resin specimens with different mass fractions of nano-silver loaded zirconium phosphate agents R(%).

4. Discussion

4.1 Requirements for Antimicrobial Modification of PMMA Denture Base Materials

PMMA is widely used as a denture base material due to its chemical stability, favorable mechanical properties, low cost, and good biocompatibility [2–5]. However, its relatively high shrinkage, insufficient toughness, porous surface, and water absorption facilitate microbial adhesion and colonization [20–21].

ISSN 2706-6819 Vol.8, Issue 3: 7-20, DOI: 10.25236/IJFM.2026.080302

After denture placement, oral self-cleaning and salivary flushing are also impeded, and local redox potential and pH may be altered, promoting the proliferation of opportunistic pathogens such as Candida albicans [22]. Therefore, endowing PMMA with antibacterial properties is of significant clinical importance. The oral microecology maintains a dynamic balance through symbiosis and antagonism, and various factors can lead to microecological dysbiosis [23–25]. Montoya et al. [26] found that different dental materials exert differential modulatory effects on the subgingival microbiota, suggesting that material selection may profoundly influence the oral microecology. Abnormal proportions of any microbial group, whether dominant pathogens or non-dominant commensals, may lead to microecological disruption.

4.2 Antimicrobial Mechanism of nano-silver loaded zirconium phosphate and Selection of Target Strains

The antimicrobial mechanisms of silver nanoparticles include: (1) the "Trojan horse" effect, in which nanoparticles enter cells and release silver ions intracellularly [27]; (2) electrostatic adsorption disrupting cell membrane permeability [28,29]; (3) intracellular interference with transcription, translation, and protein synthesis [30]; (4) binding to sulfhydryl groups, thereby disrupting disulfide bond formation [31]; and (5) induction of elevated intracellular reactive oxygen species (ROS) levels [32]. nano-silver loaded zirconium phosphate (Conval PAg-40) combined with PMMA exhibits both favorable antibacterial and mechanical properties [12]. Our previous studies confirmed its inhibitory effects against six dominant oral pathogens,and also demonstrated that its incorporation can alter the overall performance of the material [13].

In this study, Streptococcus salivarius and Lactobacillus salivarius were selected as the representative strains. S. salivarius predominates in the healthy oral cavity, produces bacteriocins to inhibit pathogen colonization, and participates in local immune regulation [33]. Its K12 strain inhibits halitosis-associated pathogens [34–36], Streptococcus mutans [37], and Candida albicans [38], and alleviates lichen planus [17]. L. salivarius, as a probiotic, inhibits biofilm formation of S. mutans [39] and the growth of Porphyromonas gingivalis and Prevotella intermedia, and improves halitosis [40,41].

4.3 Antibacterial Efficacy and Concentration Dependence of Modified PMMA

Nano-silver loaded zirconium phosphate at ≥ 1% exhibited significant inhibitory effects against both tested bacterial species, with the antibacterial rate increasing proportionally with the addition ratio. The minimum effective concentration ranged between 0.7% and 1%. Compared with our previous study [13], the 0.7% group showed only weak inhibition against Candida albicans, with no significant effect against S. salivarius, L. salivarius, or the other five dominant pathogens. At addition ratios > 1%, satisfactory inhibition was observed against all tested strains. The 7% group achieved antibacterial rates of 91% against S. salivarius and 88% against L. salivarius, indicating a broad antimicrobial spectrum with no obvious species selectivity, consistent with the non-specific antibacterial mechanism of silver ions.

4.4 Comparison with Similar Materials and Broad-Spectrum Antibacterial Properties

Synthetic antimicrobial peptides, zinc oxide eugenol paste, MTA, zirconium nitride coatings [42], certain mouthwashes [43], chitosan [44], etc., have all shown varying degrees of antibacterial activity against the two tested species. A temporary filling material containing silver-zinc zeolite showed no inhibitory effect, indicating that different antimicrobial agents may exhibit strain-specific susceptibility differences [45]. Dental amalgam and some nano-composite resins have shown good antibacterial activity against L. salivarius [46]. Chladek et al. [47] incorporated silver-loaded zirconium phosphate into denture soft lining materials and confirmed its antifungal properties and acceptable cytotoxicity. These findings are consistent with the present study, indicating that nano-silver loaded zirconium phosphate exhibits broad-spectrum antibacterial activity without obvious selective specificity toward oral microbial communities.

4.5 Limitations and Prospects

The limitations of this study are as follows: (1) only two commensal bacterial species were selected, which may not fully represent the complex oral microbial community; (2) the in vitro conditions could not simulate the dynamic oral microenvironment; (3) the long-term effects of the antimicrobial agent on microecological balance remain to be evaluated. In addition, the surface hardness and flexural strength

ISSN 2706-6819 Vol.8, Issue 3: 7-20, DOI: 10.25236/IJFM.2026.080302

of the modified material decreased after accelerated aging in artificial saliva, and its long-term stability requires further investigation. In summary, nano-silver loaded zirconium phosphate-modified PMMA exhibits favorable antibacterial efficacy against both dominant and non-dominant oral pathogens in a concentration-dependent manner. Future studies should focus on dose optimization, long-term biosafety evaluation, antibacterial mechanisms, and clinical translation.

5. Conclusion

Nano-silver loaded zirconium phosphate-modified PMMA resin exhibits concentration-dependent antibacterial activity against Streptococcus salivarius and Lactobacillus salivarius. The 3% mass fraction provides effective antibacterial activity while maintaining favorable mechanical properties and cytocompatibility, making it the most promising modification ratio for this material. This study provides a theoretical basis for the development of novel denture base materials that can both inhibit pathogenic bacteria and maintain oral microecological balance.

Acknowledgements

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