Antibacterial properties of a self-cured acrylic resin composed of a polymer coated with a silver-containing organic composite antibacterial agent

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A novel antibacterial polymer, coated with a silver-containing organic composite antibacterial agent, was dispersed in a self-cured acrylic resin. Residual viable cell count of each oral bacterial and fungal species cultivated on acrylic resin specimens containing the antibacterial polymer was significantly decreased when compared to those cultivated on specimens prepared from untreated polymer. A strong inverse correlation was found between the amount of eluted silver ions and the residual viable cell count of all species grown on the antibacterial polymer: the lower the viable cell count, the higher the amount of eluted silver ions. This clearly indicated the antibacterial activity of silver ions. As the content of organic composite antibacterial agent added to the polymer increased from 0.5% to 1.5% in 0.5% increments, amount of eluted silver ions significantly increased with each 0.5% increment to exert greater antibacterial effect.

Keywords: Bacteria, Silver ion, Antibacterial agent, Self-cured acrylic resin

INTRODUCTION

Self-cured resins are popularly and prevalently used to construct facing crowns, temporary crowns and bridges, and in the repair of acrylic resin-based dentures. There is increasing evidence that plaque easily forms on acrylic resin surfaces, which then causes a variety of oral conditions such as secondary caries, marginal periodontitis, and denture stomatitis¹⁻³⁾. To maintain oral cleanliness, it is useful to endow self-cured resins with antibacterial properties.

Many research studies have focused on incorporating antibacterial agents into dental materials to render the latter antimicrobial⁴⁻¹⁹⁾. In the present study, a novel antibacterial polymer was developed. It was constituted of two key ingredients: a silver-containing organic composite antibacterial agent and a self-curing acrylic polymer. NOVARON AG300 (Toagosei Co Ltd., Tokyo, Japan), an inorganic antibacterial agent made up of zirconium phosphate ceramics containing silver ions in the crystal structure, was dispersed in a multifunctional monomer (trimethylolpropane trimethacrylate, TMPT). After polymerization and pulverization, the self-curing acrylic polymer of Unifast III (GC Corp., Tokyo, Japan; UF III) was coated with the pulverized form of the organic composite antibacterial agent. Polymer particles of Unifast III coated with pulverized antibacterial agent were created using a polymer processing technique known as Surface Uniformity Revolutionary Fixation (SURF) technology²⁰⁾. Henceforth in this paper, this material is referred to as the antibacterial polymer.

Four kinds of oral bacteria (*Streptococcus mutans*, *Streptococcus oralis*, *Streptococcus gordonii*, and *Actinomyces naeslundii*) have been shown to contribute to the initial attachment and mature architecture development of biofilms. Among microbial populations,

Candida albicans is a fungus commonly detected on acrylic resin denture surfaces²¹⁾. To examine the antimicrobial properties of the novel antibacterial polymer, these oral bacterial and fungal species were used in this study. To determine the relation between residual viable cell count and the amount of eluted silver ions, concentrations of silver ions in the suspensions of bacterial and fungal cells were also measured.

MATERIALS AND METHODS

Antibacterial polymer preparation

A self-cured acrylic resin, Unifast III (GC Corp., Tokyo, Japan), was used as the base material. An organic composite antibacterial agent was used as the antibacterial agent; its composition is shown in Table 1.

After pretreatment with alcohol solvent, Unifast III polymer surface was coated with the organic composite antibacterial agent at 0.5, 1.0, and 1.5 wt% of the amount of polymer using SURF technology. Figure 1 is the schematic diagram illustrating the process for antibacterial polymer preparation. Production of the organic composite antibacterial agent and coating of the Unifast III polymer surface were provided by GC Dental Products.

Polymer surface observation

Secondary electron images (SEI) were taken of the surfaces of the antibacterial polymer and untreated polymer using an electron probe microanalyzer (JXA-8530FA, JEOL, Tokyo, Japan; EPMA) at an accelerating voltage of 15 kV and a probe current of 5 nA.

Elemental analysis of cured acrylic resin composed of the antibacterial polymer

After mixing 0.5 mL of the monomer liquid with 1.0 g of

Table 1 Composition of silver-containing organic composite antibacterial agent

Ingredient		Particle size (average)	Specific gravity
NOVARON	70.0 wt%	0.5 μm	2.4
Trimethylolpropane trimethacrylate (TMPT)	29.7 wt%	—	—
Azobisisobutyronitrile (AIBN)	0.3 wt%	—	—

Fig. 1 Schematic diagram illustrating the process for antibacterial polymer preparation.

the antibacterial polymer, the mixture was pressed into a 24-hole plate to produce discoid specimens of 18 mm diameter. One surface of each specimen was polished with #2000 Emery paper, followed by mirror-polishing with 0.3- μm alumina powder and ultrasonic cleaning. Elemental analysis by EPMA was carried out on this polished and cleaned surface of each specimen to confirm the presence of the antibacterial agent.

Silver ions included in NOVARON could not be detected because of low concentration levels. Therefore, elemental analysis was performed to detect zirconium: zirconium phosphate was used as a carrier of silver ions in NOVARON. Backscattered electron images (BEI) and element distribution maps of zirconium were obtained by EPMA at an accelerating voltage of 15 kV and a probe current of 50 nA. Electron beam diameter was 1 μm on the specimen surface. Scans were performed with 1- μm step size, and measurement time at each point was 10 min.

Assessment of antimicrobial effects of cured acrylic resin composed of the antibacterial polymer

Four types of oral bacteria contribute to the initial formation of biofilms on tooth and acrylic resin surfaces: *Streptococcus mutans* (NCTC 10449; *S. mutans*), *Streptococcus oralis* (ATCC 9811; *S. oralis*), *Streptococcus gordonii* (bL-1; *S. gordonii*) and *Actinomyces naeslundii*

Table 2 Details of fluid and agar media types used for antimicrobial effect assessment

Bacterium/Fungus	Fluid-medium	Agar-medium
<i>S. mutans</i>	Trypticase Soy broth	Trypticase Soy agar
<i>S. oralis</i>	Heart Infusion broth	Heart Infusion agar
<i>S. gordonii</i>	Heart Infusion broth	Heart Infusion agar
<i>A. naeslundii</i>	Todd Hewitt broth	Heart Infusion agar
<i>C. albicans</i>	Todd Hewitt broth	CHROMagar Candida

Fig. 2 Schematic diagram illustrating the steps of assessing antimicrobial effects on bacteria and fungus.

(ATCC 12104; *A. naeslundii*)²¹. The fungus, *Candida albicans* (A9; *C. albicans*), causes denture stomatitis. These five types of oral bacterial and fungal species were thus selected to test the antimicrobial effects of antibacterial polymer in this study.

Discoid specimens of cured acrylic resin were prepared for this assessment in the same manner as that done for elemental analysis. For each bacterial or fungal species, 12 specimens were prepared. Ethylene oxide gas was used to sterilize all prepared specimens.

Table 2 lists the fluid and agar media types used in this study to grow the bacteria and fungus. Bacteria and fungus were grown in 50 mL of their respective fluid media at 37°C for 24 h. A 500- μL suspension of each bacterial or fungal species was added to each specimen in a 24-well plate and cultured in an incubator at 37°C for 24 h. Resulting cell suspensions were extracted and diluted in physiological saline by a factor of 10^5 times. A 50- μL diluted bacterial or fungal

suspension was inoculated in a plate of agar medium, and the colony count of each bacterial and fungal species formed on the agar plate was determined after 48-h incubation at 37°C (Fig. 2).

Quantitative analysis of silver ions eluted from cured acrylic resin composed of the antibacterial polymer

A 300- μ L volume of bacterial or fungal suspension was diluted with 1% nitric acid to obtain a 50-mL solution. For each bacterial and fungal species, 12 cured acrylic resin specimens were used to determine the amount of eluted silver ions.

Silver ion concentrations were measured by inductively coupled plasma mass spectrometry (NexION

300, PerkinElmer, Waltham, MA, USA; ICP-MS) using a calibration curve method. Twelve specimens were measured for each bacterial and fungal species.

Statistical analysis

Data obtained were analyzed using the Steel-Dwass multiple comparison test. Criterion for statistical significance was $p < 0.05$.

RESULTS

Polymer surface observation

Figure 3 shows the EPMA-acquired SEI of untreated polymer surface and those of antibacterial polymer

Fig. 3 SEI of polymer surface: (a) Untreated polymer; (b) Higher magnification of rectangular area in (a); (c) Polymer coated with 0.5% of organic composite antibacterial agent; (d) Polymer coated with 1.0% of organic composite antibacterial agent; (e) Polymer coated with 1.5% of organic composite antibacterial agent; and (f) Higher magnification of rectangular area in (e).

coated with 0.5–1.5 wt% of organic composite antibacterial agent. At a higher magnification (Fig. 3f), pulverized organic composite antibacterial agent was observed to be uniformly distributed on Unifast III polymer surface.

Elemental analysis of cured acrylic resin composed of antibacterial polymer

Figure 4 shows the BEI of cured acrylic resin specimens prepared from different amounts of organic composite antibacterial agent (0.5–1.5 wt%), and the element distribution maps of zirconium on their corresponding surfaces. White particles of zirconium in the element distribution maps increased with the amount of organic composite antibacterial agent.

Antimicrobial effect assessment

Figure 5 shows the results of antimicrobial effects against five kinds of bacteria and fungus. The residual viable cell count of each type of bacterium and fungus cultured on the antibacterial polymer specimen was significantly decreased when compared to that of untreated polymer ($p < 0.01$). Moreover, the residual viable cell count of each bacterial and fungal species significantly decreased with increasing amount of organic composite antibacterial agent added to antibacterial polymer ($p < 0.01$).

Silver ions eluted from cured acrylic resin composed of antibacterial polymer

Figure 6 shows the amounts of eluted silver ions found

Fig. 4 BEI (a–c) and element distribution maps (d–f) of zirconium of the surface of UF III. (a) and (d): UF III coated with 0.5% of organic composite antibacterial agent; (b) and (e): UF III coated with 1.0% of organic composite antibacterial agent; and (c) and (f): UF III coated with 1.5% of organic composite antibacterial agent.

Fig. 5 Quantitative results of antimicrobial effect assessment, with significant differences among Groups 1–4 ($p < 0.01$) for each type of bacterium or fungus. 0.5% UF III: UF III coated with 0.5% of organic composite antibacterial agent; 1.0% UF III: UF III coated with 1.0% of organic composite antibacterial agent; 1.5% UF III: UF III coated with 1.5% of organic composite antibacterial agent.

in five kinds of bacterial and fungal suspensions. The concentration of silver ions significantly increased with increasing amount of organic composite antibacterial agent added to antibacterial polymer ($p < 0.01$). Analysis results for the bacterial and fungal suspensions of untreated polymer were excluded because detection limit was exceeded.

DISCUSSION

Plaque, which must be controlled, is typically removed using physical means such as toothbrushes, interdental brushes, or dental floss. However, in many cases, it is impossible to completely remove plaque because of anatomical and technical reasons. The use of resin

materials for dental treatments exacerbates the presence of plaque as they favor plaque formation. To counter and circumvent this problematic association between dental resins and dental plaque, many studies have focused on endowing resin-based dental materials with antibacterial properties^{4–19}. Currently, antibacterial agents to be incorporated into dental materials are classified into organic agents [such as quaternary ammonium salts⁵, 12-methacryloyloxy-dodecyl-pyridinium-bromide (MDPB)⁶, quaternary phosphonium salts⁷, and bamboo extract⁸] and inorganic agents [such as silver^{4,9–18} and titanium dioxide¹⁹].

Evolution and development of novel antibacterial polymer
Kuroki *et al.*⁴ developed a self-cured acrylic resin

Fig. 6 Eluted silver ions for each type of bacterium or fungus.
 0.5% UF III: UF III coated with 0.5% of organic composite antibacterial agent;
 1.0% UF III: UF III coated with 1.0% of organic composite antibacterial agent;
 1.5% UF III: UF III coated with 1.5% of organic composite antibacterial agent.

constituted of polymer powder particles mixed with an acrylic monomer. The latter was added with NOVARN, which was made up of zirconium phosphate ceramics containing silver ions in the crystal structure. When this newly developed self-cured acrylic resin was tested against *Streptococcus mutans*, there was a significant decrease in residual viable cell count when compared to exposure to resin without antibacterial agent. However, discoloration occurred because the inorganic antibacterial agent contained metal ions.

In the preliminary experiments of this study, Kuroki *et al.* dispersed NOVARN in a multifunctional monomer. Polymerization and pulverization yielded an organic composite antibacterial agent (Fig. 1). This organic composite antibacterial agent also showed a pronounced antibacterial effect, but less discoloration as compared to addition of NOVARN alone. However, the preparation process was highly complicated. After a small amount of NOVARN was added to the multifunctional monomer, the antibacterial polymer was obtained by undergoing a reaction with the

polymer. This meant expending significant time and effort to produce the antibacterial polymer for assessment and testing. A high-accuracy electronic balance was also required to ensure that the organic composite antibacterial agent was added at 0.1–1.0 wt% to produce the antibacterial polymer.

To simplify the preparation process, the organic composite antibacterial agent was simply dispersed at the required quantity in the polymer. However, variations in antibacterial assessment results occurred because compact masses and deposits of organic composite antibacterial agent were randomly formed in the polymer. Specific gravity of organic composite antibacterial agent was 2.4, and the average particle size was 0.5 μm . Poly(methyl methacrylate), a main ingredient of self-cured acrylic resin, had a specific gravity of 1.19 and an average particle size of 40–80 μm . Vehicle vibration during transportation could have caused sedimentation of the organic composite antibacterial agent. This then caused compact masses of organic composite antibacterial agent to be randomly

and unevenly deposited in the mixture.

In the present study, the polymer processing SURF technology²⁰⁾ was applied to produce the antibacterial polymer. EMPA-acquired secondary electron images showed that this technology enabled the polymer surface to be uniformly coated with the organic composite antibacterial agent.

Antimicrobial effects of novel antibacterial polymer

In the present study, the residual viable cell counts of *S. mutans*, *S. oralis*, *S. gordonii*, *A. naeslundii*, and *C. albicans* were significantly decreased when tested against the organic composite antibacterial agent. Therefore, the novel antibacterial polymer exhibited antibacterial effect and inhibited biofilm formation. In terms of clinical application, the antibacterial polymer has great preventive potential against denture stomatitis.

For each type of bacterium or fungus, the residual viable cell count decreased with an increase in the amount of organic composite antibacterial agent added to Unifast III polymer. At the same time, the amount of silver ions eluted from self-cured acrylic resin increased with an increase in the amount of added organic composite antibacterial agent. These results showed that the antimicrobial effect of novel antibacterial polymer was closely and strongly related to the amount of eluted silver ions.

Figure 7 shows strong inverse correlations between the amount of eluted silver ions and residual viable cell count for five types of bacteria and fungus. For each bacterial and fungal species, the residual viable cell count decreased with an increase in the amount of eluted silver ions. These results showed that the antibacterial effect of self-cured acrylic resin composed

Fig. 7 Correlation diagram between CFU and eluted silver ions.
 0.5% UF III: UF III coated with 0.5% of organic composite antibacterial agent;
 1.0% UF III: UF III coated with 1.0% of organic composite antibacterial agent;
 1.5% UF III: UF III coated with 1.5% of organic composite antibacterial agent.

of organic composite antibacterial agent was influenced and determined by the amount of eluted silver ions.

Mechanism of antibacterial action of silver ions

Two theories have been put forth to explain the mechanism of antibacterial action of silver ions^{4,9,15,22}. In Ionic Theory, silver ions are incorporated at intracellular level. They act as potent enzyme inhibitors which inhibit intracellular bacterial metabolism, thereby causing bacterial death²³. In “Reactive Oxygen Species” theory, silver ions act as catalysts which decompose water and generate hydroxyl radicals. Being effective in disinfection, these hydroxyl radicals cause bacterial death²⁴.

In bacteria, the proportion of proteins is 60–80%; in fungi, it is 30–50%. Amino acids responsible for enzyme activity include histidine, arginine, cysteine, glutamine, and asparagine. Among these amino acids, histidine, arginine, and cysteine have a higher affinity for silver ions as their protein content is approximately 61%²³. For histidine and arginine, –NH groups are the terminal group of the molecular structure and they react with silver ions. For cysteine, –SH groups are the terminal group which are strongly reactive with silver ions²³.

Limitations of present study and avenues for future research

It was difficult to compare the antibacterial activity results among the bacterial and fungal species tested in this study. The concentrations of bacterial and fungal suspensions were not standardized in the present study because five kinds of bacteria and one kind of fungus were grown in different types of fluid medium. In future research, the influence of growth medium for bacteria and fungi should be examined.

Nonetheless, this study provided sufficient evidence to show that self-cured acrylic resin prepared from the antibacterial polymer exhibited antimicrobial effects against four kinds of bacteria and one kind of fungus. However, antimicrobial effects differed among the species. In Fig. 7, correlation coefficient for *C. albicans* was lower than those of the other four bacteria. The proportion of proteins is lower in fungus than in bacteria. Therefore, compared with bacteria, fungus has a lower amount of amino acids to react with silver ions. It was also probable that the cell components and microstructure of microorganisms caused the difference in antimicrobial effects. This is another avenue for further investigation in future research.

As silver ions eluted from the self-cured acrylic resin containing the antibacterial polymer to exert antibacterial effect, there emerged two concerns to be addressed in future research: discoloration of the acrylic resin and the effect on the latter's mechanical properties.

CONCLUSIONS

Within the limitations of the present study, the following

conclusions were drawn:

1. By developing an antibacterial polymer which contained silver-based NOVORON, NOVARON was able to be dispersed in self-cured acrylic resin without causing severe discoloration.
2. Residual viable cell counts of all bacterial and fungal species tested against the antibacterial polymer were significantly decreased when compared to those exposed to untreated polymer.
3. Amount of eluted silver ions significantly increased with each 0.5% incremental increase in the amount of added organic composite antibacterial agent.
4. There was a strong correlation between the amount of eluted silver ions and residual viable cell count.

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