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Anti-biofilm Effects of Resin-Modified Glass-Ionomers Incorporated with Silver Nanoparticles and Sodium Fluoride

Kavya Gopalakrishnan[1], Ajay Ramesh[2], Abirami Devendran[3], Sankarganesh Palaniyandi[4], Lokesh Elumalai[5], Krishnasamy Loganathan[6], Pugazhenthi Elumalai[1], Ganesh Kumar Anbazhagan[7]*

[1]Department of Microbiology, Hindustan College of Arts & Science, Padur, OMR Chennai – 603 103, Tamil Nadu, India.

[2] Department of General Surgery, Saveetha Medical College and Hospital, Saveetha Institute of Medical and Technical Sciences, Chennai – 602105.

[3]Department of Microbiology, Vivekanandha College of Arts and Sciences for Women, Ellayampalayam, Tiruchengode 637 205, Tamil Nadu, India.

[4]Department of Food Technology, Hindustan Institute of Technology and Science, Padur, Chennai – 603103, Tamil Nadu, India.

[5]Department of Microbiology, Periyar University, Salem – 636 011, Tamil Nadu, India.

[6]Department of Biotechnology, Hindustan College of Arts & Science, Padur, OMR Chennai – 603 103, Tamil Nadu, India.

[7]Department of Microbiology, Saveetha Medical College and Hospital, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai – 602 105, Tamil Nadu, India.

Abstract

*The glycocalyx or biofilm is essential for microbial survival. But dental biofilms cause cavities in teeth. The current study planned to incorporate silver nanoparticles and sodium fluoride in resin-modified glass-ionomer cement to test anti-biofilm efficacy**.** Silver nanoparticles (NAg) were chemically reduced by sodium borohydride. At 0.15% mass fraction, NAg was added in commercial resin-modified glass-ionomer cement (GC) and in this blend, 4 mass fractions (5%, 10%, 20%, and 30%) of NaF were mixed. The metabolic activity of biofilm, which shows the metabolic activity was decreased by NaF (P > .05). The biofilm CFU count shows the CFU counts of GC+NAg+20%NaF were decreased to 10^6 CFU/disk (P<.05). The cell viabilities of GC+NAg+20%NaF were79.7%,75.6%,73% for days 1, 4, and 7 respectively, which were lower than GC control (P < .05) but higher than GC+NAg (P < .05). The use of NAg reduced cell viability at all three-time points (day 1, day 4, and day 7), which was partially reversed by the addition of NaF.*

Keywords: *Biofilm, Glass-Ionomer, Orthodontic Cement, Silver Nanoparticles, Sodium Fluoride*.

Introduction

The advancement from tying down orthodontic connections to teeth using circumferential groups to theimmediate holding of individual orthodontic sections has happened during the beyond 35 years. Dental caries is the most prevalent and multifactorial oral disease and is still considered a serious worldwide oral health problem. Antibiotics, metal ions, and fluorides in various vehicles have been used as alternatives to the traditional filling approach for preventing and arresting caries. Topical agents, such as silver diamine fluoride (SDF), have been concluded as effective, efficient, equitable, and safe caries-preventive agents. SDF therapy is simple and non-invasive [1]. Clinical trials and lab studies have supported the success of 38% SDF in arresting dental

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****Corresponding Author: drgkanbazhagan@gmail.com***

caries and inhibiting the growth of cariogenic pathogens [2]. Enamel demineralization, or white spot lesions (WSL), is a common complication found in patients using orthodontic devices, such as orthodontic braces. A meta-analysis reported that the incidence of new carious lesions formed during orthodontic treatment in patients was 45.8% and the prevalence of lesions in patients undergoing orthodontic treatment was 68.4%. Against caries orthodontic cement is a promising measure to battle white spot sores (WSLs) because of its autonomy of patient consistency and its vicinity to biofilms [3]. The developed biofilm burns through carbs and produces natural acids, which further outcome in lacquer demineralization showing as WSLs. Accordingly, an ideal orthodontic concrete to battle WSLs ought to have protein-repellent properties to diminish the salivary protein adsorption and antibacterial capacity to restrain the corrosive creation [4].

Nanotechnology has been applied to deal with the introduction of dental materials is known as nano- dentistry. Silver has trustworthy antibacterial properties and makes less bacterial impediment than anti-toxins [5]. The solidification of nano-sized silver particles (NAg) into dental materials could enough smother the advancement of oral microbes. A possible way in which to address the displeasing aesthetic effect of SDF is to use a fluoride solution containing silver nanoparticles (AgNPs). Fluoride has been shown to enhance the remineralization of caries. Hence, fluoride solutions containing AgNPs may be used to control caries without aesthetically undesirable side effects [4]. Resin-modified glass ionomer cement has longer working times and undergoes rapid setting after light curing. *Staphylococcus aureus (S. aureus)* has been considered the oral pathogen involved in the development of dental caries. Silver nanoparticles possess a broad spectrum of antibacterial, antifungal and antiviral properties. Sodium fluoride is used to prevent caries development and arrest early enamel and even soft dentine caries through the promotion of remineralization of carious tooth substance. Biofilms can form on both biotic and abiotic surfaces and are common causes of chronic infections including dental plaques. *S. aureus* is a presumed pathogen for many oraldiseases, such as oral mucositis, periodontitis, peri-implantitis, endodontic infections and even dental caries. The potential presence of S. aureus is especially important in dental infections due to its increased resistance. Therefore, it is very logical to check the status of microbial resistance against the commonly used antibiotics for the treatment of dental infections that occur by *S. aureus* [6].

Materials and Methods

Synthesis of Silver Nanoparticles (NAg)

The silver nanoparticle synthesis was carried out by adding the chemical-reducing agent sodium borohydride to the silver nitrate solution. In brief, under magnetic stirring conditions, 30ml of 0.0020M sodium borohydride was added with 10ml of 0.0010M silver nitrate through a burette or dropper. The colour of the reaction medium was changed to yellow.

Incorporation of NAg and NaF into Resin Modified Glass Ionomer

Commercially available resin-modified glass-ionomer cement (GC) was used as the parental system. NAg was added into GC at a mass fraction of 0.15%. Mass fractions greater than 0.15% were not used to avoid compromising the mechanical properties of the parental system based on preliminary experiments. Sodium fluoride (NaF) was obtained commercially, and four mass fractions of NaF were incorporated into NAg-containing GC: 5%, 10%, 20%, and 30%. Mass fractions greater than 30% were not used because of reductions in enamel bonding strength.

UV - Vis Spectroscopy

Chemically reduced silver nanoparticles can be easily observed by ultraviolet-visible (UV-Vis) spectroscopy. The chemo-reduction of the Ag+ ions in solutions was monitored by periodic sampling of aliquots (1 mL) of the aqueous component and measuring the UV-Vis spectra of the solution. UV-Vis spectra of these aliquots were monitored as a function of the time of reaction on a spectrophotometer in the 400–600 nm range operated at a resolution of 1 nm.

TEM Analysis

The transmission electron microscopy (TEM) technique was used to visualize the morphology of the Ag NPs. The 200 kV High-resolution transmission electron microscope. TEM grids were prepared by placing 5 μL of the AgNP solutions on carbon-coated copper grids and drying them under the lamp. Additionally, the additional presence of metals in the sample was analyzed by using HR-TEM.

Preparation of GIC Disks

The following four groups were included in the subsequent experiments: TB control**,** glass-ionomer cement (GC) control, GC+NAg, and GC+NAg+20%NAC (N-acetylcysteine)**.** The cover of a 96-well plate was used as the mould to prepare cement specimens. Cement pastes were placed in each well of theplate cover and light-cured for 1 minute. The cement disks were approximately 8 mm in diameter and 0.6 mm in thickness. The cured disks were immersed in distilled water and stirred using a magnetic barat 200 rpm for 1 hour to remove the initial burst of uncured monomers.

Inhibition of Bacteria by Orthodontic Cement

Staphylococcus aureus was used to evaluate the antibacterial ability of orthodontic cement because itis the primary bacteria causative of dental caries. The *S. aureus* stock was added into brain-heart infusion (BHI) broth and incubated at 37°C anaerobically overnight. The *S. aureus* suspension was diluted to approximately 10 7 colony-forming units (CFU)/mL to prepare the inoculation. Each cement disk was placed in a well of a 24-well plate and immersed in 1.5 mL of inoculation medium. The cement disks were incubated at 37° C in 5% CO_2 for 24 hours. The disks were then transferred to a new 24-well plate containing 1.5 mL fresh BHI broth in each well and incubated at 37° C for another 24 hours. A total of 48 hours of incubation was able to form a mature biofilm on cement disks.

Biofilm Metabolic Activity Assay

The 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) is a colourimetric assay that measures the enzymatic reduction of MTT. The MTT assay was conducted to evaluate the metabolic activity of biofilms grown on the cement disks. Briefly, each disk with 2-day biofilm was immersed in 1 mL of MTT solution and incubated at 37°C for 1 hour. The specimens were then transferred to a new24-well plate containing 1 mL of dimethylsulfoxide (DMSO) in each well and incubated in the dark for20 minutes. After that, the absorbance of the DMSO solution at 540 nm was determined.

Biofilm CFU Count

The 2-day biofilm was harvested in phosphate-buffered saline by scraping from the cement disks followed by sonication and vortexing. The bacterial suspensions were serially diluted and spread onto BHI agar plates. The plates were incubated at 37°C for 48 hours. The CFU counts were determined by counting the colony number on the BHI plates.

Cytotoxicity Analysis

After obtaining informed consent oral submucous cells were obtained from healthy human adults. The fibroblast was obtained by

digestion with the enzyme collagenase and by using Dulbeccos Modified Minimum Essential Medium (DMEM) supplemented with 20 per cent fetal bovine serum. The tryptic activity of trypsin was neutralized by the addition of culture media containing 10 per cent fetal bovine serum. The fibroblast was resuspended in fresh DMEM containing 20 per cent fetal bovine serum and 10% DMSO. Each cement disk was placed in a 48-well plate and immersed in 500 μL of Dulbecco's Modified Eagle Medium (DMEM), yielding the mass ratio of cement surface area to solution volume tobe approximately 1.8 cm^2 /mL, following the recommendation of the International Standards Organization (ISO). The disks were incubated at 37° C in 5% CO_2. The culture medium was replaced daily. The extracts were collected on days 1, 4, and 7 and stored at 28°C for cytotoxicity analysis.

Results

Synthesis of AgNPs

In this study, using silver nitrate as the starting material and sodium borohydride as a reducing agent synthesized Ag NPs were prepared. Which is a chemical reduction method, the reaction of sodium borohydride and silver nitrate showed a yellow colour appearance (Fig. 1).

Figure 1. Chemical Synthesis of Silver Nitrate

Physical Characterization

Synthesized silver nanoparticle solution may be easily observed by ultraviolet-visible (UV-Vis) spectroscopy. The UV-Vis spectrum of NAg showed an absorption peak at 420nm (Fig. 2).

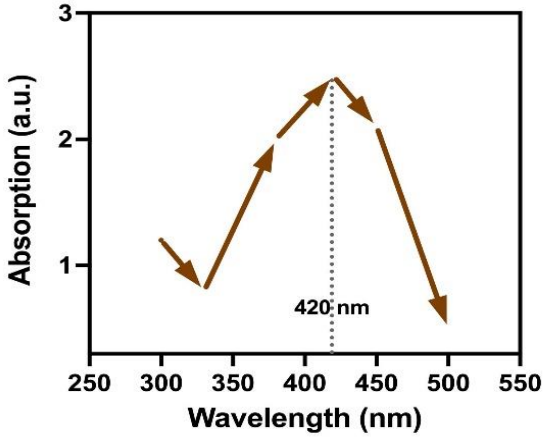

Figure 2. UV-VIS Spectral Analysis of Chemically Synthesized AgNPs

When TEM analysis was performed to determine the morphology of the Ag NPs, it was found that the synthesized NAg is composed of fine spherical particles with an average size of approximately 20 nm (Fig 3). Formation of *S.aureus* biofilms in the prepared cement materials (Fig.4).

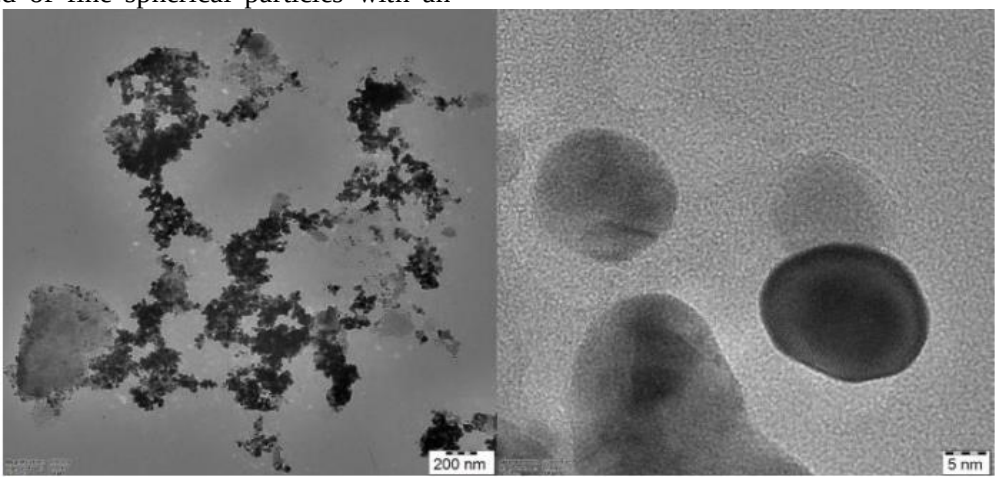

Figure 3. TEM Analysis of Chemically Synthesized AgNPs

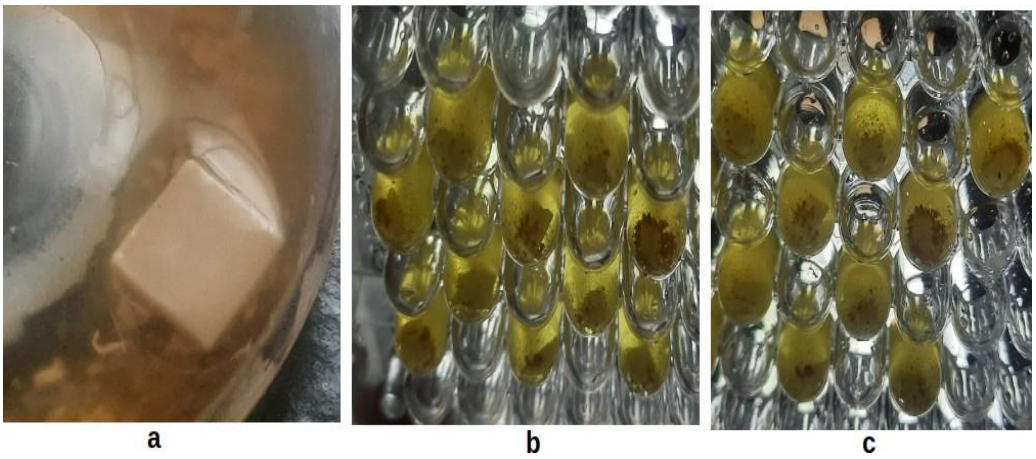

Figure 4. Biofilm Formation of S. Aureus Over the GIC Disks

Staphylococcus aureus was used to evaluate the ability of orthodontic cement to inhibit bacteria, the results showed that 20% GC+Nag+NaF inhibited bacteria to a greater extent, where a clear zone of inhibition was found (Fig5 a). In the GC+NAg+NaF against *Staphylococcus aureus*, the zone of inhibition was high in 20% of GC+NAg+NaF (Table 1). GC control had the highest metabolic activity and the metabolic activity was significantly reduced (Fig 5 b). When assays were performed to determine the metabolic activity CFU counts in GC control were close to 10^7 CFU/disk, which was slightly higher than that of GC+NAg ($P < .05$). The CFU counts of GC+NAg+NaF were found to decrease to 10^6 CFU/disk ($P<.05$). (Fig 5 c). The cell viabilities against the extracts of orthodontic cement were performed to determine the cytotoxicity, the results revealed the eluents of orthodontic cements reduced cell viability as compared to the control (Fig 5 d). The cell viability of GC+NAg+15% NaF were 79.7%,75.6%, and 73% for days 1, 4, and 7 respectively, which were lower than TB control and GC control ($P < .05$) but higher than GC+NAg ($P < .05$). Day 7 has shown a sensitive assay with excellent linearity up to ~10 cells per well.

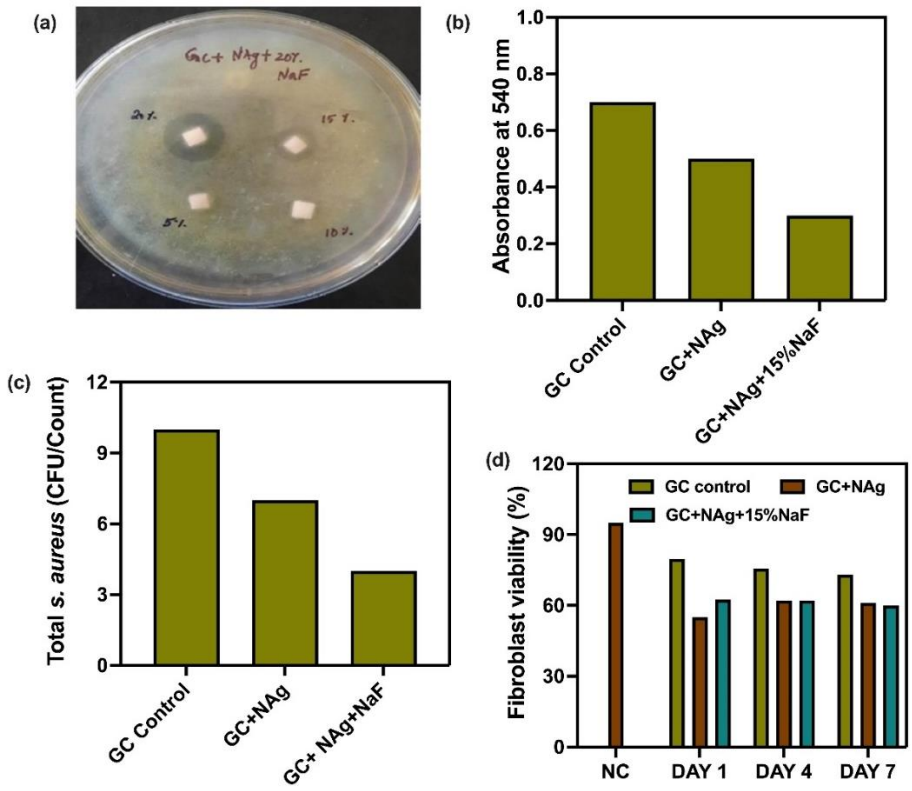

Figure 5. Overall Results

Table 1. Inhibition of Staphylococcus Aureus

S. No	GC + Nag + NaF (%)	Observation
1.	5%	No inhibition
2.	10%	No inhibition
3.	15%	Found to inhibit
4.	20%	Found to inhibit

Discussion

AgNPs were prepared by the chemical reduction method and during the synthesis process, the reductant (C6H5O7Na3) directly reduced Ag+ to generate metallic Ag atoms. The produced Ag atoms then acted as nucleation centres and catalyzed the reduction of the remaining metal ions present in the solution. At the beginning of the process, the newly reduced Ag atoms acted as the nuclei of the nanoparticles. With further processing time, these nuclei grew continuously [7]. Indication of the formation of AgNPs was observed by the change in the solution colour. During this process, the solution went through several colour changes from light yellow- yellow-greenish, before it stabilized. These colour changes indicate the growth of the AgNPs [8].

Synthesis of silver nanoparticles has become possible using (NaBH4) as a reducing agent and using AgNO3 as a reductant. These gave a dark yellowish colour when synthesized by a protective layer of borohydride ions. The Ag solution became yellowish because of the

absorption of wavelength at 386nm. As Silver nanoparticles are very delicate to the absorption of light, they interact with light due to a very high dielectric constant that makes the light response occur in the visible region [9]. Thus, silver nanoparticle absorption and scattering properties can be tuned by controlling the particle size, shape, and the local refractive index near the particle surface. Silver nanoparticles are harmful to the environment, due to which mesocosms are created to protect them [10].

A major concern for adding bioactive agents into orthodontic cement is the reduction of bonding strength. The minimum SBS recommended for clinical use is approximately 7.8 MPa, which is high enough to prevent accidental debonding but not too high to hinder the removal of brackets after treatment. In this study, the SBS of GC+NAg+20%NaF was 10.25 MPa. Which is high enough to prevent accidental debonding but not too high to hinder the removal of brackets after treatment. The previous study showed the SBS of GC+NAg+20%NAC was 8.25 MPa. This value was slightly higher than the minimum recommended SBS, indicating that it was acceptable for orthodontic practice [11]. However, future studies are still needed to improve bonding strength to guarantee acceptable behaviour in clinical practice. In contrast to a recent study in which increased adhesion of sealer was observed after adding NAC. This difference could be attributed to two factors. First, enamel was used in this study, whereas the previous study used dentin. Second, a bracket-tooth model was used to test the SBS in this study, whereas the previous study used a push-out test to evaluate adhesion [13-15].

In this study, the antibacterial activity of GC against *s aureus* was enhanced by adding Nag, when the mass fraction of NAg reached 0.20% where 0.15% NAg was used. It is believed that the antibacterial action of the Ag ion is due to its ability to inactivate the vital enzymes of bacteria, which inhibits DNA replication, thus causing bacteria death. Consistent with previous studies demonstrating the antibacterial capability of NAg against oral bacteria, the antibacterial activity of GC against *S mutants* was enhanced by adding NAg in this study. Significant reductions in mechanical properties were observed when the mass fraction of NAg reached 0.20% in the pilot study. Thus, 0.15% NAg was used to achieve a balance between antibacterial capability and mechanical properties [10].

The efficacy of NAC in inhibiting biofilm metabolism is well documented, although the mechanisms have not been fully elucidated. Current study suggests the antibacterial ability of NAC is likely related to the inhibition reaction with bacterial cell proteins, and disturbance of intracellular redox equilibrium. In this study, adding NaF into Nag-containing cement reduced the metabolic activity and CFU counts of *S aureus* biofilm by 20.5%, 32.2%, and 69.1%, respectively, indicating the combined use of NAg and NaF is more effective in inhibiting bacterial metabolism than using NAg alone [11].

The increasing presence of MRSA in the oral cavity is an immense public health threat that cannot be downplayed, given its potential for enhanced MRSA transmission. Moreover, it introduces new dimensions to the already intensified debates on whether or not to administer antibiotic prophylaxis to at-risk dental procedure candidates. Probably, the choice needs to be made on a case-by-case basis [16-18]. It follows then that newer therapeutic agents are needed more urgently than previously. Admittedly, there is very limited data to inform on the interaction of *S. aureus*, and therefore MRSA, with the oral microbiota, and the extent to which the oral cavity mediates *S. aureus*- and MRSA-caused endocarditis as a sequel to dental procedures [19]. Additionally, it is largely unclear whether the presence of

MRSA in the oral cavity reflects disease or carriage. Subsequent studies in the area could focus on filling these identified knowledge gaps. Principally, researchers undertaking MRSA carriage studies may need to concurrently screen for oral and nasal colonization [20].

Conclusion

This study successfully developed an orthodontic cement with antibacterial capabilities and acceptable biocompatibility by incorporating NAg and NaF into an orthodontic cement. Incorporating Sodium fluoride (NaF) and nano silver particles (NAg) into orthodontic cement might be an effective and safe approach to inhibit bacteria in orthodontic practice.

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Conflict of Interest

The authors declare no competing interest.

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