Bioglass Influence on Tooth Surface Biofilm Eradication to Control Dental Caries

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**Abstract: Aim**: To investigate the role of silver-integrated calcium fluoride bioglass in inhibiting the growth of oral pathogens. **Materials and method**: The synthesized silver-integrated CaF2 bioglass was prepared using sol-gel method. Then evaluated against the common oral pathogen *Streptococcus mutans* to assess the antimicrobial properties of the bioglass using crystal violet assay together with minimum inhibitory concentration (MIC). The research data underwent analysis against pure microbial cell growth ang Ag-CaF2-Bioglass. **Results**: The results revealed that Ag-CaF2-Bioglass effectively killed Streptococcus mutans among the studied oral microbial strains. The testing data from crystal violet assay and MIC values demonstrated superior antimicrobial behavior of silver-infused bioglass relative to the control thus proving effective bacterial growth reduction. **Conclusion**: Ag-CaF2-Bioglass exhibits improved capability to inhibit the growth of Streptococcus mutans, which triggers this material for future dental applications to fight against oral infections to improve oral health. The research requires extended investigation for better understanding of long-term operation and safety implications.

**Keywords**: Calcium fluoride; Silver; Bioglass; *Streptococcus mutans;* Antimicrobial

# INTRODUCTION

Oral bacteria primarily colonize and cause disease in the oral cavity because they have very particular adhesion mechanisms. Nonetheless, oral infections are recognized to be the cause of infectious endocarditis and can cause systemic illness [(Aparna et al., 2021; Ganapathy & Professor and Head of Department of Prosthodontics, 2021; Verma & Muthuswamy Pandian, 2021)](https://paperpile.com/c/mOlfrC/wl4Tj+pCG1u+IV6yy). The oral cavity is the source of pathogens collected from an infected location because certain oral bacteria are not restricted to the mouth but can be found in other parts of the body [(Ganapathy & Professor and Head of Department of Prosthodontics, 2021; Gendron et al., 2000; Pandiyan et al., 2022; Poornima et al., 2021)](https://paperpile.com/c/mOlfrC/tDyPk+5rCQp+yFsQT+gn1Bx).

Bioactive glass was first developed for treating bone defects. It has the ability to bond with bone and promote bone growth, showing promising bioactivity and biocompatibility for forming connections with both hard and soft tissues [(Dai et al., 2020)](https://paperpile.com/c/mOlfrC/lmQ4T). The process by which bioactive glass promotes bone regeneration occurs in stages, starting with the precipitation of calcium phosphate, followed by the formation of bonelike apatite on its surface, which then binds to hard tissues [(Stoor et al., 1998a)](https://paperpile.com/c/mOlfrC/qwST)[(Stoor et al., 1998b)](https://paperpile.com/c/mOlfrC/CQjm)[(Stoor et al., 1998a)](https://paperpile.com/c/mOlfrC/qwST). Bioactive glass can mineralize dentin tubules, providing long-term relief from tooth pain and hypersensitivity caused by external stimuli [(Merchant et al., 2025; Shenoy et al., 2022, 2023)](https://paperpile.com/c/mOlfrC/TKlrp+MYFJ4+isZF8). The remineralization process starts with the material dissolving in an aqueous solution, leading to a rise in pH [(Chokkattu et al., 2022; Merchant et al., 2022; Ramamurthy et al., 2022)](https://paperpile.com/c/mOlfrC/r3eWo+h4eB5+Ahv9c). This elevated pH encourages the precipitation of hydroxyapatite (HA), the primary component of mineralized enamel and dentin. Calcium and phosphate ions from the bioactive glass and saliva provide the necessary substrates for mineralization [(Jones, 2013)](https://paperpile.com/c/mOlfrC/YWUTs). It was believed that calcium, fluoride, and phosphate reacted when bioglass applied topically and that is advantageous[(Jain & Verma, 2022; Marya et al., 2022; Wadhwani et al., 2022)](https://paperpile.com/c/mOlfrC/4hhNJ+cYz6r+G5h42). The possibility of it offering direct protection to the enamel surface or supplying free fluoride ions for later integration into the hydroxyapatite lattice [(Rølla, 1988)](https://paperpile.com/c/mOlfrC/go5df). In order to improve tooth remineralization, the nano-CaF2 can be utilized as an efficient anti-caries agent by raising the labile fluoride concentration in oral fluid. Additionally, it can be very helpful in treating dentin permeability reduction [(Sun & Chow, 2008)](https://paperpile.com/c/mOlfrC/Bd3oH). Many calcium phosphate technologies have been created to improve fluoride's capacity to stop demineralization and encourage remineralization [(Cochrane et al., 2014)](https://paperpile.com/c/mOlfrC/6JbFj). Calcium fluoride-like substance, makes up the majority of the fluoride on teeth after topical administration, and it is fairly stable in the mouth.[(Rølla & Saxegaard, 1990)](https://paperpile.com/c/mOlfrC/lx59a).

Dentine caries could be stopped and dentine collagen breakdown could be avoided with the supplementary administration of AgNO3. The remineralization of teeth is influenced not just by fluoride content but also by the availability of calcium and phosphate ions [(Yu et al., 2018)](https://paperpile.com/c/mOlfrC/K9g38). Silver diamine fluoride (SDF) has demonstrated the ability to prevent the development of complex cariogenic biofilms on dentin surfaces. Clinical trials have confirmed its effectiveness in both preventing and halting the progression of caries. SDF offers a non-invasive treatment option for carious lesions by avoiding the need to excavate superficial infected layers, thereby reducing the risk of exposing the pulp [(Zhao et al., 2017)](https://paperpile.com/c/mOlfrC/LlIjb). A biocompatible solution containing sodium fluoride and silver nanoparticles coated with polyethylene glycol was created. This solution exhibits antibacterial properties against *Streptococcus mutans* without causing tooth discoloration, making it suitable for use as an anti-caries treatment [(Yin et al., 2020)](https://paperpile.com/c/mOlfrC/HgwvJ).

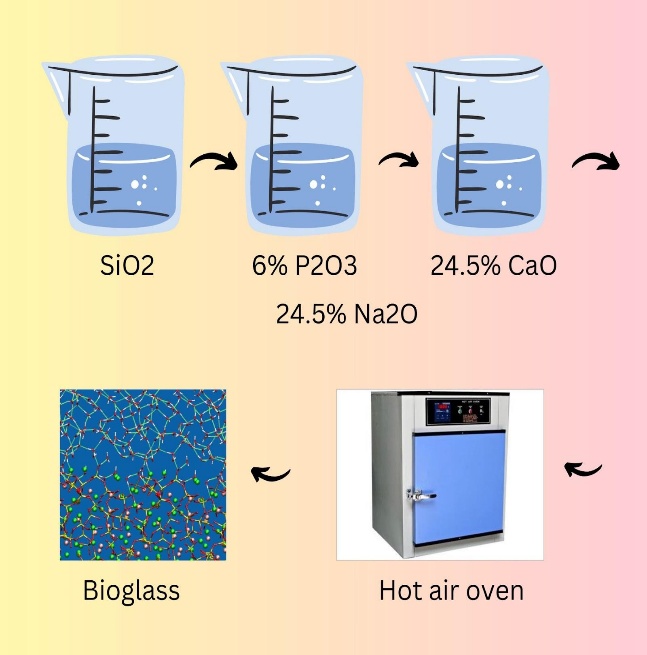
# MATERIALS AND METHODS

## Materials

SRL supplies both CaF2 with 97% purity and molecular weight 78.08 and CaO with 95% purity and molecular weight 56.08. The AgNO3 with the molecular weight of 169.87 was also purchased from SRL with the purity of 99.9 %. NaNO3 and Ortho-phosphoric acid (85% concentration) are supplied by EMPLURA, while Tetraethyl orthosilicate is obtained from Sigma-Aldrich. Finally, Calcium Nitrate Tetrahydrate (molecular weight 236.15) is also sourced from EMPLURA.

## Bioglass of synthesis

To synthesize silver-infused calcium fluoride bioglass was developed, SiO2 (45%), P2O5 (6%), CaF2 (5%), CaO (24.5%), Ag (2.5%), and Na2O (24.5%) were utilized. TUsing ethanol and double-distilled water the components were mixed to form an even blend, which we termed as sol like paste. A paste was prepared using a teflon beaker, followed by drying and sintering at 700 ° for achieving proper densification and crystallization of the bioglass. After cooling, the bioglass. In vitro remineralization studies were conducted on extracted human dentin samples or simulated dentin substrates to evaluate the bioglass's potential for remineralization. Ethical considerations were addressed by obtaining informed consent and adhering to relevant guidelines for the use of human samples [(S et al., 2022)](https://paperpile.com/c/mOlfrC/yXhTN) [(Chitra et al., 2020)](https://paperpile.com/c/mOlfrC/Wo1UU).



**Figure 1:** Picture representing the synthesis of bioglass.

## Dentin slab preparation

The dentin slabs were prepared by carefully sectioning human teeth to expose the dentin surface. To induce demineralization, the slabs were immersed in a hydrochloric acid (HCl) solution and then thoroughly rinsed to remove residual acid. The demineralized dentin slabs were subsequently treated with 17% ethylenediaminetetraacetic acid (EDTA) for five minutes, followed by another rinse with double-distilled water to open the dentinal tubules and eliminate remaining mineral traces.

## Mineralization protocol

The remineralization potential of silver-infused calcium fluoride bioglass was tested using demineralized dentin slabs from human teeth. The prepared demineralized dentin slabs were then subjected to a remineralization test, where the silver-infused calcium fluoride bioglass was applied directly to the surface and brushed twice daily. Samples containing artificial saliva, bioglass were incubated for further observation. After seven days, the samples were analyzed to calculate the results.

## Minimal inhibitory concentrations

Minimum inhibitory concentration was performed to determine antibacterial activity of synthesized silver infused calcium fluoride bio glass. Minimum Inhibitory Concentration is defined as the minimal concentration of the drug that inhibits the visible growth of microorganisms under overnight incubation. MIC of silver infused calcium fluoride bio glass for *S. mutans* by broth microdilution technique (10-1 to 10-5) was carried out with the help of BHI broth according to the methods described by CLSI. The absorbance of samples was measured in a microplate reader (BioTek, Synergy) at the wavelength of 595 nm. BHI broth medium and S. mutans-inoculated BHI broth without drugs were maintained as blank and control, respectively [(Selvaraj et al., 2024)](https://paperpile.com/c/mOlfrC/4zpG3) [(Shivalingam et al., 2024)](https://paperpile.com/c/mOlfrC/Vpvmi).

## Crystal violet assay

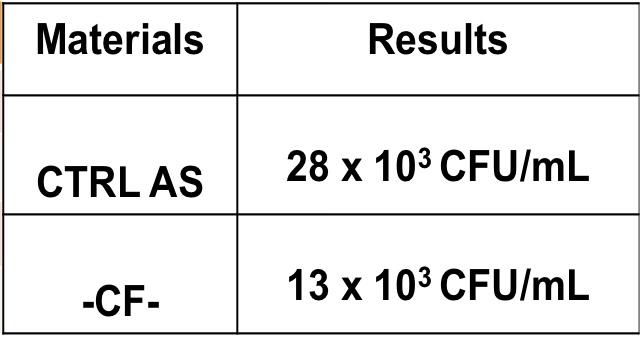
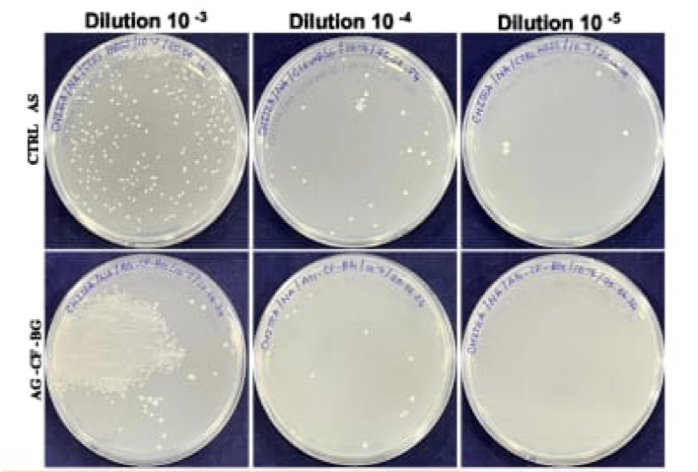
For crystal violet assay gently wash the sample with PBS for 3 to 4 times . Add 0,1% or 0.2% of crystal violet solution for 20 min . Then remove the dentin samples from the crystal violet solution. Then gently wash the dentin again with PBS. Transfer the stained dentin to 95% ethanol which is incubated for 30 min . Then transfer the solution in a microtiter plate . Measure it in a spectrometer at 590 nm. The positive control is the control group and the negative control is those without bacterial inoculation . Biofilm mass can be calculated by subtracting negative control from adsorbance .

# RESULTS

CTRL (Control): The result shows 28 x 10^3 CFU/mL, indicating a higher number of colonies and thus, more bacterial growth.

CF-BG (Calcium fluoride -infused Bioactive Glass): The result shows 13 × 10^3 CFU/mL, indicating a lower number of colonies, thus, less bacterial growth compared to the control. This shows that the CF-BG treatment has a significant inhibitory effect on bacterial growth compared to the control.The MIC for *Staphylococcus aureus* was 25 µg/mL (Chehelgerdi et al., 2023). The addition of silver nanoparticles can reduce Staphylococcus aureus biofilm formation [(El-Wassefy et al., 2018)](https://paperpile.com/c/mOlfrC/mPUdr). The number of *Streptococcus mutans* counts in the Log 10 CFU of Groups 1 to 3 (i.e., treated with silver nitrate ) are significantly less than that of Group 4 which is in the presence of water.[(Yu et al., 2018)](https://paperpile.com/c/mOlfrC/K9g38). The CFU analysis of the Streptococcus mutans biofilm revealed that incorporating nCaF2 into a nanocomposite had no antibacterial effect unless dimethylamino dodecyl methacrylate was also included (Saadh et al., 2024). However, further findings showed that adding dimethylamino dodecyl methacrylate to the nCaF2 nanocomposite resulted in a significant reduction in the CFU count for the Streptococcus mutans biofilm, with a decrease of 6 logs compared to other groups [(Alhussein et al., 2023)](https://paperpile.com/c/mOlfrC/qlVRY).

## Minimal Inhibitory Concentration (MIC)



(a) (b)



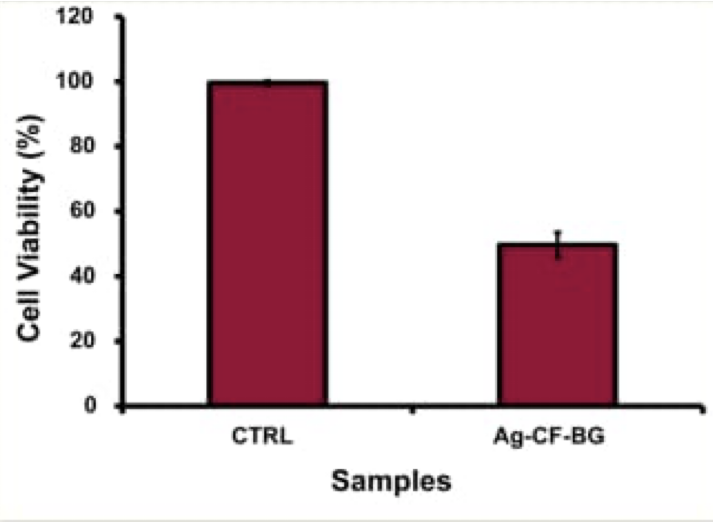
(c)

**Figure 2:**  (a)(b)(c) Minimal inhibitory concentration of the silver infused calcium fluoride bioglass compared to the control group.

## Crystal Violet

The bar graph shows the results of crystal violet assay, which measures cell viability. The two samples compared are control, which is pure dentin slabs with microbes and calcium fluoride infused bioactive glass, which is calcium fluoride treated dentin slabs with microbes. In the control group the cell viability is near 100% indicating that the control group cells are healthy and unaffected. In CF-BG the cell viability is significantly lower, around 45%, suggesting that the CF-BG treatment substantially reduces cell viability.

This shows that CF-BG has a cytotoxic effect, leading to a reduction in cell viability compared to the control and can target pathogenic viruses. The optical density measured by crystal violet staining decreased as the concentration of monocalcium phosphate increased [(Alostad, 2020)](https://paperpile.com/c/mOlfrC/kyViM). Following treatment with immunoglobulin-loaded amorphous calcium phosphate, the CFU count and adhesion rate of Streptococcus mutans were significantly lowered. The crystal violet staining appeared lighter, and staining of Streptococcus mutans indicated an increase in dead cells [(Alostad, 2020; Yan et al., 2022)](https://paperpile.com/c/mOlfrC/kyViM+fEA4p). Crystal violet staining results demonstrated that irresistible-16, at concentrations of 0.061 and 0.122 μM, showed significant anti-biofilm activity against Streptococcus mutans [(Hu et al., 2022)](https://paperpile.com/c/mOlfrC/kiSCR) .



**Fig 3:** Bar graph showing the crystal violet assay of the silver infused calcium fluoride bioglass compared to the control group.

# DISCUSSION

The results of our study illustrate a significant improvement in tooth remineralization when Vitamin D is combined with Bio Glass supplementation. This finding proposes that this combination may be a promising methodology for improving the normal remineralization process of teeth [(Elliott & FitzSimons, 2009)](https://paperpile.com/c/mOlfrC/7QXMl). These findings are consistent with earlier research demonstrating the role of Vitamin D in enhancing calcium absorption and promoting enamel remineralization [(Aydin & √ñzdemir, 2022)](https://paperpile.com/c/mOlfrC/7xXci). Bio Glass, on the other hand, is recognized for its ability to release calcium and phosphate ions, creating an ideal environment for enamel remineralization [(Chokkattu et al., 2023; Solanki et al., 2023; Sreevarun et al., 2023)](https://paperpile.com/c/mOlfrC/f52VU+Op3SG+O4aAO). The combination of these two components may synergize, enhancing each other's remineralization potential [(Herzog, 2015; Ramadoss et al., 2022)](https://paperpile.com/c/mOlfrC/LBZl9+hCIjR).

Silver-infused calcium fluoride bio glass plays a significant role in inhibiting growth of oral pathogens due to its unique antibacterial properties [(*Anti-Inflammatory Potential Mouthwash Formulated Using Clove Ginger Mediated Zinc Oxide Nanoparticles: Vitro Study*, n.d.; Laghari et al., 2023; Muthuswamy Pandian et al., 2022)](https://paperpile.com/c/mOlfrC/P9zBJ+svix8+14qZZ). Silver ions have broad-spectrum antimicrobial efficacy which disrupt bacterial cell membranes and interfere with their metabolic processes leading to cell death.

When incorporated into bio glass ions are gradually released, providing a sustained antibacterial effect[(Adel et al., 2023; Singh et al., 2024; Subramanian & Harikrishnan, 2023)](https://paperpile.com/c/mOlfrC/rd1TQ+h71Yz+TxYPx). Calcium fluoride enhances this action by releasing fluoride ions, which strengthen tooth enamel and create an unfavorable environment for bacterial colonization. The combination of silver and fluoride in the bio glass targets harmful bacteria and supports overall oral health by preventing demineralisation and promoting remineralisation of the teeth. This dual-action mechanism makes silver-infused calcium fluoride bio glass a promising material in dental applications.

# CONCLUSION

Silver-infused calcium fluoride bio glass shows great potential in inhibiting the growth of oral pathogens. This innovative material offers a novel and effective strategy for combating these harmful microorganisms. With ongoing research and development, silver-infused calcium fluoride bio glass could greatly enhance dental care and improve patient outcomes.

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