Formulation and Assessment of Silver Infused Calcium Fluoride Bioglass for Remineralization of Dentin

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**Abstract:** Bioactive glass (BAG) has revolutionized dental care, prompting research into innovative solutions for remineralizing dentin. This study introduces a novel silver-infused calcium fluoride bioglass (Ag-CaF2-BG) designed to produce an effective remineralization of dentin. By combining specific components, including calcium fluoride and silver nitrate, the bioglass was synthesized. The synthesised Ag-CaF2-BG was characterised using XRD (X-ray diffraction), FE-SEM (Field Emission Scanning Electron Microscopy), EDS (Energy Dispersive Spectroscopy), and Raman spectroscopy. Subsequently, demineralized human dentin slabs were treated with bioglass to assess its remineralization ability. The bioglass was found to include critical phases that support remineralization. Scanning electron microscopy (SEM) imaging revealed a characteristic flake-like morphology, while energy-dispersive X-ray spectroscopy (EDS) confirmed significant components within the bioglass. Examination of dentin subjected to Ag-CaF2-BG treatment through SEM reveals a refined and cohesive surface texture, marked by closely packed remineralized mineral crystals that signify improved adhesion and structural enhancement.Conclusion Overall, Ag-CaF2-BG demonstrates promising potential for dentin remineralization. Future research endeavors should concentrate on optimizing the composition of the bioglass and **validating its safety and efficacy through clinical trials.**

**Keywords**: Dentin remineralisation, silver infusion, Calcium fluoride bioglass.

# Introduction

Dental caries, characterized by demineralization of tooth structure, remains a prevalent oral health concern globally, with 54.16% overall prevalence in the Indian population[(Pandey et al., 2021)](https://paperpile.com/c/7guoRD/P4VG). It poses significant challenges for clinicians in managing its progression and sequelae, compromising the quality of life to a greater extent. While preventive measures such as fluoride-containing dental products have shown efficacy in reducing caries incidence[(Munteanu et al., 2022)](https://paperpile.com/c/7guoRD/EWMj), the quest for novel remineralization agents persists to enhance therapeutic outcomes.In the pursuit of advancing dental care, researchers continuously explore innovative materials and techniques to address the prevalent issue of dentin demineralization. Traditional restorative approaches often involve the removal of decayed dentin followed by the placement of synthetic materials[(Pavithra et al., 2023; Shenoy et al., 2023; Thomas & Jain, 2023)](https://paperpile.com/c/7guoRD/UlyT+ScAi+yMpS). However, such interventions may compromise the structural integrity of the tooth and fail to address the underlying issue of demineralization [(Abou Neel et al., 2016)](https://paperpile.com/c/7guoRD/sVw5).The development of remineralizing agents presents a paradigm shift in dental care, aiming to restore the mineral content of demineralized dentin and halt the progression of carious lesions.Among the array of promising solutions, bioglass-based formulations have emerged as a compelling avenue for promoting remineralization—a crucial process in combating tooth decay and preserving dental health[(Ramadoss et al., 2022)](https://paperpile.com/c/7guoRD/yVAI4). As the first generation of materials sought bio-inert properties to avoid host inflammatory responses, the emergence of bioactive glass (BAG) opened new possibilities. It has been demonstrated that BAG-loaded dental resin-based composite materials, particularly those with high BAG loading, have exceptional mineralization capabilities indicating improved ion release, pH elevation, and apatite production[(Yun et al., 2022)](https://paperpile.com/c/7guoRD/26heB). Resin composites loaded with BAG showed antibacterial and biocompatibility in vitro. We have proposed in this paper, a novel formulation and characteristics of Calcium fluoride bioglass with silver infusion (Ag-CaF2-BG). By harnessing the combined benefits of remineralisation promotion and potent antimicrobial properties, this innovative bioglass presents a promising solution to fortify dentin structure and elevate oral health standards [(Doshi et al., 2023; Lampl et al., 2023; Pandiyan et al., 2023)](https://paperpile.com/c/7guoRD/L9Ph+q0BS+R9Di).The antibacterial qualities of silver have long been known to exist; it inhibits the growth and activity of cariogenic bacteria, which are responsible for the development and progression of tooth caries[(Fakhruddin et al., 2020)](https://paperpile.com/c/7guoRD/BPQxU). Their remarkable capacity to infiltrate bacterial cell walls and modify membrane structure ultimately results in cell demise. Meanwhile, calcium fluoride bioglass, renowned for its biocompatibility and remineralization potential, serves as a reservoir for calcium and fluoride ions, essential components for enamel and dentin remineralization[(Abdul Jalil et al., 2020; Li et al., 2023)](https://paperpile.com/c/7guoRD/Tgsj4+q2PcR). By combining these two components, Ag-CaF2-BG offers a multifaceted approach to caries management, addressing both bacterial proliferation and mineral loss in the tooth structure [(Janani et al., 2021; Kachhara et al., 2021; Subramanian et al., 2023)](https://paperpile.com/c/7guoRD/Gc8Ry+nKjDb+gE6A7).Despite advancements in dental materials science, several knowledge gaps persist regarding the formulation and assessment of Ag-CaF2-BG for dentin remineralization. Addressing these gaps is paramount to harnessing the full therapeutic potential of this innovative biomaterial in clinical practice. Therefore, this study aims to investigate the formulation parameters, antimicrobial efficacy, and remineralization potential of Ag-CaF2-BG and its suitability for dentin remineralization in the context of dental caries management.

# Materials and methods

## Materials

All the chemicals used are analytical grade and are used without any additional purification. Tetraethyl orthosilicate silica (TEOS), with a molecular weight of 208.33 g/mol, was procured from Sigma-Aldrich (United States) to be used as the precursor of silica. Chemical compounds, including calcium fluoride CaF2 (97% purity with a molecular weight of 78.08 g/mol) was procured from SRL, India. Silver nitrate (AgNO3) is also procured from SRL India with the molecular weight of 169.87 g/mol and 99.9% purity. sodium nitrate (NaNO3) with the M.W of 84.99 g/mol, orthophosphoric acid (H3PO4) with the M.W. of 98 g/mol, with a purity of 85%; calcium nitrate (Ca(NO₃)₂) with the M.W of 236.15 g/mol were purchased from Merck (India).

## Synthesis of the Bioglass

silver-infused calcium fluoride bioglass (Ag-CaF2-BG) was synthesized to analyze the remineralisation potential of demineralised dentin. To synthesise bioglass TEOS [SiO2 (45%)], orthophospharic acid [P2O5 (6%)], CaF2 (5%), Calcium nitrate [CaNO3 (24.5%)], silver nitrate [AgNO3 (2.5%)], and sodium nitrate [NaNO3 (24.5%)] were utilized. TEOS was dissolved in an ethanol and double-distilled water mixture. 6% H3PO4 and 5% CaF2 were separately prepared and included in TEOS to form a solution. After complete gelation of the solution, Ca(NO₃)₂ and NaNO3 were individually dissolved and drop-wise added to the above-mentioned solution. Then, 2.5% of AgNO3 was dissolved and added to the bioactive solution mixture (as shown in Fig. 1). Ageing time was provided for 24 h, followed by drying and sintering (700°C for 3 h) in order to properly maintain the density and crystalline properties of the bioglass.

## Characterisation tools

To analyse the properties of synthesised materials, XRD (X-ray diffraction), FE-SEM (Field Emission Scanning Electron Microscopy), EDS (Energy Dispersive Spectroscopy), and Raman spectroscopy were used. X-ray diffraction (BRUKER, D8 Advances) used to study the crystalline parameters of the material. FE-SEM (JEOL, JSM –IT 800) is used to analyze the morphological properties of material and dentin. Energy Dispersive Spectroscopy (Oxford Instruments) was used to evaluate the elemental composition of the material. Raman spectroscopy was employed to analyze the functional group properties of the materials; Confocal Raman Microscope with AFM was used from WITec, Alpha 300RA.

## Preparation of demineralized dentin

In-vitro remineralization studies were performed using extracted human dentin samples or simulated dentin substrates, where the bioglass was applied to assess its remineralisation potential. Ethical considerations were taken into account for the use of human samples, including obtaining informed consent and adhering to relevant ethical guidelines. The results of this study hold promise for developing effective strategies for remineralising dentin and improving dental healthcare. In this study, the remineralisation capability of Ag-CaF2-BG was evaluated using demineralised dentin slabs from human teeth. In order to reveal the dentin surface, the dentin slabs were produced by meticulously sectioning human teeth. The dentin slabs were submerged in a solution of hydrochloric acid (HCl), and any remaining acid was thoroughly rinsed away. This process was used to promote demineralization. The demineralised dentin slabs were next treated with 17% ethylenediamine tetraacetic acid (EDTA) for five minutes and then washed once more with double-distilled water to produce open dentinal tubules and eliminate any leftover mineral traces. After putting the silver-infused calcium fluoride bioglass directly to the surface and brushing it twice a day, the prepared demineralised dentin slabs were then put through a remineralisation examination. The aim of this research is to assess how effectively the bioglass can support the remineralisation of dentin, which could have important implications for dental health and potential applications in restorative dentistry.

# Results

## Structural properties of silver-infused calcium fluoride bioglass (Ag-CaF2-BG)

XRD patterns used to analyse the crystal structure of the material revealed the two main crystalline phases in the silver-infused calcium fluoride bioglass (as shown in Fig. 2a). The first phase identified was Na2Ca2Si3O9, indicating the formation of sodium-calcium silicate in the bioglass composition. The second phase identified was CaSO4Si2O7F2, suggesting the presence of calcium sulphate and silicofluoride in the bioglass structure. These findings indicate the successful formation of the targeted phases in the silver-infused calcium fluoride bioglass, which may have an impactful role in promoting remineralisation of dentin and potentially improving dental health. Similarly, in fluoride bioglass, the fluorapatite phase was predominant at the sintering temperature of 800 °C[(Verné et al., 2008)](https://paperpile.com/c/7guoRD/Xc3UH)Raman spectroscopy is a non-invasive analytical technique that explains the molecular vibrations within the silver-infused calcium fluoride bioglass. Shedding light on its molecular structure and chemical composition explains the vibrations of functional groups. The analysis revealed specific vibrational patterns associated with the bioglass composition (as shown in Fig. 2b), including a prominent peak at 1085 cm-1 signifying the silica (Q4 Si-O-Si) vibration, and another distinctive peak at 945 cm-1, indicating the phosphate peak of PO43-, which indicates the formation of bioactive glass structure. Q4 Si-O-Si vibrations at the wavelength of cm-1 778 denote the varying structures of the silica network. The shift in peaks indicates the successful integration of calcium fluoride and silver in the glass structure. This feature emphasises its potential to facilitate dentin remineralisation and its prospective applications in restorative dentistry. [(S et al., 2022)](https://paperpile.com/c/7guoRD/9jdGp)

## Morphological and elemental analysis

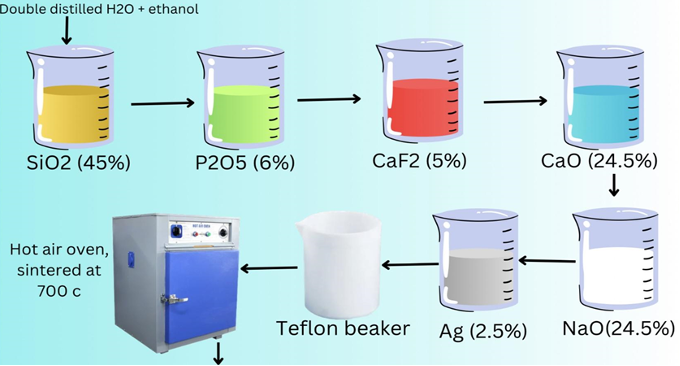
FE-SEM imaging of the Ag-CaF2-BG revealed a flake-like morphology (as shown in Fig. 3a). This means that the surface of the bioglass material appeared to have thin, flat, and plate-like structures resembling flakes. Almost homogeneous flakes were obtained through FE-SEM. According to literature, porous structures were formed in bioglass after the sintering flake crystals were developed due to the heat treatment [(Xin et al., 2010)](https://paperpile.com/c/7guoRD/dKzmc)(10).The EDS examination of the Ag-CaF2-BG (as shown in Fig. 3b) revealed substantial components, including 15.6% silver, 12.8% calcium, and 9.2% fluoride, validating the effective production of the material. These components are essential for their biological uses, particularly in tissue engineering and dentistry. Along with that, carbon (29.2%), oxygen (29.2%), sodium (10. %), silica (3.8%), and phosphate (2.2%) were observed, which explains the formation of bioactive glasses.

## Dentin remineralisation studied

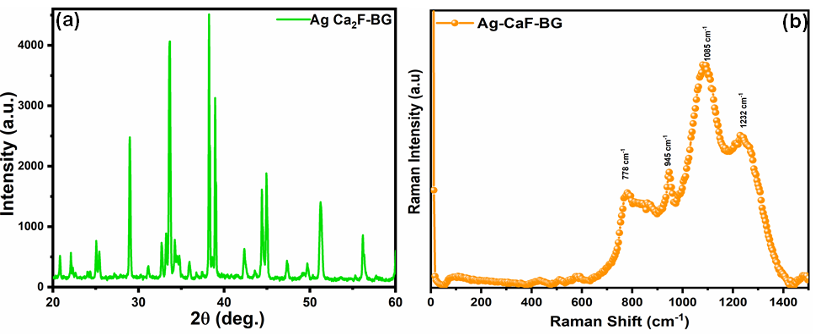
Surface characteristics of demineralised dentin (as shown in Fig. 4a) were seen during the SEM investigation. The most noticeable of them were surface imperfections, which were represented by pits, craters, and uneven terrain. Additionally, open dentinal tubules were clearly visible, indicating greater sensitivity and porosity. Dentin's once distinct structure looked to be destroyed, and the dentin matrix's weakness was apparent.Examination of dentin subjected to Ag-CaF2-BG treatment through SEM (as shown in Fig. 4b) reveals a refined and cohesive surface texture, marked by closely packed remineralised mineral crystals that signify improved adhesion and structural enhancement. Open tubules were precipitated by hydroxyapatite minerals with the incubation of Ag-CaF2-BG. Significant mineral formation with the treatment of Ag-CaF2-BG explicates the rapid remineralisation behaviour of that respective material.Demineralised dentin (as shown in Fig. 5a). showed 17% calcium and 6.8% phosphate, along with carbon (62.1%) and oxygen (14.1%), which indicated a poor appetite on the tooth surface with evolved carbonate. Hence, this EDS spectrum clearly authenticates the poorly formed mineral apatite, which explains the damage in dentin in terms of sensitivity. On the other hand, Ag-CaF2-BG applied dentine (as shown in Fig. 5b) revealed an increase in the weight percentage of elements such as calcium and phosphate, which is 30.3% and 12.2% when compared to demineralised dentine, which is a sign of dentine remineralisation.Hence, from this morphological and elemental analysis, the rapid mineralisation efficiency of Ag-CaF2-BG could be elicited. Occluded tubules explain the mineral apatite precipitation with the support of an elevated calcium phosphate percentage in the elemental composition analysis.

# Discussion

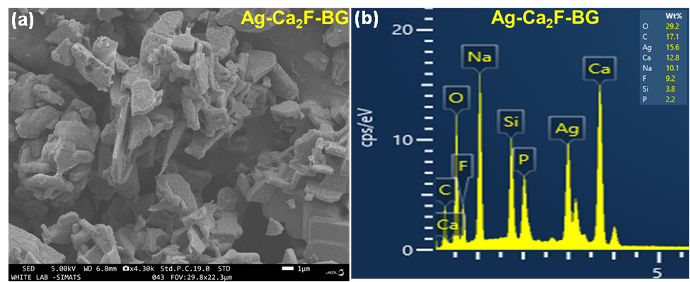
Dentin remineralisation entails a multifaceted interplay of chemical processes orchestrated to reverse the detrimental effects of demineralization. The complete mechanical recovery of the demineralised dentin would result from the regrowth of extrafibrillar and intrafibrillar linear minerals between the fibrils (12). The amorphous form and bioactive composition of calcium fluoride bioglass allow it to have tight interactions with the collagen fibrils found in demineralised dentin. Essential ions, like calcium and fluoride, released by the bioglass serve as catalysts for nucleation and growth, fostering the formation of hydroxyapatite crystals on the dentin surface(Rafi et al., 2024). These ions are crucial to the remineralisation process (13[(Skallevold et al., 2019)](https://paperpile.com/c/7guoRD/SkVMz).Calcium fluoride bioglass (CaF2-BG), a bioactive glass composition incorporating calcium fluoride as a key constituent, presents a compelling amalgamation of biocompatibility, bioactivity, and remineralisation potential [(Shah, 2016)](https://paperpile.com/c/7guoRD/tPHt). The composition's capacity to interact with physiological fluids and promote the development of a physiologically active hydroxyapatite layer on dentin surfaces highlights its bioactivity (Tuluwengjiang et al., 2024). This hydroxyapatite layer, akin to the natural mineral component of dentin, serves as a scaffold for further mineral deposition, thereby promoting dentin remineralization. A study on fluoride-containing bioactive glasses found that substituting CaF2 for CaO increased silicate network connectivity, resulting in improved cell viability and proliferation for mouse osteoblast cells and antibacterial action against E. coli [(Prasad et al., 2020)](https://paperpile.com/c/7guoRD/eubM).Furthermore, the incorporation of calcium fluoride within the bioglass matrix imbues the material with distinct advantages. Calcium fluoride, renowned for its high solubility in acidic environments, augments the material's ability to neutralise acidic byproducts of bacterial metabolism, thereby mitigating the demineralisation cascade inherent to dental caries [(Li et al., 2023)](https://paperpile.com/c/7guoRD/Tgsj4). Moreover, calcium fluoride's propensity to release fluoride ions further fortifies its remineralising efficacy, as fluoride ions actively participate in the formation of fluorapatite, a highly stable and acid-resistant mineral phase integral to dental hard tissue integrity[(Gandhi et al., 2021; Katyal et al., 2023; Priyadharshini et al., 2023)](https://paperpile.com/c/7guoRD/QZWuX+dPlsb+xh2xe).The formulation and assessment of silver-infused calcium fluoride bioglass (Ag-CaF2-BG) for dentin remineralisation present a promising avenue in contemporary dental research. In comparing its efficacy with other remineralisation agents, particularly silver diamine fluoride (SDF) and similar materials, several key considerations emerge. Firstly, the incorporation of silver into calcium fluoride bioglass introduces antimicrobial properties, enhancing its potential to combat caries-causing bacteria within dentin lesions. While SDF also exhibits antimicrobial activity (16, 17[(Piovesan et al., 2021)](https://paperpile.com/c/7guoRD/MRVh8), the potential for staining and discolouration associated with its use (18) may limit its widespread acceptance, particularly in aesthetic regions. In contrast, Ag-CaF2-BG offers a dual benefit of antimicrobial action coupled with the biocompatibility and translucency characteristic of fluoride-based materials, thus presenting a more aesthetically favourable option for remineralisation therapy [(Chokkattu et al., 2023; Dharman et al., 2023; Govindaraj & Shanmugam, 2023)](https://paperpile.com/c/7guoRD/DGJQf+KgLy1+hQnnN).Furthermore, the release kinetics of fluoride ions from the bioglass (Ag-CaF2-BG) play a crucial role in promoting remineralization. The amorphous nature of bioglass facilitates controlled and sustained release of fluoride ions, fostering the deposition of fluorapatite crystals within demineralised dentin matrices. This sustained release mechanism contributes to long-term remineralisation efficacy, surpassing the transient effects observed with conventional fluoride treatments or SDF application.Moreover, the structural compatibility of calcium fluoride bioglass with the dentin matrix offers distinct advantages in promoting remineralization. By closely mimicking the composition of hydroxyapatite, the primary mineral phase in dentin, bioglass fosters intimate integration with dentin surfaces, facilitating the nucleation and growth of new mineral crystals (20). This structural affinity enhances the efficacy and durability of remineralised dentin tissues, surpassing the adhesive limitations often encountered with SDF application [(Rajeshkumar & Lakshmi, 2021; Sivakumar et al., 2021)](https://paperpile.com/c/7guoRD/Zn0VC+Yw8c3).The study validates the remineralisation efficacy of calcium fluoride bioglass, facilitating mineral growth within demineralised dentin. Its incorporation in remineralisation protocols presents a targeted approach for intrafibrillar mineral promotion. Ag-CaF2-BG emerges as a promising alternative to SDF, circumventing discoloration issues and potential bonding complications in composite resin restorations. This novel approach addresses SDF limitations, fostering improved dental care and reinforcing tooth structures. It augments mechanical recovery and mitigates bonding concerns for subsequent composite resin restorations.



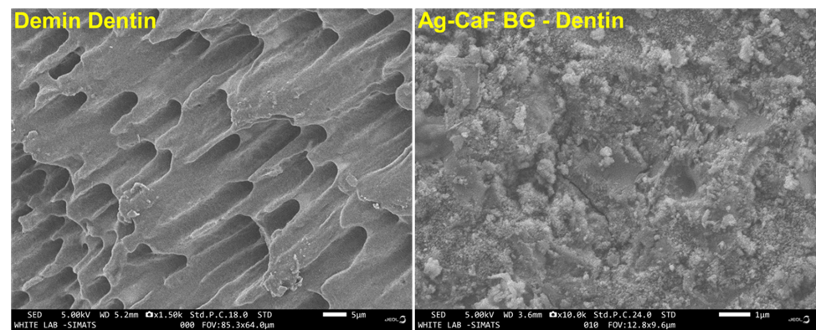
**Fig. 1.** Represents the synthesis methodology of silver infused calcium fluoride bioactive glasses (Ag-CaF2-BG).



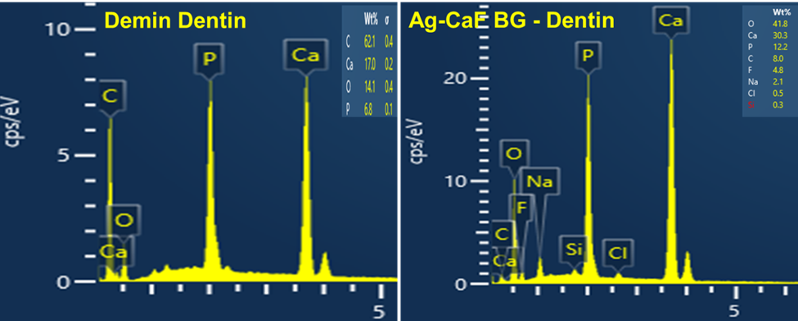
**Fig. 2.** Crystalline patterns and functional group properties of silver infused calcium fluoride bioglass (a) XRD and (b) Raman spectroscopy.



**Fig. 3.** Morphological and elemental properties of silver infused calcium fluoride bioglass (a) FE-SEM and (b) EDS spectra.



**Fig. 4.** Morphological analysis of demineralized and remineralized dentin, open dentin tubules was apparent in demin dentin, occluded tubes were prominent in silver infused calcium fluoride bioglass treated dentin slabs.



**Fig. 5.** Elemental analysis of demineralized and remineralized dentin, rapid increase of calcium and phosphate was persisted in calcium fluoride bioglass treated dentin slabs.

# Conclusion

The research on silver-infused calcium fluoride bioglass (Ag-CaF2-BG) has shown promising results, confirming its efficacy for dentin remineralisation through XRD spectra and Raman spectroscopy analysis. SEM imaging revealed its potential for enhancing structural integrity, while EDS examinations substantiated its essential composition. These findings have immediate applications in addressing dentin demineralisation, tooth sensitivity, and dental caries, with future research focusing on clinical trials to validate safety and practicality in dental healthcare settings.

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