Micro Organism’s Viability and Quantitative Estimation of S. Mutans With Interaction of Sr Infused Bioglass

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**Abstract:** The researcher evaluates the *Streptococcus mutans* survival rates as well as determining its population count during strontium-infused bioactive glass (Sr-BG) exposure. The researchers produced Sr-BG as an advanced material which displayed increased functionality and improved mechanical capabilities for dental usage. The examination of surface topography showed Sr-BG had a smoother finish compared to regular samples and cell survival tests established that Sr-BG decreased cell counts to about 75-80% of initial values but still preserved adequate cell compatibility. Sr-BG exhibited considerable bacterial blocking features against *S. mutans* implying its capability to stop biofilm development for enhanced oral health. The obtained data indicates that Sr-BG shows potential as a material for future applications within dental treatment fields.

**Keywords:** Bioglass; Strontium; Biofilm; *S. mutans*; Antibacterial.

# INTRODUCTION

Bioactive glass grafts are commonly used in dentistry and orthopaedic applications because they undergo surface reactions when exposed to bodily fluids that result in the creation of a hydroxycarbonate apatite (HCA) layer and a strong link with the living bone Protelos®, a medication licensed for the treatment and prevention of osteoporosis, contains strontium ranelate (SrR), which is reportedly the active ingredient.[(Dharman, 2021; “Strontium-Substituted Bioactive Glasses in Vitro Osteogenic and Antibacterial Effects,” 2016)](https://paperpile.com/c/HnJ2x0/eNmRF+CG7s)

Adding Sr2+ ions to silicate-based glasses has garnered significant attention among the several therapeutic components for use in bone restoration. An alkaline earth metal called strontium (Sr) is typically found in the human skeleton. This element has a high bone-seeking property and can be substituted in the calcium (Ca) locations of apatite. Over the past few decades, strontium ranelate (SrR), a type of silver, has been used to treat osteoporosis, a common bone disease. Because strontium has the capacity to restore teeth, it has also been utilized in toothpaste. Sr2+ ions can increase bone tissue density and significantly lower the risk of fracture in mammals by promoting osteoblast activity and suppressing osteoclast function.[(Lakshmi, 2021; Kargozar et al., 2019; “Sol–gel Hybrid Coatings with Strontium-Doped 45S5 Glass Particles for Enhancing the Performance of Stainless Steel Implants: Electrochemical, Bioactive and in Vivo Response,” 2015)](https://paperpile.com/c/HnJ2x0/IU4VO+zg45c+0OHB))

structural characteristics of genuine tissues while also taking into account the intricate nanostructure. The fundamental component of bone is formed type I collagen fibrils, which provide an organic substrate appropriate for the precipitation of hydroxyapatite (HA) crystals. Bone is an extremely complex, hierarchically organized structure. About 90% of the protein in natural bone tissue is composed of type I collagen, which is the most prevalent structural protein in the human body.[(Maliael et al., 2021; Montalbano et al., 2018; Maiti, 2021)](https://paperpile.com/c/HnJ2x0/R0VKx+nYGL+QfoS)

Injectable biomaterials that show promise for bone regeneration applications are bioactive glass nanoparticles (BGNPs). Due to their small size and ability to be internalized into cells to deliver therapeutic ions intracellularly, nanoparticles (NPs) have the potential to be more advantageous than microparticles. Their high surface to volume ratios also boost their rate of dissolution. The glass's ability to supply medicinal cations continuously during dissolution due to their amorphous form is one of its advantages.[(Graf et al., 2023; “In Vitro Osteogenesis by Intracellular Uptake of Strontium Containing Bioactive Glass Nanoparticles,” 2018; Tiwari & Jain, 2023)](https://paperpile.com/c/HnJ2x0/JOFO+5KFD+VXwD)

Neves et al. did a thorough investigation on the in vivo effects of Sr-doped biomaterials on bone formation and remodeling (2017). Gorustovich et al. (2008) initially described the in vivo bone response to SiO2-CaO–Na2O–P2O5 glass particles containing Sr. Regarding the 45S5 glass control system, this investigation did not find any appreciable variations in osteoconductivity. In actuality, neither the amount of Sr discovered at the contact nor its presence in the freshly created bone tissue changed the osteoconductive qualities.[(Gorustovich et al., 2010)](https://paperpile.com/c/HnJ2x0/Qqcm2)It has been noted that strontium (Sr), a divalent cation found in trace levels in tooth structure, is important for remineralization. Carbonated hydroxyapatite's (HAp) crystallinity was improved and enamel solubility was decreased when Sr and F were combined. Additionally, it has been discovered that strontium and F work in concert to strengthen remineralization and improve antibacterial activity.[(Gorustovich et al., 2010; Ramamurthy, 2021)](https://paperpile.com/c/HnJ2x0/Qqcm2+5jtn)

When calcium hydroxide is formed in reaction with water or tissue fluids, calcium silicate (CS), an alkaline pH substance that releases calcium, is created. Because of this characteristic, CS can have a buffering action that lessens the pathogenic bacteria's acidic microenvironment and minimizes mineral loss.[(Govindaraj & Dinesh, 2021; Kutsch, 2023)](https://paperpile.com/c/HnJ2x0/DS6cj+8Tmt)

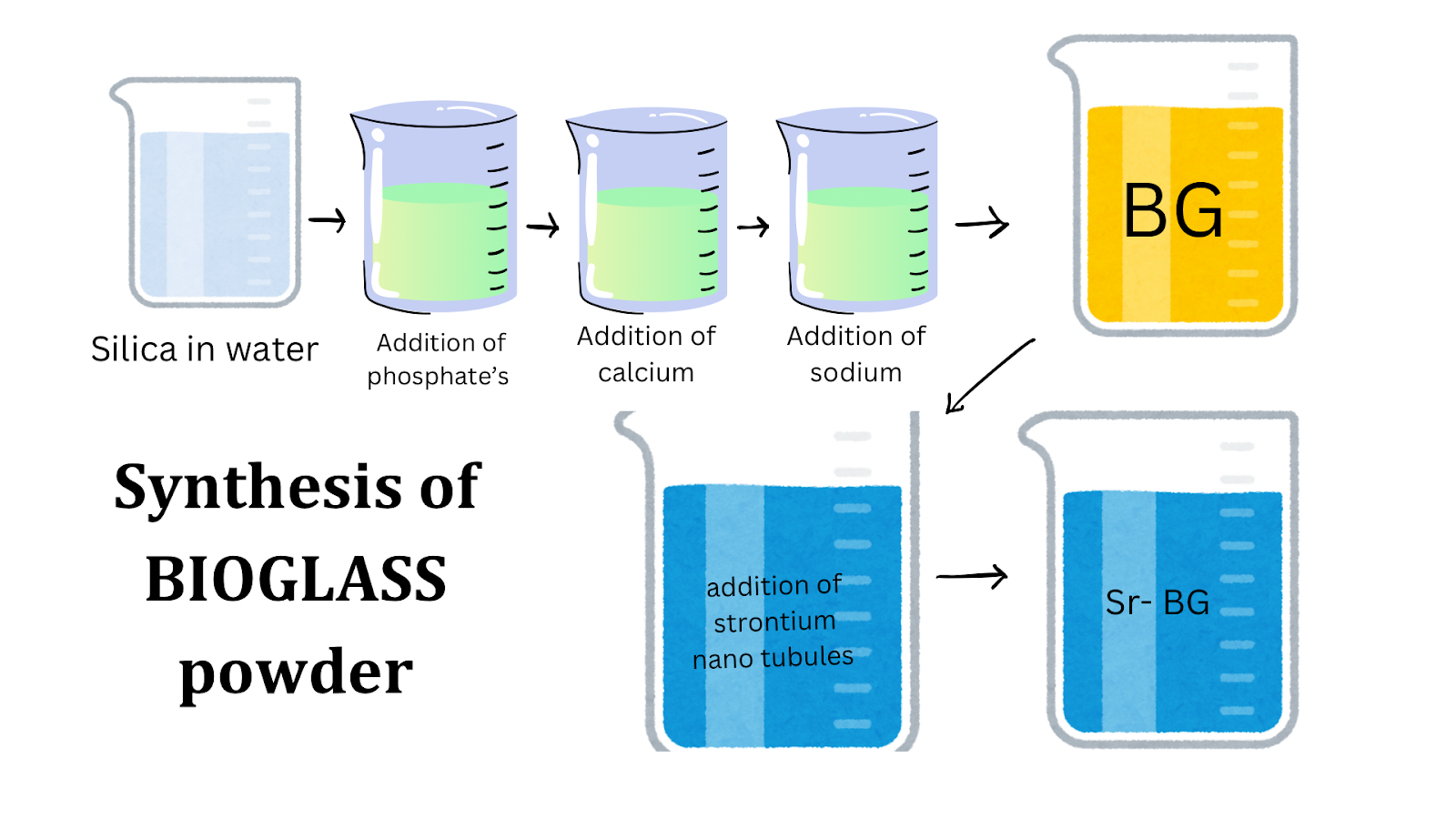
Anaerobic gram-negative bacteria make up the majority of oral biofilm, which is closely linked to the development of periodontitis and peri-implant disorders. In fact, the rate of these microorganisms rises noticeably when compared to physiological conditions. Therefore, after regenerative periodontal procedures, the colonization of such species at surgical sites is a primary concern. In fact, the majority of oral infections are caused by biofilms, and compared to planktonic bacteria, the organisms in biofilms are 1000–1500 times more resistant to antibiotics. The three recognized periodontal pathogens that are most important in the onset and progression of periodontal and peri-implant-associated bone deterioration are Aggregatibacter Actinomycetemcomitans Prevotella intermedia, and Porphyromonas gingivalis.[(*Portico*, n.d.; Sushanthi, 2021)](https://paperpile.com/c/HnJ2x0/8Uuas+my6m)

It is nonsensical to go over every one of the many varied applications connected to the term "bioactive" given the variety of dental materials that are given this designation. Conversely, it seems that concentrating on dental materials that use or are based on BAGs is a crucial endeavor in order to succinctly cover the pertinent state-of-the-art. When it comes to oral health, BAGs are typically used as implant coatings and implants themselves, as well as as periodontal or alveolar bone socket particulate fillers, maxillofacial sinus floor repairs, dentine hypersensitivity management, and additives in a variety of materials used in oral, maxillofacial, and dental procedures.[(Balaji Ganesh S & Sugumar, 2021; “Sol-Gel Bioactive Glass Containing Biomaterials for Restorative Dentistry: A Review,” 2022)](https://paperpile.com/c/HnJ2x0/O3VlF+xcjF)

Minerals are lost from the teeth's hard tissues due to dental caries, a biofilm-mediated illness. Demineralization of the teeth results from the cariogenic bacteria in biofilm producing acid for an extended period of time and creating an acidic environment in the mouth. Antiseptics like chlorhexidine are frequently used to reduce oral bacteria biofilms. However, because antibiotics are often made to work at low concentrations through a specific high-affinity antimicrobial target, which makes it relatively easy for bacteria to build resistance, they are not used clinically to eliminate cariogenic germs.[(“Antibacterial Effect of a New Bioactive Glass on Cariogenic Bacteria,” 2020)](https://paperpile.com/c/HnJ2x0/WHZaS)

# MATERIALS AND METHODS

The research utilized analytical grade chemicals as received for direct use without requiring purification steps. It obtained 98% pure tetraethyl orthosilicate from Alfa Aesar and orthophosphoric acid at 88% purity as well as 99% pure strontium nitrate and nitric acid (70%) from spectrum reagents and chemicals Pvt. Ltd. (Kerala, India) while sodium hydroxide with 98% pure was obtained from Sisco research laboratory (Tamil Nadu, India). The production of bioactive glass occurred through the sol-gel method by combining SiO2 (45%), P2O5 (6%), CaO (24.5%), Na2O (24.5%). The manufacturing procedure engaged Na2O reduction through Sr(NO3)2 additions at three separate amounts of 0.5%, 1.5% and 2.5%. Strontium nitrate at 1.5% concentration served to decrease sodium levels in selected solution locations when preparing the system. The reaction produced gel after one hour of stirring that followed the dissolution of tetraethyl orthosilicate and orthophosphoric acid with ethanol and nitric acid and double-distilled water. The solution received strontium nitrate and sodium hydroxide simultaneously. The different compositions of bioglass include 5% Sr-BG having Na2O content at 19.5%. The weight percentage solutions received each precursor over the span of half an hour time periods. The experimental procedure ran under room temperature conditions until complete gel development occurred. During overnight at 80 °C temperature all samples dried completely. A 24-hour drying period at 100 °C under hot air oven conditions allowed the removal of moisture before exposing the samples to three hours at 600 °C [(“Impact of Copper on in-Vitro Biomineralization, Drug Release Efficacy and Antimicrobial Properties of Bioactive Glasses,” 2020)](https://paperpile.com/c/HnJ2x0/637Z) [(S. & S., 2021)](https://paperpile.com/c/HnJ2x0/nA9U) [(S. & S., 2021)](https://paperpile.com/c/HnJ2x0/nA9U).

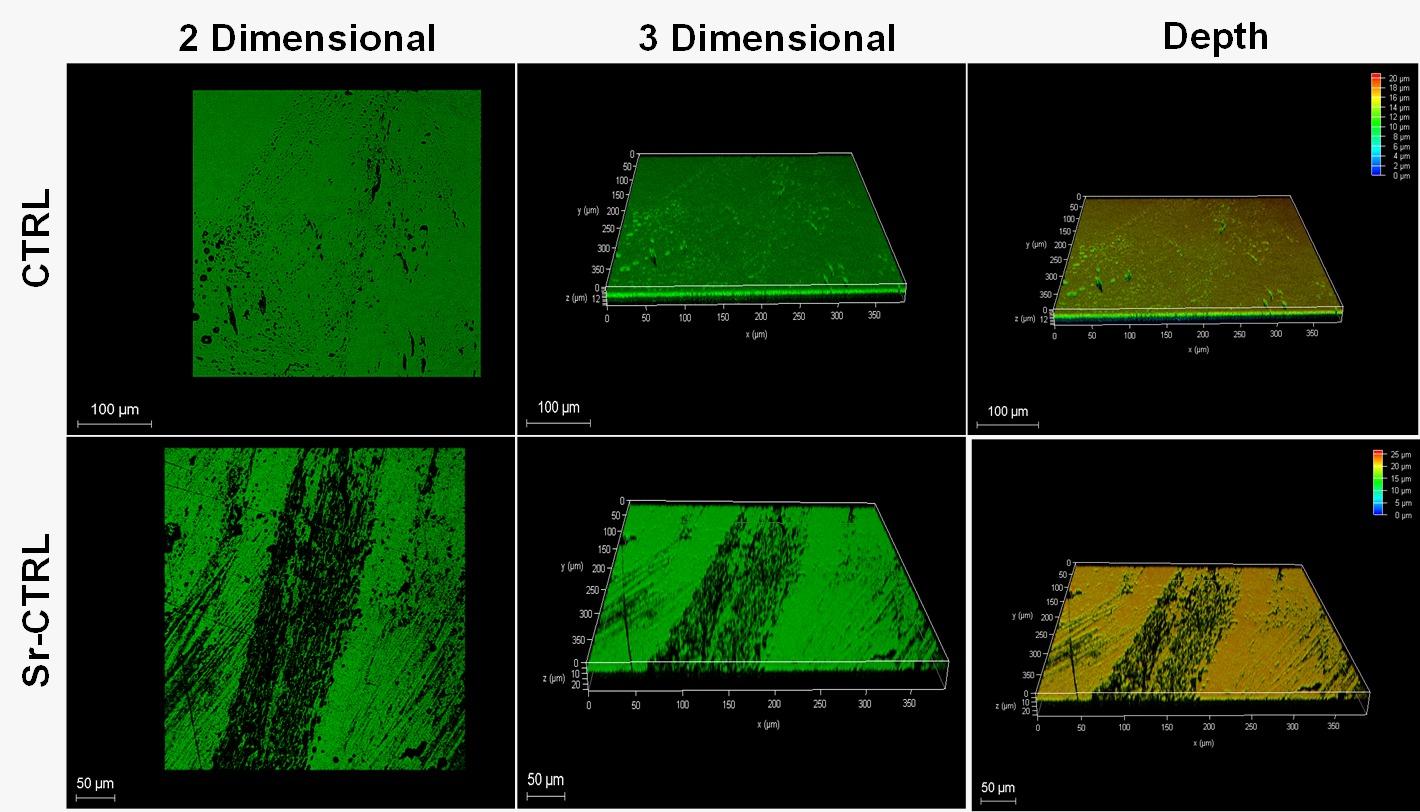


**Fig 1:** Representative diagram of Bioglass Synthesis.

# RESULTS AND DISCUSSION

# CONFOCAL LASER SCANNING MICROSCOPY

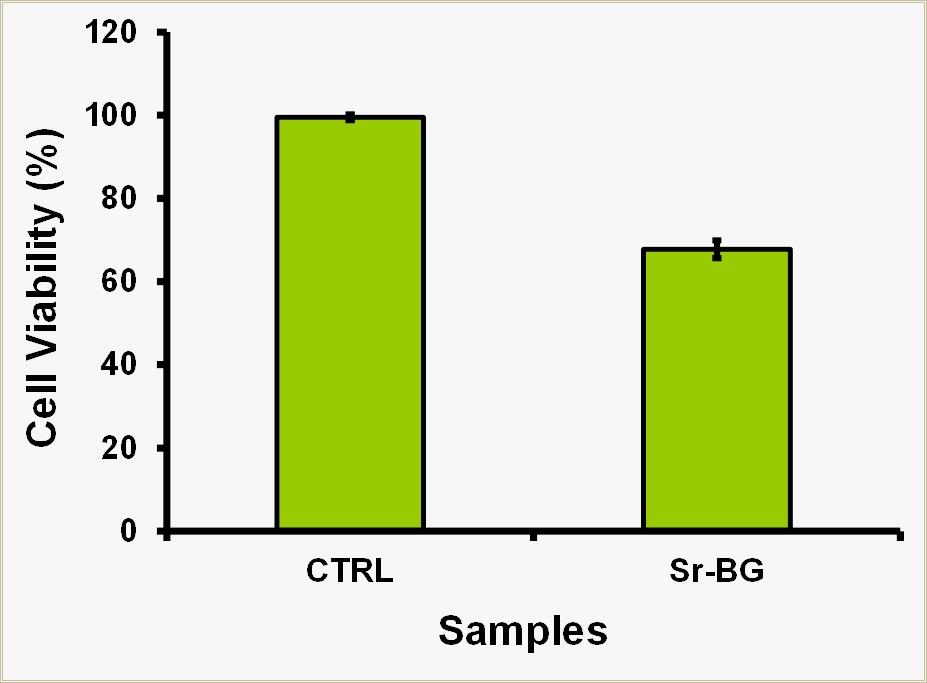
The 2D image below displays a surface that is mostly smooth with a few minor defects or dark areas. The depth map, which displays very little variation in topography, confirms that the 3D reconstruction depicts a homogeneous surface with few surface flaws.The Sr-CTRL (bottom row) image shows distinct streaks or scratches that indicate a rougher surface in the 2D image. The 3D reconstruction validates these surface imperfections by displaying more pronounced depth and structural details. With a wider variety of topographic heights than the control, the depth map emphasizes these differences.



**Fig 2:** Confocal Laser Scanning Microscopy explains the inhibition of S. mutant on Dentin surface.

# CELL VIABILITY BY MTT

These variations probably suggest that the Sr-CTRL group has experienced surface change, resulting in enhanced porosity or roughness, which can be important for enhancing cell integration or adhesion in biomedical applications. This bar graph compares the cell viability between the two sample types (CTRL and Sr-BG). The control group (CTRL) exhibits almost 100% cell viability, indicating excellent biocompatibility. In contrast, the Sr-BG group shows a lower viability, though still relatively high, around 75-80%.



**Fig 2:** MTT assay explains the inhibition of S. mutant via cell viability.

TThe following studies were performed on the samples to assess colony-forming unit count (log CFU/mL), cytotoxicity (MTT assay), to exhibit lower biofilm adhesion.[(“Biofilm Formation and Cell Viability on Monolithic Zirconia with Silver-Doped Sodalime Glass,” 2022)](https://paperpile.com/c/HnJ2x0/lUtjr)

The process starts with silica ( SiO\_2 ) being dissolved in water. Silica serves as the primary component in the bioglass structure, forming a glassy matrix that contributes to the material’s bioactivity and mechanical strength. Water acts as the medium in which the reactions occur, aiding in the dispersion and solubilization of the materials. Phosphates are introduced into the silica solution. Phosphorous compounds play a crucial role in bioglass composition because phosphorus is an essential element in bone mineralization(Chehelgerdi et al., 2023). By incorporating phosphate ions, the bioactivity of the glass is enhanced, as the bioglass mimics natural bone composition more closely. Calcium is then added to the solution. Calcium ions are essential for the bioactive properties of bioglass because they promote the formation of hydroxyapatite (a mineral found in bone) when the glass comes in contact with body fluids. This hydroxyapatite layer encourages the bonding of the bioglass with bone tissue, making calcium a key component for bioglass used in bone repair applications. Sodium is added next to the mixture. Sodium acts as a network modifier in the bioglass structure, helping to adjust the thermal and mechanical properties. The presence of sodium increases the solubility of the glass and promotes the release of calcium and phosphate ions in biological environments, enhancing bioactivity.

At this point, the combined mixture of silica, phosphate, calcium, and sodium forms what is known as “Bioglass” (BG). This material is now bioactive and suitable for applications in medical implants, particularly in bone healing (Saadh et al., 2024). Strontium nanotubes are then incorporated into the bioglass solution. Strontium ions ( Sr^{2+} ) are well-known for their role in stimulating bone formation and reducing bone resorption, which makes them highly beneficial in bioglass for bone repair. [(Harsha & Subramanian, 2022)](https://paperpile.com/c/HnJ2x0/xlov)The addition of strontium nanotubes further enhances the mechanical properties and biological performance of the bioglass. The nanotubes provide structural reinforcement, improving the material’s strength and bioactivity.

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The result of this synthesis is a modified bioglass, referred to as Sr-BG (strontium-doped bioglass). This material possesses improved properties, including enhanced bioactivity, osteoconductivity (promoting bone growth), and mechanical strength, making it suitable for bone regeneration, particularly in cases of osteoporosis or bone defects. Glasses and glass-ceramics can have their in vitro antibacterial activity assessed using a range of screening techniques. The two most popular ones are the dilution and diffusion techniques. Diffusion methods classify bacteria as sensitive, intermediate, or resistant, yielding qualitative results. It is unable to differentiate between bactericidal and bacteriostatic actions, though. Diluting techniques, on the other hand, are regarded as quantitative assays after they establish the minimal inhibitory.[(Balaji Ganesh S & Sugumar, 2021; “Multifunctional Bioactive Glass and Glass-Ceramic Biomaterials with Antibacterial Properties for Repair and Regeneration of Bone Tissue,” 2017)](https://paperpile.com/c/HnJ2x0/5ifg+xcjF) [(“Influence of Strontium on Structure, Sintering and Biodegradation Behaviour of CaO–MgO–SrO–SiO2–P2O5–CaF2 Glasses,” 2011; Jabin et al., 2021)](https://paperpile.com/c/HnJ2x0/qMa2e+XWVr)

# CONCLUSION

The CTRL sample has a smoother surface with excellent cell viability, making it suitable for environments where minimal surface roughness is needed.

The Sr-CTRL sample demonstrates a rougher surface, which could enhance cellular interaction depending on the application. While the cell viability is slightly reduced, it still remains high, suggesting that the Sr-modified material retains good biocompatibility and may offer advantages in tissue engineering or bone regeneration.

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