Bioglass-Coated Titanium Screws: Fabrication, Characterization, and Cytocompatibility for Implant Applications

Hooriyah Khan1 , S.Bavi1,a)

1Hooriyah Health Hub, Chennai, Tamil Nadu, India

**Corresponding Author:** a)[sreevybavi05@gmail.com](mailto:sreevybavi05@gmail.com)

**Abstract:** This study aimed to fabricate and evaluate bioglass-coated titanium screws for potential use in implant applications, focusing on their surface morphology, biomineralization properties, and cytocompatibility. Bioglass was synthesized via the sol-gel method using calcium nitrate, tetraethyl orthosilicate, orthophosphoric acid, and sodium hydroxide, with a composition of 45% SiO₂, 24.5% Na₂O, 24.5% CaO, and 6% P₂O₅. Medical-grade titanium screws were prepared through sandblasting and acid etching, coated with bioglass, and sintered at 600°C. Surface morphology and functional groups were analyzed using FE-SEM and FT-IR, respectively. Biomineralization was evaluated by immersing coated screws in simulated body fluid (SBF) for 7 days, while cytocompatibility was assessed through MTT assays with MG-63 osteoblast cells. The bioglass coating exhibited a porous structure with uniformly deposited particles, facilitating cell adhesion and proliferation. Hydroxyapatite formation was confirmed through FE-SEM and FT-IR analysis after 7 days of SBF immersion. MTT assay results demonstrated 96% cell viability, meeting ISO 10993 standards, and optical imaging revealed effective cell attachment and characteristic morphological transitions. Bioglass-coated titanium screws show excellent bioactivity and cytocompatibility, making them highly promising for dental and orthopedic implants. These coatings enhance osseointegration and biomineralization, potentially improving clinical outcomes in implantology.

**Keywords:**Titanium, Bioglass, Hydroxyapatite, biomineralization, Osteointegration, Dental implant

# INTRODUCTION

Titanium (Ti) and its alloys have been established as favorable biomaterials for implant applications due to its exceptional mechanical strength and biocompatibility [(Uskoković et al., 2021)](https://paperpile.com/c/FsQJQr/sPDr). When Ti is implanted into host tissues, it does not inherently promote osseointegration due to its classification as a bioinert material[(Aparna et al., 2021; Poornima et al., 2021; Verma & Muthuswamy Pandian, 2021)](https://paperpile.com/c/FsQJQr/jM9fy+SY2NY+FalWn). Being bioinert means that titanium does not actively interact with the surrounding biological environment to form a direct bond with bone tissue[(Aparna et al., 2021; Poornima et al., 2021; Verma & Muthuswamy Pandian, 2021)](https://paperpile.com/c/FsQJQr/jM9fy+SY2NY+FalWn);[(Merchant et al., 2022; Pandiyan et al., 2022)](https://paperpile.com/c/FsQJQr/tn0n9+M8f3P)). Instead, its surface typically forms a passive oxide layer, which, while providing corrosion resistance and stability, lacks the biological activity required to integrate seamlessly with bone. As a result, additional surface modifications, coatings, or treatments are often necessary to enhance titanium's osseointegration properties and improve its performance as an implant material [(Prasad et al., 2024)](https://paperpile.com/c/FsQJQr/wdB0).

Osseointegration which provides a stable and functional connection between bone and an implant, occurs in four distinct stages: hemostasis, inflammation, proliferation, and remodeling[(Chokkattu et al., 2022; Ramamurthy et al., 2022)](https://paperpile.com/c/FsQJQr/8Nxw+JwWt)). These stages collectively ensure the formation of a direct, structural, and functional interface between the implant and the surrounding bone tissue, which is essential for the long-term success of implants, particularly in dental applications [(Shirazi et al., 2022)](https://paperpile.com/c/FsQJQr/qC1Z). Extensive research has been conducted to explore various strategies for enhancing the surface characteristics of dental implants to promote faster and more effective osseointegration[(Jain & Verma, 2022; Marya et al., 2022)](https://paperpile.com/c/FsQJQr/12tAa+2mjLd) [(Wadhwani et al., 2022)(Wadhwani et al., 2022)](https://paperpile.com/c/FsQJQr/tXFi)[(Adel et al., 2023)](https://paperpile.com/c/FsQJQr/uABPR). Among these strategies, the use of bioactive glass coated Ti dental screws has emerged as a promising approach to improve the surface properties of implants, providing enhanced biocompatibility, increased surface roughness for better cellular adhesion, and improved mechanical stability[(Subramanian & Harikrishnan, 2023)](https://paperpile.com/c/FsQJQr/LPqhs)[(Solanki et al., 2023)](https://paperpile.com/c/FsQJQr/vSKS)[(Chokkattu et al., 2023)](https://paperpile.com/c/FsQJQr/UPMQy). These materials also provide a bioactive surface that can stimulate bone growth and integration, overcoming some of the limitations associated with traditional implant materials like titanium [(Ganapathy et al.,2021; Liang et al., 2023)](https://paperpile.com/c/FsQJQr/IQD5+gOZa).

Bioactive glass, with its inherent antimicrobial properties, provides a novel approach to minimize the risk of infections associated with dental implants[(Laghari et al., 2023; Ramakrishnan et al., 2023)](https://paperpile.com/c/FsQJQr/LcLWX+cOSji)[(Muthuswamy Pandian et al., 2022)](https://paperpile.com/c/FsQJQr/z6DL) [(Muthuswamy Pandian et al., 2022; Ramakrishnan et al., 2023)](https://paperpile.com/c/FsQJQr/z6DL+LcLWX). By undergoing extensive mechanical testing, corrosion resistance evaluations, and long-term observational studies, bioactive glass demonstrates potential as a transformative material in dental implantology[(Merchant et al., 2022)](https://paperpile.com/c/FsQJQr/M8f3P)[(Sreevarun et al., 2023)](https://paperpile.com/c/FsQJQr/dfr5). Compared to other biomaterials, bioactive glasses coated Ti screw offer superior potential for enhancing the integration of metal implants with surrounding tissues. This is achieved through the formation of an apatite layer at the interface between the bone and the implant[(Ganapathy et al., 2021)](https://paperpile.com/c/FsQJQr/4aEF). This bioactive layer mimics the natural mineral composition of bone, promoting a stronger and more biologically harmonious connection. By fostering this interface, bioactive glasses improve the stability and longevity of metal implants, making them highly effective in applications requiring seamless bone integration [(Lam et al., 2020)](https://paperpile.com/c/FsQJQr/y10Y).

Through precise experimentation and comprehensive analysis, our research seeks to contribute to the advancement of implant technology through bioglass composite coated through sol gel method on the Ti screw, envisioning a future where dental implants not only restore function but also actively foster biological interactions for sustained oral health. This is particularly critical for patients with congenital tooth absence or tooth loss due to trauma or caries, where implant stability is essential for proper functionality.

Our objective is to present compelling empirical evidence supporting the hypothesis that bioactive glass coatings significantly enhance the speed and robustness of the osseointegration process. This is achieved through detailed investigations, including in-vitro experiments and cell culture studies, which underscore the potential of bioactive glass to revolutionize dental implant surfaces and improve clinical outcomes.

# MATERIALS AND METHODS

## PREPARATION OF BIOGLASS

Bioglass was prepared using precursor chemicals such as, calcium nitrate tetrahydrate (CaNO3), tetraethyl orthosilicate (TEOS), orthophosphoric acid, and sodium hydroxide (NaOH), all procured from Sigma Aldrich, India. Fabrication was performed using Sol-gel method in a beaker. The target weight percentage of components being 45% SiO₂, 24.5% Na₂O, 24.5% CaO, and 6% P₂O₅.To initiate the process, in a Teflon beaker, calcium nitrate, sodium hydroxide, and orthophosphoric acid were mixed and stirred thoroughly to achieve a homogeneous gel solution with a pH of 11. Ethanol and nitric acid were used as catalysts to facilitate the hydrolysis process [(Lung et al., 2021)](https://paperpile.com/c/FsQJQr/Da8r). The resulting sol was then aged for 24 hours at room temperature to complete the gelation process. This step ensured the formation of a stable gel structure suitable for further processing.

## PREPARATION OF Ti-SCREW COATING

Medical-grade titanium screws were procured from Ti Anode India Pvt. Ltd., Chennai. The screws, measuring 9 mm in length and 2 mm in diameter, underwent sandblasting to create a porous surface structure. This was followed by an acid etching treatment to remove any residual debris from the screw surface. Afterward, the screws were thoroughly washed with water and subjected to ultrasonic cleaning to ensure complete surface preparation.

The screws were then coated with the previously prepared sol-gel solution using a withdrawal speed of 20 mm/sec. Once coated, the samples were allowed to dry at room temperature before being sintered at 600°C in an oven to enhance the coating's adhesion and stability [(Sarian et al., 2022)](https://paperpile.com/c/FsQJQr/KGF3).

## SURFACE CHARACTERIZATION STUDIES

The morphological studies of coated surfaces were evaluated in FE-SEM with EDX analysis (JEOL ITM800). The functional group was confirmed by the FT-IR spectrum Alpha II Bruker using the KBR grid in the wave number range of 4000 cm-1 to 400 cm-1 [(Bargavi et al., 2020)](https://paperpile.com/c/FsQJQr/1uBP).

## *In-vitro* BIOMINERALIZATION STUDIES

To evaluate the biomineralization properties of the coated sample, it was immersed in simulated body fluid (SBF) solution for 7 days. The SBF solution was prepared by sequentially dissolving the following chemicals in 1000 mL of deionized distilled (DD) water: 8.0 g of NaCl, 0.35 g of NaHCO₃, 0.24 g of KCl, 0.22 g of K₂HPO₄·3H₂O, 0.30 g of MgCl₂·6H₂O, 0.27 g of CaCl₂, 0.07 g of Na₂SO₄, and 6.05 g of Tris buffer. The pH of the solution was adjusted to 7.4 using 1 M HCl.

After the 7-day immersion period, the sample was removed, dried at room temperature, and analyzed using Field Emission Scanning Electron Microscopy (FE-SEM) and Fourier Transform Infrared (FT-IR) spectroscopy to study its surface morphology and chemical properties [(Lung et al., 2021)](https://paperpile.com/c/FsQJQr/Da8r).

## MTT ASSAY

For the cell proliferation assay, MG63 Osteoblast cells cultured in the mixture of Dulbecco’s Modified Eagle’s Medium (DMEM) with 10% of fetal bovine serum (FBS) and 1% antibiotic were seeded at a density of 1x105 cells per well, coated with novel bioactive glass coated Titanium in 24-well plates at a temperature of 37°C with 5% CO2. Upon reaching confluence, the samples underwent UV treatment and were subsequently transferred to a fresh 24-well plate for an incubation period of one to three days. Following the designated incubation time, the samples were extracted from the wells and washed in phosphate-buffered saline (pH 7.4) and serum-free DMEM. A 0.5% MTT solution (5 mg/ml) was added at a volume of 10 μl per well, and the cells were subjected to a 4-hour incubation phase. Post-incubation, each well received 1 ml of DMSO. DMSO was also used as a control when measuring absorbance at 570 nm.

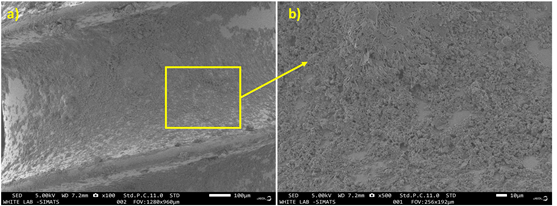
The values obtained from these measurements were employed to identify concentration needed to inhibit 50% of the organisms (IC50). Cell viability was determined using the formula:

% cell viability = (A570 of treated cells / A570 of control cells) × 100 [(Bargavi et al., 2020)](https://paperpile.com/c/FsQJQr/1uBP).

# RESULTS

## SURFACE MORPHOLOGY STUDIES

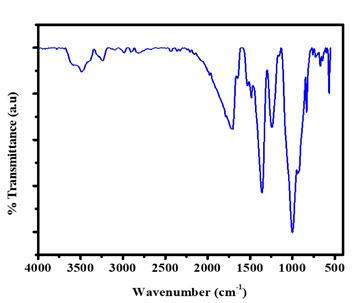
The surface properties of the Ti screw, as observed through SEM, reveal a thickness of 2 mm and a length of 9 mm, with a bioglass coating applied to the surface, as depicted in Fig. 1. The morphological analysis indicates that the coated particles are uniformly deposited on the screw, with the surface exhibiting porosity, agglomerates, and white particles. At a scale of 100 micrometers, these features are distinctly visible. Further, the Fig. 1b image at a 10-micrometer scale highlights a small plate-like structure. These surface characteristics are crucial for the osteointegration process, significantly influencing cell adhesion and proliferation.



**Figure 1**. a) 100 and b) 10 micrometers of SEM image in bioglass coated Ti-screw

## FT-IR SPECTRA

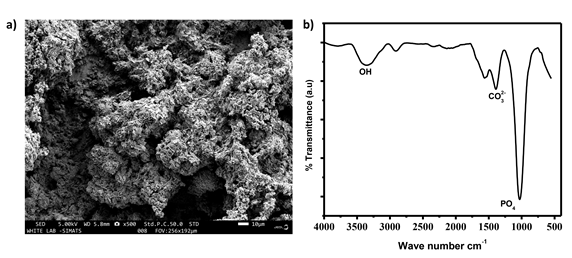
The presence of functional groups was analyzed within the wavenumber range of 4000–500 cm⁻¹, as shown in Fig. 2. A small peak corresponding to the hydroxyl group was observed in the range of 3700–3200 cm⁻¹. A broad peak around 1600 cm⁻¹ was attributed to the symmetric vibration band of water molecules. The stretching band of calcium carbonate (C=O) appeared at 1400 cm⁻¹. Peaks corresponding to the phosphate band (P-O) and silica (Si-O-Si) were identified at 1020 cm⁻¹. Additionally, a characteristic base metal oxide peak was observed at 530 cm⁻¹. These findings confirm the functional group composition of the bioglass coating on the titanium screw.



**Figure 2.** FT-IR spectra of bioglass coated Ti-screw

## IN-VITRO BIOMINERALIZATION STUDIES

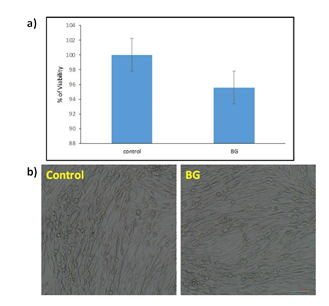
In vitro biomineralization studies were conducted by immersing the coated samples in SBF solution for 7 days to evaluate the formation of hydroxyapatite. The bioglass surface facilitated the nucleation of hydroxyapatite, as confirmed through FE-SEM and FT-IR analyses, presented in Fig. 3. The surface topography of the hydroxyapatite, shown in Fig. 3a, revealed a spike-like morphology. At 10-micrometer magnification, the surface exhibited needle-like structures along with a porous nature. Fig. 3b further validated these observations through functional group analysis. After 7 days of immersion, the presence of phosphate and calcium carbonate was confirmed, with the asymmetric vibration of the phosphate peak appearing at 1020 cm⁻¹ and the carbonate peak at 1400 cm⁻¹. Additionally, a broad peak corresponding to the hydroxyl group was observed in the 3500–3200 cm⁻¹ range. These results demonstrate the bioglass's ability to nucleate hydroxyapatite within the initial 7-day period, as confirmed by FT-IR spectra.



**Figure 3.** Biomineralization process of the hydroxyapatite; a) surface morphology through FE-SEM and b) FT-IR spectra of bioglass after 7 days immersion in SBF solution

## MTT ASSAY

Cytocompatibility tests were conducted on bioglass-coated Ti screws, as illustrated in Fig. 4. The cell proliferation rate was assessed after 24 hours of exposure to the bioglass-coated samples, revealing a significant increase within this time frame. The bioglass demonstrated compatibility with cells, meeting ISO 10993 standards for biological evaluation of medical devices (>76%), with a cell viability of 96% compared to 100% in the control. The MG-63 cells retained their characteristic morphology, transitioning from an elongated to a polygonal shape with spindle-like features. Optical imaging revealed that cells adhered effectively to the coating layer on both the control and bioglass-coated samples after 24 hours. These results confirm that the material possesses biocompatibility and bioactive properties, making it suitable for implant applications.



**Figure 4.** a) Cell viability percentage and b) optical image of control and coated Ti-screw sample

# DISCUSSION

The original bioactive glass composition, 45S5, was formulated by weight as 45% SiO₂, 24.5% Na₂O, 24.5% CaO, and 6% P₂O₅. It was commercially trademarked as Bioglass®, and this composition remains the basis for many commercially available products today [(Song et al., 2023)](https://paperpile.com/c/FsQJQr/vJBu). In this study screw coated bioglass were analysed for dental application. The surface morphology analysis using SEM demonstrated that the bioglass-coated titanium screw exhibited a porous and uniformly distributed layer of bioglass particles to facilitate the osseointegration and biomineralization process. The presence of agglomerates and plate-like structures indicates effective deposition of the bioglass coating. The porosity observed on the coated surface plays a crucial role in enhancing osteointegration by providing an ideal microenvironment for cell attachment, proliferation, and bone formation. The plate-like and needle-like structures, seen at higher magnifications, are indicative of hydroxyapatite nucleation, which is essential for bioactivity in implant applications. Similar to our study, Bioactive glass 13-19 synthesized via sol gel method tailored in the range 5–43 wt.% by controlling heat treatment parameters, showed the presence of different morphologies in the glass-ceramic microstructure as a function of heat treatment [(Nawaz et al., 2021)](https://paperpile.com/c/FsQJQr/dUxv). Ting et al. has reported 58S glass, with a nominal composition of 60 mol% SiO₂, 36 mol% CaO, and 4 mol% P₂O₅, as one of the earliest sol–gel-derived bioactive glass compositions developed and commercialized by NovaBone Products LLC (Alachua, FL, USA). Hydroxyapatite (HA) was found to form within the 58S glass during sol–gel synthesis, following thermal stabilization at 700 °C [(Ting et al., 2017)](https://paperpile.com/c/FsQJQr/DHoz). In another research, biological characterization of  bioactive glasses in the systems of (60-x) SiO2–36CaO–4P2O5–xB2O3, and (60-x) SiO2–31CaO–4P2O5–5SrO-xB2O3(x = 0, 1, 5, 10 and 15 mol%) through the incorporation of strontium and boron (up to 10 %) and synthesized through sol-gel technique possessed the highest bioactivity, owing to faster ion release and superior ability to induce apatite precipitation making BG-5S10B a highly promising option for use in bone tissue engineering applications [(Niazvand et al., 2024)](https://paperpile.com/c/FsQJQr/JoqG).The functional group of the coating was identified by FT-IR spectra. The FTIR analysis confirmed the presence of key functional groups, including hydroxyl, phosphate, carbonate, and silica peaks, within the bioglass-coated layer. The hydroxyl group peak in the 3500–3200 cm⁻¹ range and phosphate and carbonate peaks at 1020 cm⁻¹ and 1400 cm⁻¹, respectively, are critical indicators of the bioglass's bioactive potential. These functional groups contribute to hydroxyapatite formation, demonstrating the bioglass coating's ability to mimic the mineral composition of natural bone [(Bargavi et al., 2020)](https://paperpile.com/c/FsQJQr/1uBP). The broad peaks further validate the presence of water molecules and hydroxyl groups, enhancing the material's compatibility for biomedical use.In the biomineralization process, it is widely recognized that HAp forms on the surface of bioactive glasses. The mechanism, dissociation of cations from the surface of the bioactive substances and an increase in the overabundance degree of the fluid surrounding them about the components of hydroxyapatite. Simultaneous dissolution of silicate ions from the bioglass to the forming silanol groups, which are critical to nucleate sites to produce HAp [(Ramadoss et al., 2022)](https://paperpile.com/c/FsQJQr/wlkL). A silica-rich layer is produced when the hydrated silica (SiOH) that develops on the glass surface is rearranged by the polycondensation of nearby silanols during the dissolving process that occurs before the accumulation of calcium and phosphate ions (Rafi et al., 2024). The hydrated silica ions accelerate the mechanism of calcium hydroxyapatite layer nucleation and growth [(Filip et al., 2022)](https://paperpile.com/c/FsQJQr/3Fb0).The 7-day immersion in SBF confirmed the bioglass coating's ability to nucleate hydroxyapatite, as evidenced by the spike-like and needle-like hydroxyapatite morphology observed through SEM and corroborated by FTIR spectra. The functional group analysis revealed critical bands corresponding to phosphate, carbonate, and hydroxyl groups, further supporting hydroxyapatite formation. These results highlight the bioactive properties of the bioglass coating, which promotes mineralization processes vital for successful osseointegration in implants [(Bargavi et al., 2020)](https://paperpile.com/c/FsQJQr/1uBP).The MTT assay results confirmed the excellent cytocompatibility of the bioglass-coated samples, with a cell viability rate of 96% compared to 100% in the control group, demonstrating compliance with ISO 10993 standards (Tuluwengjiang et al., 2024). The enhanced proliferation rate of MG-63 cells within 24 hours highlights the coating's ability to support and promote cellular activity. In a comparative study by N. Rohr et al., the bioglass and zirconia samples were evaluated using human osteoblast cells, showing a lower percentage of cell growth [(Rohr et al., 2020)](https://paperpile.com/c/FsQJQr/OmQz). In contrast, our results demonstrate a significant increase in cell viability, attributed to the formation of a porous morphology on the bioglass-coated surface. Furthermore, optical imaging confirmed effective cell attachment, with MG-63 cells retaining their typical morphology and transitioning from elongated to polygonal and spindle-shaped forms. These findings validate the bioglass's bioactivity and compatibility, making it a promising candidate for use in dental and orthopedic implants.

# CONCLUSION

The combined results from the surface morphology, FTIR spectra, in vitro biomineralization, and MTT assay demonstrate that the bioglass coating on titanium screws with sol-gel coating offers a bioactive and biocompatible solution for implant applications. Its ability to promote hydroxyapatite formation, support cell attachment, and maintain high cell viability makes it a suitable material for enhancing the success rates of osseointegration and implant longevity.

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