Copper-Doped 45S5 Bioactive Glasses for Regenerative Applications

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**Abstract:** The intention is to synthesise copper doped bioactive glasses for regenerative application.The capacity of bioactive glasses to bond with living tissues strengthens both bone regenerative processes and tissue integration. These glasses become more effective in regeneration when treated with copper ions. Both antimicrobial functions and increased osteogenesis and angiogenesis develop from copper within the materials. Copper-doped bioactive glasses demonstrate great potential for regenerative use because they integrate bioactivity and antimicrobial properties along with osteogenic behavior.Bioactive glass was prepared using the sol-gel process with the following ingredients: SiO2 (45%), P2O5 (6%), CaO (24.5%), and Na2O (24.5%). Copper nitrate was used as a precursor. The prepared Cu-BAG samples were subjected to various characterization procedures such as FE-SEM, EDS. Raman spectroscopy is carried out to assess bioactivity. Hemocompatibility assay was performed to assess the biocompatibility of Cu-BG samples with erythrocytes. *Klebsiella, Staphylococcus aureus (S. aureus)* bacterial species were utilised to analyse the antimicrobial activity of bioactive materials.Copper-doped bioactive glasses in regenerative applications indicate their potential as promising biomaterials for bone regeneration, dental treatments, wound healing, and other tissue engineering applications.The research examined the characterization and evaluation process of copper-doped bioactive glasses when used for regenerative purposes. The positive findings came from multiple tests including Raman spectrum, FE-SEM, EDA, blood compatibility, and bacterial susceptibility tests which demonstrated the prospects of these glasses.

**Keywords:** Bioactive glasses, biocompatibility, healing, antimicrobial activity.

# Introduction

Bioactive glasses were discovered in 1969 and provided for the first time an alternative to nearly inert implant materials. Bioglass quickly, firmly, and steadily bonded itself to the host tissues. They are effectively utilized for bone regeneration and were the first artificial materials to demonstrate a bonding to bone. They can break down in the body at a pace that corresponds with the creation of new bone because they promote the proliferation of bone cells by releasing ions and apatite crystallisation on their surface.There are three primary mechanisms at work: ion exchange, dissolution, and precipitation. At the bioactive glass surface, cations from the glass, including Na+ and Ca2+, interact with H+ or H3O+ from the surrounding solution to cause ion exchange. The breakdown of networks happens when hydroxyl ions (OH−) disrupt Si–O–Si connections. [(Harsha & Subramanian, 2022; Hench & Jones, 2015)](https://paperpile.com/c/GY5kBV/Y3sq+Di22) When the network breaks down, soluble silica in the form of silicic acid [Si(OH)4] is released into the solution. A silica-rich gel layer is produced when the hydrated silica (Si–OH) that has developed on the glass surface is rearranged as a result of neighbouring silanols polycondensing. A calcium-phosphate rich layer (CaP) is formed on the surface by the precipitation of calcium and phosphate ions that are released from the glass and those that are in the solution.[(Ajay, Rakshagan, et al., 2022; Brauer, 2015; Deepika et al., 2022)](https://paperpile.com/c/GY5kBV/Ryft+Cfip+tl2w)Within the dental field, bioactivity is defined by its clinical use, ranging from the capacity to stimulate dentin and enamel surface HA reprecipitation to cellular effects resulting from the release of physiologically active chemicals and ions. For more than 20 years, bone grafts using BG have been used. It encourages the development of new bone over its surface and possesses excellent osteoconductive qualities. The creation of apatite is the result of these biogas being included into dental adhesives. BAG compositions attach to collagen fibres and deposit hydroxyapatite, obstructing the dentinal tubules and causing therapeutic relief.[(Ajay, Sasikala, et al., 2022; Bejarano et al., 2015; Solanki et al., 2022)](https://paperpile.com/c/GY5kBV/vExO+jCG9+5s2w) Elevations in BAG levels are correlated with higher tubule occlusion. Rinsing makes it simple to remove BAG that has been applied on dentin discs alone. When BAG was used in toothpaste instead of silica, it offered resistance against pH rinses and brushing off the occluded tubules. Enzymatic degradation at the dentin interface was eliminated and dentin remineralization was promoted by resin composites containing fluoride and BAG. The samples were kept in artificial saliva for three and thirty days to compare the remineralizing abilities of F-BAG and BAG resin composites. In addition to showing the highest dentin remineralization, F-BAG also showed the lowest amount of dentin collagen network breakdown caused by enzymes. [(Ajay, Suma, et al., 2022; Cacciotti, 2017; Chidambaram et al., 2022; Maiti , 2021)](https://paperpile.com/c/GY5kBV/OMkS+wRKq+VUK2+nzd8)

# Materials and methods

## Preparation of bioactive glass

All the reagents and chemicals were used is of analytical grade and used without additional purification. 98% pure [tetraethyl orthosilicate](https://www.sciencedirect.com/topics/materials-science/tetraethyl-orthosilicate) was purchased from Alfa Aesar, 88% orthophosphoric acid, 99% pure calcium nitrate and nitric acid (70%) were purchased from spectrum reagents and chemicals Pvt. Ltd. (Kerala, India), [sodium](https://www.sciencedirect.com/topics/materials-science/sodium) hydroxide with 98% pure was purchased from Sisco research laboratory (Tamil Nadu, India). Sol-gel method was utilised to prepare bioactive glass, using SiO2 (45%), P2O5 (6%), CaO (24.5%), Na2O (24.5%) was used for bioglass preparation. In which, sodium was simultaneously decreased by incorporating 0.5%, 1.5% and 2.5% of copper nitrate in the corresponding site. After one hour of stirring in ethanol, nitric acid, and double-distilled water, tetraethyl orthosilicate and orthophosphoric acid were entirely dissolved and formed a gel-like network. In addition, sodium hydroxide and calcium nitrate were added to the previously described solution. The percentage of sodium was substituted, as previously noted, by 0.5%, 1.5%, and 2.5% of copper nitrate. The percentages of Na2O (24%) and Cu (0.5%) were labelled as 0.5Cu-BG, followed by Na2O (23%) and Cu (1.5%) as 1.5Cu-BG, and Na2O (22%) and Cu (2.5%) as 2.5Cu-BG. Every 30 minutes, a new precursor was added after each one had been independently dissolved (all the precursors were estimated by weight percentage). The experiment was run at room temperature until the gel completely formed. The samples were then dried at 80 °C for an entire night. Additionally, all of the samples' moisture content was effectively eliminated by drying them in a hot air oven for 24 hours at 100 °C, followed by a 3-hour heat treatment at 600 °C [(Chitra et al., 2020; S et al., 2022)](https://paperpile.com/c/GY5kBV/C0pzZ+ZXgU6).

## Materials characterization

The crystalline phases of the copper doped bioactive glass were studied by X-ray diffraction (XRD, − PANalytical Instruments, The Netherlands). Utilising Cu-Kα1 radiation (λ = 1.5406 Å) at a scan rate of 10°/min, the XRDA 3.1 program was utilised to compute the resulting cell characteristics. Using field emission scanning electron microscopy (FE-SEM, Hitachi SU-6600, Japan), the morphology of the synthesised bioactive materials was observed, and Image-J software was then used to determine the particle size. Using a 532 nm wavelength excitation source, micro Raman spectra were obtained with a confocal Raman microscope (RAMAN 11i - Nanophoton). The vibrational modes of the materials are confirmed through Fourier transform infrared spectroscopy with JASCO FT/IR-6600 equipment. The measurement of Cu-BG elemental composition occurred through an inductively coupled plasma optical emission spectroscopy (ICP-OES) spectrometer (Perkin Elmer Optima 5300 DV). A Quantachrome Nova-1000 surface analyzer which operated at liquid nitrogen temperature identified the porosity of copper-bioactive compounds. A nitrogen adsorption-desorption isotherm served as the analysis method for measuring porosity properties in the bioglass samples. A plot prepared using the BJH method was used to determine pore size distributions.

## Hemocompatibility assay

Hemocompatibility assay was applied to evaluate the biocompatibility of Cu-BG samples with erythrocytes. Blood was drawn from the volunteers and test tubes of their blood were preserved by adding EDTA (ethylene diamine tetra acetic acid) in order to lessen blood coagulation. They were centrifuged for 10 minutes at four degrees Celsius and they were obtained to separate red blood cells (RBCs). They were rinsed twice or three times with phosphate buffer saline (PBS, pH 7.4), after that. The hemocompatibility assay was used to estimate the lytic nature of erythrocytes in presence of BAG and Cu-BG samples and compared to positive and negative controls, respectively. These samples were analyzed tripplicate and altogether, there are a total of three test samples analyzed. During the incubation period, the experiment went on for one hour at 37 °C. The samples were centrifuged again, and the OD readings were 540 nm. The hemolysis % was calculated using the formula.

hemolysis%= sample absorbance - negative control X 100

Positive control - negative control

## Antibacterial effect

*Klebsiella, Staphylococcus aureus (S. aureus)* bacterial species were utilised to analyse the antimicrobial activity of bioactive materials. Minimal inhibitory concentration (MIC), zone of inhibition and antibacterial activity at different pH was performed to estimate the antimicrobial efficacy with the presence of [nanomaterials](https://www.sciencedirect.com/topics/materials-science/nanocrystalline-material). Bacteria were inoculated individually with 20 mL of Luria-Bertani (LB) broth media. After the revival of the culture, a loopful of culture was introduced into the sterile fresh broth and incubated at 37 °C in an orbital shaker. After reaching the mid log phase, the cells were centrifuged (4 °C, 4000 rpm for 10 to 15 min), then obtained pellets were washed several times using PBS. Further, the cells were used to analyse the antibacterial activity with the presence of our materials.The samples (PBS) were well sonicated using a bath sonicator, the ratio of the samples (1 mg) with PBS, sterile broth and cell suspension used to be at 8:1:1. Then the materials were incubated with the presence of bacteria for 6 h. Thereafter serially diluted to 10 dilutions, after that, 10−5, 10−6 and 10−7 dilutions were smeared into the agar poured, prepared plates (Mueller-hinton (MH) agar). Furthermore, the plates were incubated overnight to count the MIC, in which bacteria without material is used as negative control (complete bacterial growth). Similarly, MH agar poured plates were used to enumerate the zone of inhibition, wells were created in the microbial species spreader plates. Tetracycline is utilised as a positive control, while samples containing 20 mg/mL of BAG, 0.5Cu-BG, 1.5Cu-BG, and 2.5Cu-BG were introduced into the remaining four wells. next an overnight incubation period, the zone was photographed the next day, and MIC photographic pictures representing 10−7 dilutions were displayed.

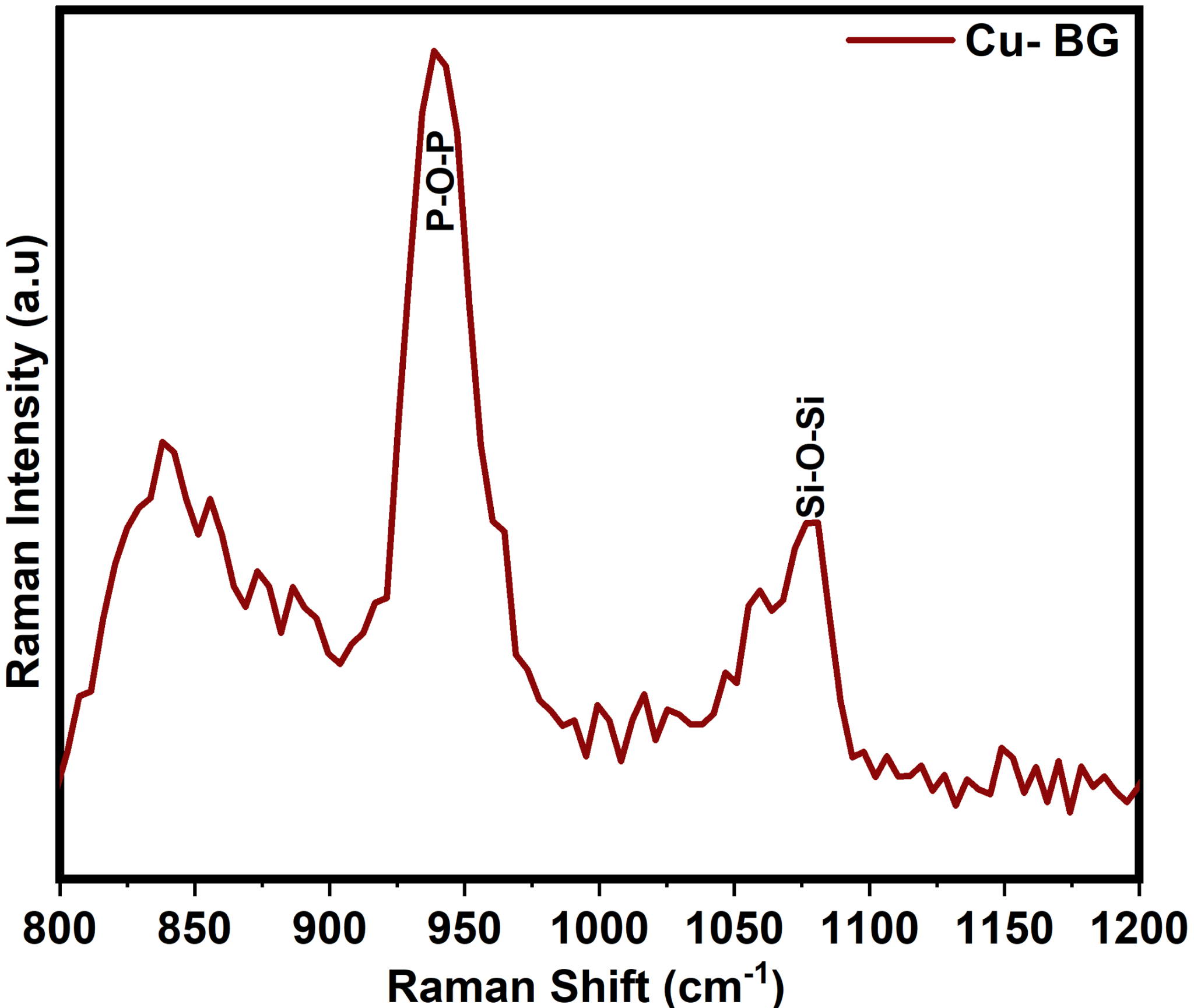
Bacterial survival% = bacterial absorbance with bioactive materials X 100

Bacterial absorbance without materials

# Results & Discussion

## Raman Spectroscopy

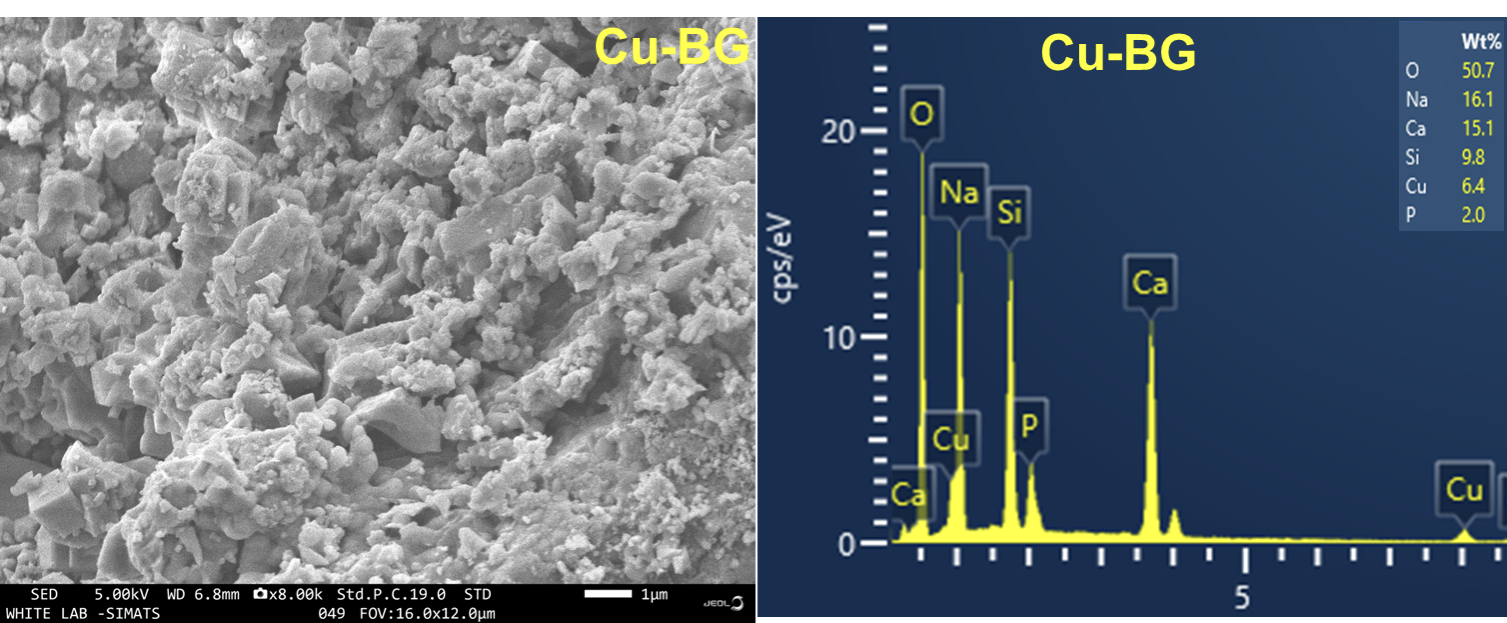
Raman spectroscopy is a potent analytical method for in depth kinetic as well as vibrational mode analysis of molecules. Interactions of the sample with the vibrational and rotational energy level of the molecules result in the part of the dispersed light travelling through a monochromatic light, most commonly produced by a laser, changing in frequency. It is able to perform substance identification, molecular structure characterization for chemicals, chemical reaction and molecular property investigations. Phosphate and silica vibrations authenticates the formation of bioglass. [(Ajay, Suma, et al., 2022; Ganapathy et al., 2022; Katyal et al., 2021; Maiti , 2021)](https://paperpile.com/c/GY5kBV/gI0E+VUK2+nzd8+DLQI)



**Fig.1.** Raman spectra of Cu-BG.

## FE-SEM and EDS Analysis

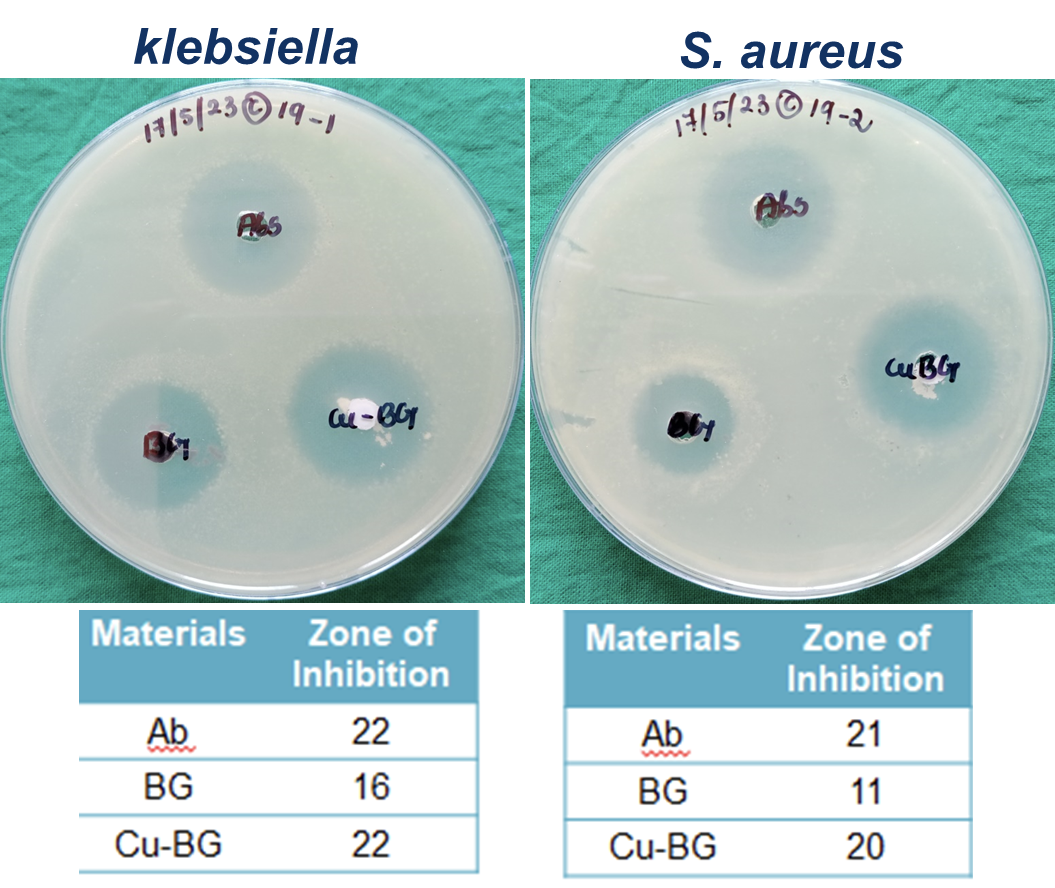
A FESEM can provide high resolution pictures of a material’s microstructure and surface morphology. FESEM can give information about glass matrix size, shape, distribution, porosity in copper doped bioactive glasses (8) or details about structural flaws, material roughness (Rafi et al., 2024). When used with EDS, FESEM can be used simultaneously for elemental composition analysis of the material as well as imaging. EDS can be used both to quantify copper ion presence and to confirm their existence in the glass matrix for copper doped bioactive glasses. In addition to detecting the other elements present in the composition of the glass, EDS can detect oxygen, silicon, calcium, phosphorus, and other elements necessary in the formation of bioactive glasses. [(Balaji Ganesh S & Sugumar, 2021; Chitra et al., 2019; Jabin et al., 2021; 2021; Sushanthi, 2021)](https://paperpile.com/c/GY5kBV/sn1g+gJsr+ZEin+x90V+QBdy). Non- homogeneous spherical morphology with Si, Ca, P, Na, Cu, C, and O was obtained from EDS spectra.



**Fig.2.** (a)FE-SEM, (b) EDS of Cu-BG.

## Antibacterial activity

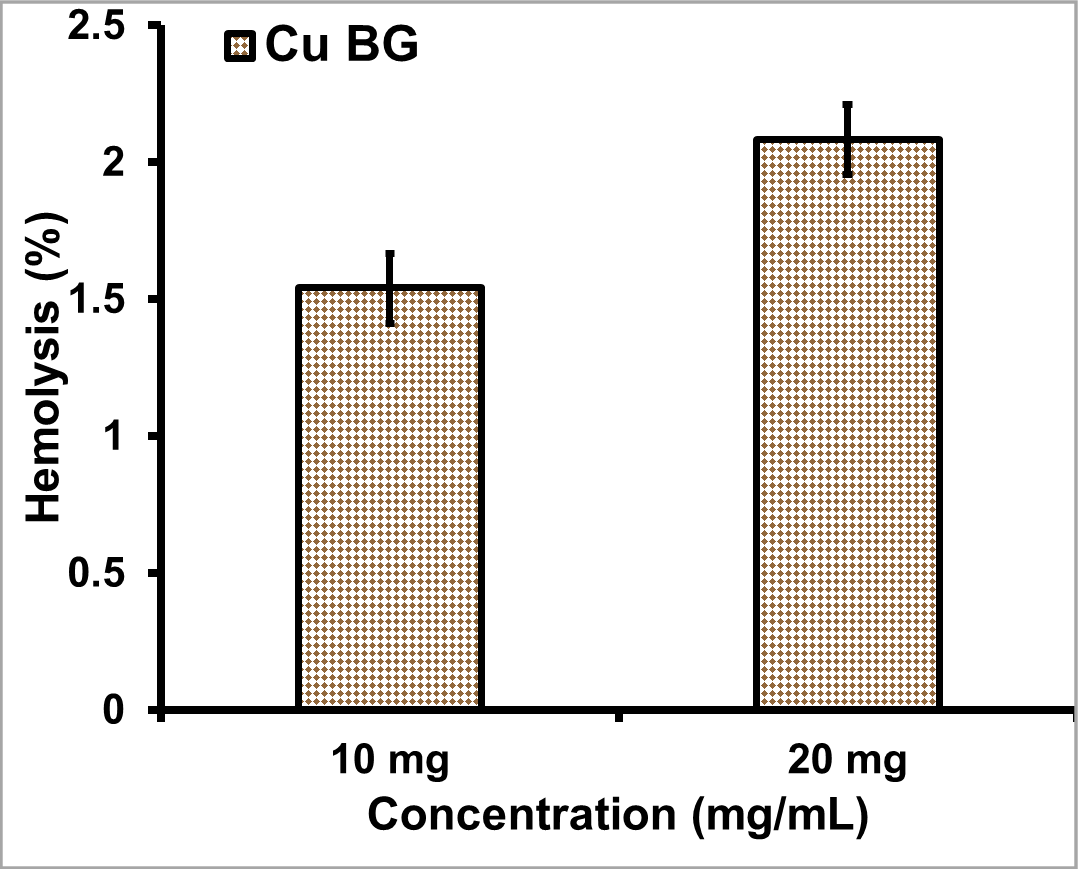
Antibacterial Susceptibility Test was carried out to determine its antimicrobial activity with both S. aureus and Klebsiella being used as test organisms (Tuluwengjiang et al., 2024). To ensure that the final bacterial concentration per well is similar in all wells, aliquots of the bacterial culture were pipetted to each well containing the sterilized bioactive glass samples, which were then placed into wells of a sterile microplate.[(Dharman, 2021; Erol-Taygun et al., 2013; Govindaraj & Dinesh, 2021; Ramamurthy, 2021; Tiwari & Jain, 2023)](https://paperpile.com/c/GY5kBV/UDzH+10bV+nTcH+qkTG+Oldx)In order to observe the baseline bacterial growth and the bioactive glass’s ability to inhibit bacterial development, a control is made. After the incubation period, inhibition zones are seen in each group. Measurements of the zones, amount of bacterial growth or comparison to other drugs with known activity can be used to calculate the antibacterial activity. [(Erol-Taygun et al., 2013; Skallevold et al., 2019)](https://paperpile.com/c/GY5kBV/UDzH+OPCZt)



**Fig.4.** Anti-bacterial activity of Cu-BG.

## Blood compatibility Assay

Hemocompatibility testing is done to assess the safety and compatibility of copper-doped bioactive glasses with blood components. Human blood was collected from volunteers. anticoagulants such as ethylenediaminetetraacetic acid (EDTA) or citrate were used to prevent blood clotting during the experiment[(*Adhesion of Microflora and the Role of Denitrifies in Colour Stability on Provisional Crowns: An in-Vitro Study Research Article*, n.d.; Baino et al., 2018; Graf, S.,Thakkar, D., Hansa, I., Pandian, S.M., Adel, S.M., n.d.)](https://paperpile.com/c/GY5kBV/5cJX+KwWX+eFS4).This procedure indicates their good compatibility with blood components and suggests their potential suitability for various biomedical applications, such as implant materials, drug delivery systems, and tissue engineering scaffolds. [(Polini et al., 2013; Tüfekçi et al., 2014)](https://paperpile.com/c/GY5kBV/Va5t+wIw0)



**Fig.5.** Blood compatibility of Cu-BG.

The use of copper doped bioactive glasses to achieve various regenerative applications including bone regeneration, dental treatments, wound healing and drug delivery systems has been demonstrated in recent research. This has been the main focus in the study of the utilization of copper doped bioactive glasses for bone tissue engineering application. They have been shown to promote bone regeneration in animal models and to promote the osteogenic differentiation of stem cells. (Bio)active copper glasses and their nanoparticles have potential in accelerating wound healing and tissue regeneration in the acute and chronic wound models [(Lakshmi, 2021; Pantulap et al., 2021a)](https://paperpile.com/c/GY5kBV/20Vc+CXcL)and have been shown to enhance angiogenesis and to promote wound closure in the diabetic wound healing. Bioactive glasses have also been considered for use in drug delivery applications in regenerative medicine where the release is under control. Their ability to release therapeutic chemicals such as growth factors or antimicrobial agents in a controlled manner for stimulating tissue regeneration or the arrest of infections were recently studied [(Nandi et al., 2016; Pantulap et al., 2021b)](https://paperpile.com/c/GY5kBV/S8j8+SIzd). In recent years, it was attempted to compare the mechanical strength, antibacterial properties, and structural integrity of several transition metal ions (such as Ag, Cu, and Fe ) doped mesoporous nano bioglass for use in bone tissue engineering. It was found that application of various transition metal ions affected the glass texture, dissolving behavior, mechanical strength and antibacterial activity.

# Conclusion

To sum up, there is great potential for a variety of regenerative applications including copper-doped bioactive glasses, such as bone regeneration, dental treatments, wound healing, and drug delivery systems. Bioactive glass matrices become more bioactive when supplied with copper ions while gaining antimicrobial properties that also enhance osteogenesis and promote vascular development together with inflammatory response regulation. The material demonstrates its tissue regeneration and healing capabilities through positive indications that reduce infection risk and inflammatory responses.

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