

*Running Title: Application of gold nanoparticles in bioactive glass for biomedical applications. | Type of study: Original Research*

## GOLD INCORPORATED BIOACTIVE GLASS FOR BIOMEDICAL APPLICATIONS

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### ABSTRACT

#### Introduction

Bioactive glasses are a group of surface reactive glass ceramic biomaterials and include the original bioactive glass, Bioglass. The biocompatibility and bioactivity of these glasses has led them to be used as implant devices in the human body to repair and replace diseased or damaged bones. Most bioactive glasses are silicate-based glasses that are degradable in body fluids and can act as a vehicle for delivering ions beneficial for healing. A bioactive material can interact with the biological environment to elicit a specific biological response, such as the formation of a hydroxyapatite layer with a bond forming between the tissue and material. The most bioactive glass has a superior surface area with a higher dissolution rate and thus faster apatite formation. In addition, they have been shown to increase the mechanical properties of such composite for natural bones and provide biomimetic nano-structuration enhancing cell adhesion. Gold nanoparticles provide an outstanding material for study due to the fact that they are one of the most stable, non-toxic, and easy to synthesize nanoparticles and exhibit various fascinating properties like assembly of various types and quantum size effect. Gold nanoparticles exhibit the potential to be utilized as a neuroprotective agent in the future.

#### Materials and methods

SiO<sub>2</sub> (45%), P<sub>2</sub>O<sub>5</sub> (6%), CaO (24.5%), and Na<sub>2</sub>O (24.5%) sources were utilized to synthesize bioactive glass by sol-gel method. The copper doped bioactive glass was characterized to study their crystalline phases by X-ray diffraction and FT IR analysis for functional group analysis. Hemocompatibility assay was performed to assess the biocompatibility of Au-BG samples with erythrocytes to estimate the lytic behavior of erythrocytes in the presence of BAG and Au-BG samples compared to positive and negative controls, respectively. To analyse the antimicrobial activity of the BAG and Au-BG with *Enterococcus faecalis* and *Streptococcus mutans*. Minimal inhibitory concentration (MIC), zone of inhibition and antibacterial activity at different pH was performed to estimate the antimicrobial efficacy with the presence of nanomaterial. The hardness of Au-BG samples and BAG samples were analysed using the Vickers hardness test and checked for its mechanical stability.

#### Results and discussion

The crystalline structure of the materials was analyzed using XRD, which revealed the presence of the Na<sub>2</sub>Ca<sub>2</sub>Si<sub>3</sub>O<sub>9</sub> crystalline phase, indicating the existence of our nanoparticles. FT IR analysis was performed to assess the properties of functional groups, showing P-O-P stretching at 575 cm<sup>-1</sup> and Si-O-Si vibrations at 1000 cm<sup>-1</sup>, confirming the presence of BIOACTIVE glasses. Hemocompatibility is a key assessment for determining the blood compatibility of

materials. According to international standards, a hemolysis rate of 5% is acceptable, and our gold-infused Bioglass exhibited a hemolysis rate of 4.5%, indicating that this material is suitable for biomedical applications.

### Conclusion

The study indicates that Gold incorporated bioactive glass has a wide potential for its application in the biomedical field due to the superior properties of the compound. Furthermore it could combat microbial diseases and depicted high mechanical stability. It could be applied for use in inflammatory disease and diseases with oxidative stress by checking for the antioxidant and anti-inflammatory properties.

**Keywords:** Bioactive glass, Gold nanoparticles, Biomedical applications, Hemocompatibility, Antimicrobial activity, Sol-gel method.

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### INTRODUCTION:

Research into dental materials has significantly increased over the last century. [1] In dental tissue engineering, inorganic synthetic materials like hydroxyapatite (HA), beta-tricalcium phosphate ( $\beta$  TCP), and silicate and phosphate glass compositions have been employed, while natural biomaterials like collagen, fibrin, chitosan, hyaluronic acid, alginate, and agar, as well as organic synthetic biomaterials like polylactic acid (PLA), polyglycolic acid (PGA), poly lactide-co-glycolic acid (PLGA), and polycaprolactone (PCL) have been employed. [2] Tissue engineering and tissue regeneration have been the focus of recent biomaterials research. [3] One of the biomaterials that has transformed contemporary biomaterial-driven regenerative medicine is bioactive glass, which has created novel biomedical uses like drug delivery and soft tissue repair. Several areas of dentistry, including periodontics, orthodontics, endodontics, oral and maxillofacial surgery, and restorative and aesthetic dentistry, use bioactive glasses. [4] Additionally, the composition of certain dental and oral care products includes bioactive glasses. In the human body, bioactive glasses have been used as dental implants to replace and repair broken bones. [5] Air abrasions, dental adhesives, periodontal disease, root canal therapy, maxillofacial surgeries, dental restorations, enamel remineralization, and dentin hypersensitivity are some additional dental conditions for which bioactive glasses are used. [6] The widespread use of bioactive glasses in dentistry necessitates the development of extensive resources and methods for supplying the necessary bioactive glasses. After being exposed to bodily fluids, [7] bioactive glasses dissolve and develop apatite crystals on their surface, which enables them to form a chemical bond with the apatite crystals found in tooth and bone tissues. [8] In addition to being a form of ceramic with certain ceramic-like qualities, bioactive glass is highly biocompatible. Because they lack free electrons in

their structure to transfer heat or electricity, ceramics—brittle, inorganic, non-metallic biomaterials made of metal-oxygen ionic bonds—are poor thermal conductors. [9] Furthermore, bioactive glass has a number of desirable qualities, such as antimicrobial and biocompatibility, which make it a good material for tissue engineering scaffolds. As one of the most basic groups of animals, marine sponges generate a variety of bioactive substances that are employed in many medical fields. [10] The anti-tumor, anti-viral, anti-inflammatory, and antibiotic properties of these substances have been demonstrated in numerous studies. Additionally, some marine sponge species have been used to extract bioactive glasses because of the mineral content of their biosilica-based structural skeletons. [11] In ancient Roman times, gold nanoparticles (AuNPs) were utilized to stain glasses for aesthetic reasons. Over the past fifty years, dependable and high-yielding techniques for the synthesis of AuNPs, including those with spherical and nonspherical shapes, have been developed. [12] The resulting AuNPs have special characteristics, including a high surface area to volume ratio, optical and electronic features that depend on size and shape, and surfaces that are easily modified with ligands that contain functional groups like thiols, phosphines, and amines that have an affinity for gold surfaces. By anchoring the ligands with these functional groups, more functionality can be added by adding other moieties like proteins, antibodies, and oligonucleotides. [13] The creation of these gold nanoconjugates has made it possible to conduct a wide range of research, such as bioelectronics, nanoparticle arrangement into dimers and trimers onto DNA templates, programmed assembly and crystallization of materials. [14] Gold nanoparticles provide an outstanding material for study due to the fact that they are one of the most stable, non-toxic, and easy to synthesize nanoparticles and exhibit various fascinating properties like assembly of various types and quantum size effect. Gold nanoparticles have the ability to transport medications and nucleic acids to cancerous cells and tissues. They can also be used to

locate cancer and track medications. GNPs can administer photosensitizers in photodynamic therapy. [15] GNPs can be employed as adjuvants and carriers in vaccine development to lower toxicity, boost immunogenic activity, and offer stability during storage. GNPs are strong antimicrobial agents that have the ability to exhibit bactericidal effects on a broad range of microorganisms. [16] Gold nanoparticles exhibit the potential to be utilized as a neuroprotective agent in the future. This study aims to synthesise gold incorporated bioactive glass for biomedical applications and test for its crystalline structure, functional group analysis, hemocompatibility and antimicrobial properties.

[17] Tissue engineering has emerged as a transformative field in regenerative medicine, integrating biomaterials, cells, and engineering principles to repair or replace damaged tissues. Recent advances in this field have highlighted its growing importance in biomedical applications, particularly in the development of innovative materials for tissue regeneration and repair. [18] In dentistry and orthopedic regeneration, bioactive glasses have attracted substantial attention due to their excellent biocompatibility, osteoconductivity, and antibacterial properties. These materials have been shown to significantly enhance tissue regeneration, promote bone formation, and improve restorative dental procedures. Furthermore, the incorporation of therapeutic ions such as strontium, zinc, and magnesium into bioactive glass systems has been reported to enhance osteogenic differentiation, antibacterial activity, anti-inflammatory responses, and angiogenesis, thereby improving their overall biological performance and suitability for regenerative medicine applications.

[19] Nanotechnology has further expanded the scope of biomedical materials through the development of metallic nanoparticles. [20] Green synthesis approaches have gained considerable interest because they provide environmentally friendly and sustainable routes for nanoparticle production. Among various nanomaterials, gold nanoparticles have emerged as versatile candidates for biomedical applications owing to their unique physicochemical properties, excellent biocompatibility, and ease of functionalization. They have been widely explored for applications in diagnostics, medical imaging, targeted drug delivery, biosensing, and cancer therapy. In addition, the combination of gold nanoparticles with biocompatible materials such as polysaccharides has demonstrated enhanced therapeutic efficacy and improved biological performance.

Recent investigations have focused on developing novel synthesis methods and expanding the biomedical applications of gold nanoparticles. Green-

synthesized gold nanoparticles derived from natural plant extracts have demonstrated significant biological activities, including antimicrobial, antioxidant, and anticancer effects. Advanced synthesis techniques have also enabled the fabrication of gold-based nanostructures with controlled morphology and improved functional properties. Furthermore, antibody-functionalized gold nanobiostructures have shown considerable promise in targeted photodynamic therapy, providing improved treatment specificity and therapeutic outcomes. Collectively, these developments indicate that bioactive glasses and gold nanoparticles possess substantial potential in tissue engineering, regenerative medicine, antimicrobial therapies, drug delivery systems, and cancer treatment, contributing to the advancement of next-generation biomedical materials and therapeutic technologies.

## MATERIALS AND METHODS:

### Preparation of bioactive glass:

Analytical grade reagents and chemicals were used for this study without additional purification, Tetraethyl orthosilicate (TEOS) was procured from Alfa Ansar, ortho-phosphoric acid, calcium nitrate and nitric acid were procured from the spectrum reagents and chemicals Pvt.Ltd. SiO<sub>2</sub> (45%), P<sub>2</sub>O<sub>5</sub> (6%), CaO (24.5%), and Na<sub>2</sub>O (24.5%) sources were utilized to synthesize bioactive glass by sol-gel method.(45S5 composition) were synthesized using sol-gel method (SiO<sub>2</sub> —45%, P<sub>2</sub>O<sub>5</sub> —6%, CaO—24.5%,NaO—24.5%). TEOS were completely dissolved with double-distilled water and ethanol, nitric acid used as catalyst, this mixture stirred for 1 h, and then turned into a gel-like network. Additionally, CaNO<sub>3</sub> and NaOH dissolved in double distilled water and added to the aforementioned mixture. About 1.5% of copper source (copper nitrate) is introduced at the place of sodium by reducing the percentage as 23%, into sodium site and is named as Cu-BAG. The quantity of the precursor sources to be used was calculated in weight percentage and each respective source was dissolved separately and added into the silica network. To eliminate moisture, samples are dried in hot air oven (24 hr at 100 degree Celsius ) and finally sintered at 600 degree Celsius for 3 hours. Prepared bioactive materials (100 mg) were loaded with 50 mg of ACE and IBU. Both the drugs were solubilized in dimethyl sulfoxide (DMSO) (10 ml) and immersed for 24 h for the drug loading, and they were loaded in BAG and Cu-BAG respectively. After solubilization of ACE and IBU, BAG and Cu-BAG powders were separately incubated and agitated using an orbital shaker for 24 h to load drugs on the voids of bioactive materials. Detailed protocol of drug loading and release kinetics was reported in our previous research article. Drugs

(ACE-IBU) loaded bioactive materials (BAG/ACE-IBU) and (Cu-BAG/ACE-IBU) were packed into prepared root canal to estimate the efficacy of filling and subsequent mineralization. These kinds of materials support root canal filling and also control inflammation as well as microbial responses in the oral environment.

### Material characterisation

Synthesized bioactive materials were investigated to identify their crystalline properties through x-ray diffraction (XRD) (PANalytical Instruments, The Netherlands). Cu-K $\alpha$ 1 radiation was utilized with the scanning rate of 10° min<sup>-1</sup>. Morphology of the copper-bioactive materials was imaged by field emission scanning electron microscope (FESEM, HITACHI SU-6600, Japan). Subsequent elemental composition was analysed by energy dispersive x-ray analysis (EDAX-Horiba). Bioactive materials sealed portions and their corresponding mineralization were also imaged by FESEM (FEI Quanta 200) and the obtained micrographs were analysed. To evaluate the particle size distribution and the morphological manifestation of copper-bioactive materials High Resolution Transmission Electron Microscopy (HR-TEM) (JEOL Japan, JEM- 2100 Plus) was utilized. Chemical composition of the copper-bioactive materials was studied by x-ray photoelectron spectroscopy (XPS) (Omicron Nanotechnology ESCA-14, Al source mediated). Micro Raman spectra were obtained by a confocal Raman microscope (RAMAN 11i—Nanophoton) using the excitation source of wavelength 532 nm. Similarly, vibrational modes were also confirmed via Fourier transform infra-red (FT-IR) analysis (Jasco FT/IR- 6600) by ATR mode with the resolution of 4 cm<sup>-1</sup>. Porosity of the copper-bioactive materials was analysed by Quantachrome Nova-1000 surface analyzer at liquid nitrogen temperature. Nitrogen adsorption-desorption isotherm measurements were carried out to understand the porosity of the samples. Surface charges were obtained from zeta potential analysis. BIOACTIVE materials were suspended in double-distilled water and sonicated by ultra Sonicator for 10 minutes before subjecting it to zeta potential measurement. To assess the sealing ability through x-ray imaging, X-mind (Acteon, Birmingham, United Kingdom) was utilized. Fracture resistance was performed to study the mechanical stability of bioactive sealants by a universal testing machine (TEC-SOL INDIA—TSLD-C-200).

### Hemocompatibility assay

Hemocompatibility assay is considered as one of the primary biocompatibility assays to assess the compatibility of biomaterials. Especially, for all the

biomaterials at the point of contact in vivo, foremost interaction will be with blood cells. Particles of bioactive glasses enter into the blood and get into contact with erythrocytes. Hence, it is essential to explore the compatibility of bioactive glasses in the bloodstream. In order to evaluate such responses with RBCs, in vitro hemolytic activity was carried out. Hemolytic activity was performed to assess the biocompatibility of the bioactive materials.

$$\text{Hemolysis (\%)} = \frac{\text{Sample absorbance} - \text{Negative control}}{\text{Positive control} - \text{Negative control}} \times 100 \quad (1)$$

Subsequently, clot lysis was carried out with fresh blood (without anticoagulant). For that, 100  $\mu$ l of the blood was dropped on the bioactive materials in watch glasses. After 10 min, the clotted blood was washed with 10 ml of double distilled water. The corresponding solution was used to found the rupture rate of hemoglobin, which was obtained by the absorption at 540 nm (Nan et al., 1994). All the materials were tested with their triplicates.

### Antimicrobial assay:

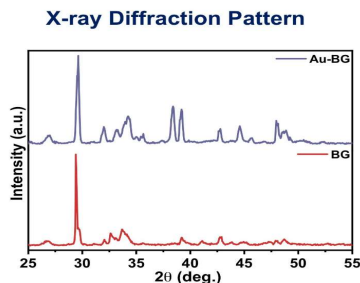
To evaluate the antimicrobial activity of bioactive glass and gold incorporated bioactive glass against *Enterococcus faecalis* and *Streptococcus mutans*, two key methods were typically employed, namely the zone of inhibition and the minimum inhibitory concentration (MIC) assay. The bacterial stains used were *Enterococcus faecalis* and *Streptococcus mutans*. Nutrient agar was used for each bacterial strain with Sterile Filter Paper Discs (for Zone of Inhibition Test), Broth for MIC Determination (Mueller-Hinton broth), Incubator (37°C for optimal growth, Micropipette and Sterile Tips, Calipers or Ruler (to measure the zone of inhibition and Sterile Saline or Phosphate Buffered Saline (PBS) (to prepare bacterial suspensions). The zone of inhibition test involves applying the antimicrobial agent to the surface of an agar plate inoculated with bacteria. The agent diffuses outward, and if it is effective, it will inhibit bacterial growth, creating a clear zone around the application site. The bacterial suspensions of *Enterococcus faecalis* and *Streptococcus mutans* were prepared in sterile saline to match the turbidity equivalent to a 0.5 McFarland standard ( $\sim 1 \times 10^8$  CFU/mL). An inoculating loop was sterilised and the bacterial suspension was streaked evenly across the surface of the agar plate, using a method like the spread plate technique. Using sterile forceps, a sterile filter paper disc was placed onto the

surface of the agar. Following this, a specific volume (e.g., 10  $\mu\text{L}$ ) of the antimicrobial agent was pipetted onto the disc or used pre-soaked antibiotic discs. The agar plates were incubated at 37°C for 18-24 hours for optimal bacterial growth. After incubation, measure the diameter of the clear zone surrounding the disc (area of inhibition) using a ruler or calipers, the zone diameters were measured (in mm). The measured zones were compared with standard zone size interpretation charts to determine the effectiveness of the antimicrobial agent (e.g., susceptible, intermediate, or resistant). For MIC (Minimum Inhibitory Concentration) assay the bacterial suspension was prepared as described in the zone of inhibition method. A series of two-fold dilutions of the antimicrobial agent was prepared in sterile broth or agar. Common concentrations range from 1000  $\mu\text{g/mL}$  to 0.5  $\mu\text{g/mL}$ , depending on the potency of the antimicrobial agent being tested. In a 96-well microtiter plate or a set of test tubes, add the appropriate amount of broth (e.g., 100  $\mu\text{L}$ ). A fixed volume of the bacterial suspension (e.g., 10  $\mu\text{L}$  of bacterial culture with  $\sim 1 \times 10^6$  CFU/mL) was added to each well/tube containing the antimicrobial agent dilutions. The microtiter plate or test tubes were incubated at 37°C for 18-24 hours. After incubation, each well was examined for bacterial growth, which can be identified by turbidity or color change if using indicator dyes. The MIC is the lowest concentration of the antimicrobial agent where no visible bacterial growth was observed.

#### Vickers Hardness test :

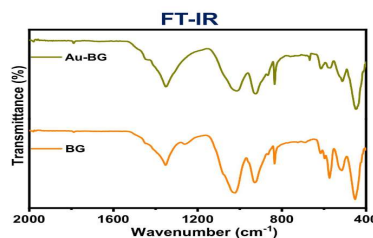
Bioactive glass is a type of glass that can bond with bone and tissue, making it ideal for use in bone regeneration and repair. It typically consists of silicate-based compositions, such as 4S5 (composed of  $\text{SiO}_2$ ,  $\text{Na}_2\text{O}$ ,  $\text{CaO}$ , and  $\text{P}_2\text{O}_5$ ). Pure bioactive glasses generally have moderate hardness, which is often in the range of 4 to 6 GPa. However, the hardness can be influenced by the composition, structure, and processing of the bioactive glass. Bioactive glasses tend to be brittle, so their hardness may not be high compared to metals or ceramics designed for wear resistance. The mechanical stability and strength of BAG and AuBG were analysed and compared using the Vickers Hardness Test.

#### RESULTS :



**Fig 1:** XRD analysis is a tool used to analyse the crystalline structure of the materials; presence of  $\text{Na}_2\text{Ca}_2\text{Si}_3\text{O}_9$  crystalline phase that confirms the presence of our nanoparticles.

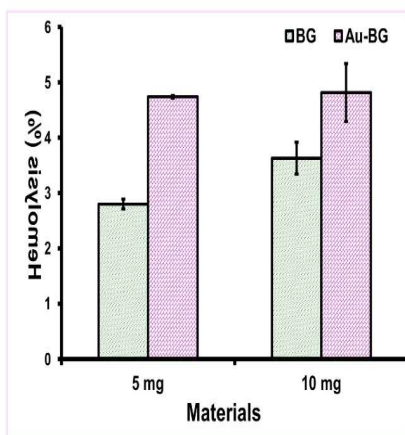
The XRD pattern compares conventional bioactive glass (BG) and gold-incorporated bioactive glass (Au-BG). Both samples exhibit distinct diffraction peaks, confirming the presence of crystalline phases within the glass matrix. The intense peak around 29° 2 $\theta$  and additional peaks between 32° and 50° indicate the formation of crystalline calcium-sodium silicate phases such as combeite. Au-BG displays sharper and more intense peaks than BG, suggesting increased crystallinity following gold incorporation. The presence of gold nanoparticles may have acted as nucleation sites during heat treatment, promoting crystal growth and structural ordering. Enhanced crystallinity is advantageous because it improves mechanical strength and structural stability while maintaining the bioactive nature of the material. Therefore, the XRD results confirm the successful incorporation of gold without disrupting the glass network and demonstrate an improvement in the crystalline characteristics of the bioactive glass.



**Fig 2:** FT IR analysis to evaluate the functional group properties that depicted P-o-P stretching at 575  $\text{cm}^{-1}$  and at 1000  $\text{cm}^{-1}$  Si-O-Si vibrations.

The FTIR spectra provide information regarding the functional groups present in BG and Au-BG. Both materials exhibit characteristic absorption bands corresponding to silicate and phosphate networks, confirming the preservation of the bioactive glass structure after gold incorporation. The broad bands observed around 1000–1100 cm<sup>-1</sup> are attributed to Si–O–Si asymmetric stretching vibrations, while peaks in the 800–900 cm<sup>-1</sup> region correspond to Si–O–Si bending vibrations. Additional bands between 500 and 650 cm<sup>-1</sup> indicate phosphate (PO<sub>4</sub><sup>3-</sup>) groups, which are essential for apatite formation and bioactivity. Slight shifts in peak position and intensity in Au-BG suggest interactions between gold nanoparticles and the glass network, resulting in modifications of network connectivity. These changes may increase ion release and enhance bioactivity. Overall, the FTIR results confirm that gold incorporation does not alter the fundamental glass structure but slightly modifies the network, potentially improving biological performance.

### Blood Compatibility

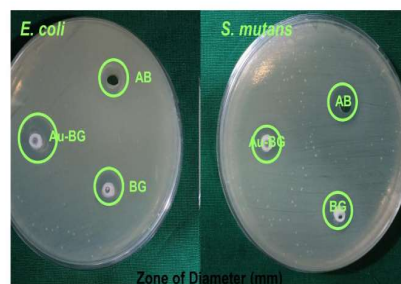


**Fig 3:** Haemocompatibility assay depicting the percentage of lysis of red blood cells by gold incorporated bioactive glass at a concentration of 5mg and 10mg.

The hemocompatibility study evaluated the effect of BG and Au-BG on red blood cell integrity by measuring hemolysis percentages at concentrations of 5 mg and 10 mg. BG exhibited hemolysis values of approximately 2.8% and 3.6%, whereas Au-BG showed values of approximately 4.7% and 4.8% at the respective concentrations. Although Au-BG demonstrated slightly higher hemolysis than BG, all

values remained below the internationally accepted threshold of 5% for biomaterials. This indicates that both materials possess acceptable blood compatibility and are unlikely to cause significant damage to erythrocytes. The results confirm that gold incorporation does not compromise hemocompatibility and supports the safe use of Au-BG in biomedical applications involving direct blood contact, such as implants, bone grafts, and dental materials.

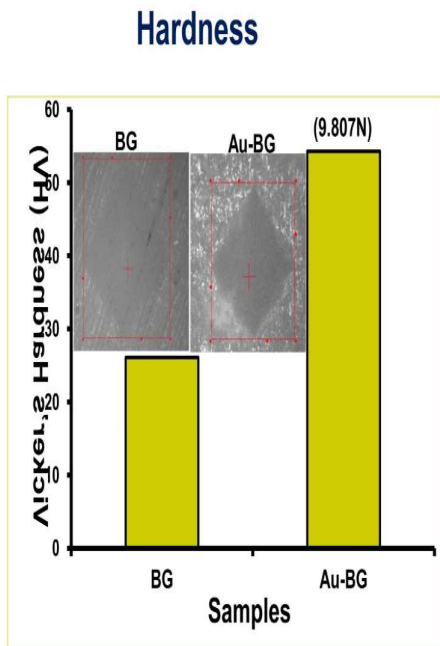
### Antibacterial Activity



Materials	<i>E. coli</i>	<i>P. mutans</i>
AB	16	16
BG	15	15
Au-BG	18	16

**Fig 4:** Analysis of antimicrobial activity of controlled group, BIOACTIVE glass and Gold incorporated BIOACTIVE glass against *Enterococcus faecalis* and *Streptococcus mutans*.

The antibacterial assay assessed the effectiveness of BG and Au-BG against *Escherichia coli* and *Streptococcus mutans* using the zone of inhibition method. Au-BG exhibited the highest antibacterial activity, producing inhibition zones of 18 mm against *E. coli* and 16 mm against *S. mutans*. In comparison, BG showed inhibition zones of 15 mm against both bacterial species, while the antibiotic control (AB) produced zones of 16 mm. The larger inhibition zone observed for Au-BG indicates enhanced antibacterial efficacy due to gold incorporation. Gold nanoparticles can interact with bacterial cell membranes, disrupt metabolic processes, and inhibit bacterial growth. The superior antimicrobial performance of Au-BG suggests its potential application in dental restorations, root canal sealers, bone implants, and wound-healing materials where prevention of bacterial colonization is essential.



**Fig 5:** Analysis of mechanical stability of BIOACTIVE glass and gold incorporated BIOACTIVE glass using Vickers Hardness test.

The Vickers hardness test was performed to evaluate the mechanical strength and resistance to surface deformation of BG and Au-BG. The hardness value of conventional BG was approximately 26 HV, whereas Au-BG exhibited a significantly higher hardness value of approximately 54 HV. This nearly twofold increase indicates that gold incorporation substantially enhances the mechanical properties of the bioactive glass. The increased hardness may result from improved crystallinity and stronger interactions within the glass network, leading to greater resistance to indentation and wear. Enhanced hardness is particularly beneficial for dental and orthopedic applications, where materials are subjected to continuous mechanical stresses. Therefore, the Vickers hardness results demonstrate that Au-BG possesses superior mechanical stability and durability compared with conventional bioactive glass, making it a promising material for load-bearing biomedical applications.

## DISCUSSION:

Bioactive glass (BAG) is a silica-based biomaterial widely used in biomedical and dental applications because of its excellent bioactivity, biocompatibility, and ability to bond with hard tissues. [21]The silica component forms an amorphous network of  $\text{SiO}_4$

tetrahedra interconnected through oxygen atoms, while network modifiers such as sodium and calcium influence the structure and bioactivity of the material. Thermal treatment promotes the formation of semi-crystalline phases, and X-ray diffraction studies have identified crystalline components such as combeite ( $\text{Na}_2\text{Ca}_2\text{Si}_3\text{O}_9$ ), high-combeite ( $\text{Na}_{5.27}\text{Ca}_3\text{Si}_6\text{O}_{18}$ ), and calcite ( $\text{CaCO}_3$ ). In copper-incorporated bioactive glass (Cu-BAG), traces of cuprorivaite are also present. Cuprorivaite is known for its synergistic effects on wound healing, angiogenesis, and antimicrobial activity, thereby enhancing the biological performance of the material.

Surface characterization using X-ray photoelectron spectroscopy (XPS) confirmed the presence of silica, phosphorus, calcium, sodium, oxygen, carbon, and copper within the bioactive glass network. A characteristic  $\text{Cu}^{2+}$  peak at approximately 942 eV in Cu-BAG confirmed the successful incorporation of copper into the glass structure. [22] High-resolution O1s spectra revealed variations in network connectivity through the presence of bridging oxygen (BO) and non-bridging oxygen (NBO) bonds. Cu-BAG exhibited a higher percentage of NBO compared to conventional BAG, indicating greater network disruption and enhanced ion release. This increased dissolution behavior facilitates apatite formation, improves sealing ability, and enhances the biological activity of the material in aqueous environments.

[23] Raman spectroscopy and FTIR analysis provided further insight into the structural modifications caused by copper incorporation. [24]Characteristic peaks corresponding to silicate and phosphate groups demonstrated alterations in glass network connectivity. Copper ions partially substituted sodium ions as network modifiers, leading to increased NBO formation and changes in crystallinity. Although the overall structural framework remained similar to conventional BAG, Cu-BAG displayed greater phosphate prevalence and enhanced dissolution properties, which are favorable for in-vitro mineralization and tissue regeneration.

Hemocompatibility testing evaluated the interaction of the prepared materials with human red blood cells. [25]The hemolysis rate of gold-incorporated bioactive glass was found to be 4.5%, which is below the internationally accepted threshold of 5% established for biomedical materials. This result demonstrates excellent blood compatibility and indicates that the incorporation of gold does not adversely affect erythrocyte integrity, making the material suitable for applications involving direct blood contact.

[26]Antimicrobial evaluation against *Enterococcus faecalis* and *Streptococcus mutans* showed enhanced antibacterial activity for gold-incorporated bioactive glass. The material produced inhibition zones of 18

mm against *E. faecalis* and 16 mm against *S. mutans*, compared with 15 mm for standard BAG.[27] The improved antimicrobial efficacy is attributed to the ability of gold ions to interfere with bacterial cell membranes and inhibit bacterial growth. Furthermore, Vickers hardness testing demonstrated superior mechanical stability and hardness in gold-incorporated bioactive glass, suggesting greater resistance to wear and deformation. Collectively, these findings indicate that modified bioactive glasses possess enhanced bioactivity, antimicrobial performance, hemocompatibility, and mechanical strength, making them promising candidates for dental restorations, root canal sealers, bone grafts, implants, and wound-healing applications.

#### LIMITATIONS AND FUTURE SCOPE :

Future studies should investigate long-term biocompatibility, toxicity profiles, stem-cell interactions, and translational clinical applications of gold-incorporated bioactive glass systems [27,28]

In the future, Gold infused nanoparticles research will examine safety, efficacy, and dose in human trials as well as validate its properties in animal models. Mechanistic research will examine its modulation of the various pathways, and studies examining combination therapy with currently available chemotherapeutic drugs may uncover synergistic benefits.

Another important area of emphasis is identifying and manufacturing particular bioactive [29]chemicals and addition of secondary metabolites for enhancing the properties. Furthermore, expanding research to encompass alternative forms to overcome various diseases and widely used in biomedical applications.

#### CONCLUSION:

The present study demonstrates that gold-incorporated bioactive glass possesses significant potential for a wide range of biomedical applications owing to its unique combination of bioactivity, antimicrobial efficacy, mechanical stability, and biocompatibility. [30]The incorporation of gold nanoparticles enhances the material's therapeutic functionality, enabling effective inhibition of microbial growth while supporting tissue regeneration and healing processes. Furthermore, its antioxidant and anti-inflammatory properties suggest promising applications in the management of inflammatory disorders, oxidative stress-related diseases, and advanced wound healing. The synergistic interaction between bioactive glass and gold nanoparticles provides a multifunctional platform capable of promoting cellular responses and improving overall biological performance. Therefore, gold-incorporated bioactive glass emerges as a highly

promising biomaterial for future applications in tissue engineering, regenerative medicine, drug delivery, and other advanced biomedical fields.

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#### CONFLICT OF INTEREST

The authors hereby declare that there is no conflict of interest in this study.

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