

DENTAL RESTORATIVE MATERIAL: MECHANICAL AND BIOLOGICAL PROPERTIES

PROJECT WORK

Submitted in partial fulfillment of the requirements for the award of
Bachelor of Science degree in Microbiology

By

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**DEPARTMENT OF BIOTECHNOLOGY
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SATHYABAMA

**INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)**

CATEGORY – 1 UNIVERSITY BY UGC

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BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of **THULASIDEVI.R (41300051)**, **RENUGA DEVI.K (41300035)** who carried out the project entitled **DENTAL RESTORATIVE MATERIAL:AND BIOLOGICAL PROPERTIES** under our supervision from January 2024 to April 2024

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We, **Thulasidevi. R** (41300051), **Renuga devi. K** (41300035) hereby declare that the project Report entitled "**DENTAL RESTORATIVE MATERIAL: MECHANICAL AND BIOLOGICAL PROPERTIES**" done by us under the guidance of Dr. S. Jayashree Assistant professor Internal guide, Department of Biotechnology and Dr. T. Stalin dhas Assistant professor (Research) External guide at centre for ocean research, is submitted in partial fulfillment of the requirements for the award of Bachelor of Science degree in Microbiology.

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ABSTRACT

Glass ionomer cement (GIC) is a widely used biomaterial important in dental restoration for dental cavities, filling, lining and restoration of adhesion. Hydroxyapatite (HAP) is a natural mineral with favorable properties in dental restoration. The oral cavity has a moist environment that poses a major challenge to the performance of restorative materials. Due to these restorative materials hydroxyapatite and magnesium oxide nanoparticles (MgO NPs) are added to enhance the material's antibacterial properties. This results in GIC chemically binding to the tooth releasing fluoride ions for longer periods and also used as an orthodontic bracket attachment. Strong bactericidal qualities of MgO NPs prevent microleakage and bacterial colonization, which cause secondary caries and, eventually, the failure of dental restorations.

This study aims at improving the mechanical properties against food-borne pathogens. The resultant GIC composite with HAP and MgO NPs will have enhanced mechanical and antimicrobial properties.

Keywords: Hydroxyapatite, Glass ionomer, Magnesium Oxide Nanoparticles, Foodborne pathogen.

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LIST OF ABBREVIATIONS

BF	Biofilm
CAP	Calcium phosphate
EDTA	Ethylene Diamine Tetra acetic Acid
EPS	Extracellular polymeric Substances
FTIR	Fourier transform infra-red spectroscopy
GIC	Glass ionomer cement
HAP	Hydroxyapatite
LMWH	Low molecular weight heparin
MgO	Magnesium oxide
nm	Nano meter
NPs	Nanoparticles
OD	Optimal density
PA	<i>Pseudomonas aeruginosa</i>
PBSS	Phosphate buffer saline solution
pH	Potential of Hydrogen
SA	<i>Staphylococcus aureus</i>
SEM	Scanning electron microscope
Ti	Titanium
TiO ₂	Titanium Oxide
UV	Ultra violet
XRD	X-ray power diffraction
ZnO	Zinc oxide
ZrO ₂	Zirconium dioxide
Zr	Zirconium

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CHAPTER 1

INTRODUCTION

1.1 NANOTECHNOLOGY

This area of study focuses on the utilization of incredibly small objects in a variety of scientific domains, including engineering, chemistry, biology, physics, and material science. Professor Norio Taniguchi first used the word "nanotechnology" in 1974. One billionth, or 10^{-9} , of a meter, is equal to one nanometer. To create nanoparticles, two methods are employed: the top-down method and the bottom-up method. In poor nations, nanotechnology is employed to cure and prevent health problems. Nanomedicine is the general word for this application.

1.2 METAL NANOPARTICLES

These submicron entities are composed of pure metals, such as cerium, iron, zinc, thallium, silver, and fluorine, or their compounds, including oxides, phosphates, fluorides, sulfides, halides, and chlorides. Nanoparticles of gold, silver, and copper exhibit resonance and a pronounced absorption in the visible spectrum. These particles are mostly employed as catalysts in the fields of electronics, optics, and fluorescent materials.

1.3 MAGNESIUM OXIDE NANOPARTICLES

Nanomaterials smaller than 100 nm are found in many different fields, including physics, chemistry, biology, optical components, cosmetics, polymer science, toxicology, pharmaceutical drug production, and mechanical engineering. The nanoparticles of magnesium oxide have no smell and no toxicity. They have a high melting point, great hardness, and high purity. The powdered form of magnesium oxide nanoparticles is white powder.

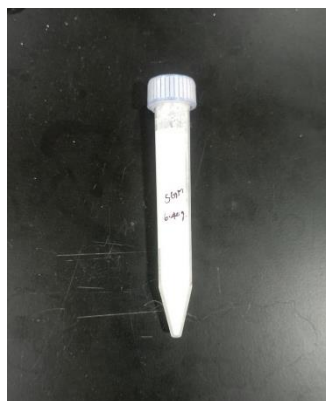


Fig1.1: Dried MgO

1.4 MAGNESIUM OXIDE NANOPARTICLES AS AN ANTIBACTERIAL AGENT

Magnesium oxide-based nanoparticles have demonstrated efficacious antibacterial capabilities against a broad spectrum of microbes for both gram-positive and gram-negative bacteria. The antibacterial mode of action of these nanoparticles is contingent upon their size and dosage. The antibacterial mechanism of MgO-NPs is explained via a variety of approximations.

1.5 HYDROXYAPATITE NANOPARTICLES

Hydroxyapatite (HAP) is a calcium phosphate molecule that shares the same hexahedral structure and 1.67 stoichiometric Ca/P ratio as bone apatite. Dental enamel and dentin are primarily constituted of carbonated calcium-deficient HAP. Under physiological conditions, HAP has remarkable thermodynamic stability in comparison to other calcium phosphates. Due to its osteogenic qualities and biocompatibility, the ability to chemically manufacture HAP has expanded its clinical applications in several medical domains. HAP synthesis can be carried out using a variety of ways, such as mechanochemical processes, wet chemical reactions, or dry procedures. These approaches enable the production of distinct compounds with varying chemical compositions, sizes, and shapes.

Because it resembles human hard tissues in terms of size, crystallography, and chemical composition, nano-sized HAP is a biomaterial of particular interest among the HAP forms. The crystal size of nano-HAP varies from 20 to 80 nm, whereas the crystal size of manufactured HAP is 25.40 nm.

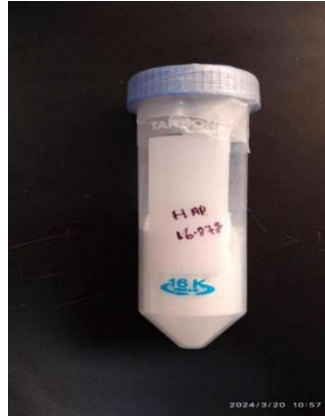


Fig1.2: Dried HAP

1.6 DENTAL IMPLANTS

In dental surgery, implants are frequently used to restore teeth. A difficulty in the field of implantology is to accomplish and preserve the osseointegration and gingival epithelial connection with implants. Earlier osseointegration of dental implants is associated with their long-term clinical success. Interactions occur between biological fluids, tissues, cells, and implant surfaces in implants. Implants come into contact with blood platelets and proteins right after implantation. The peri-implant tissue healing process will thereafter be influenced by the mesenchymal stem cells' development. Dental implant surface changes are becoming more and more common because of nanotechnologies. The use of thin calcium phosphate (CAP) coatings is an additional strategy to improve osseointegration.

1.7 DENTAL RESTORATIVE MATERIAL

Nanoparticles have a vast scope in the study field of dentistry and also have a wide area of application in dental implants. Nanoparticles are ZnO -Zinc oxide, ZrO₂ -Zirconium dioxide, MgO-Magnesium Oxide, TiO₂-TitaniumOxide, HAP-Hydroxy apatite, Ti-Titanium, Ag Silver Zr Zirconium, GIC Glass Ionomer Cement Some types of nanoparticles exhibit favorable physio-mechanical, biocompatible, and antibacterial characteristics, among others. Additionally, it addressed the role that therapeutic and restorative nanoparticles play in dentistry and how they support dental implants. In dentistry, several kinds of nanocoating can be applied extensively to improve the structural integrity of teeth. Dental implant failure can be avoided by further study and development of more biocompatible materials, which is a current area of significant research interest. A significant problem in dentistry is implant

failure brought on by cytotoxicity and biocompatibility, which can be developed or resolved by nanotechnology. (Hossain *et.al.*,2022)

1.8 GLASS IONOMER CEMENT

Glass ionomer cement (GICs) are restorative dental materials that are used in dentistry for luting and filling purposes, as well as for the attachment of orthodontic brackets. The basis of glass-ionomer cement is the reaction between an ionomer, polyacrylic acid, and silicate glass powder, or calcium alumino-fluorosilicate glass. Sometimes an acid is substituted with water, changing the material's characteristics and applications. Glass polyalkenoate, as it is chemically termed, is the powdery cement of glass particles encased in a matrix of fluoride elements that results from this process. Other comparable reactions can occur, such as the formation of a liquid glass-ionomer when tartaric acid and also used in the form of liquid.

1.9 CRYSTAL VIOLET ASSAY (BACTERIAL CYTOTOXICITY)

Biofilm (BF) is a thin layer of material that forms on a material or other interface as a result of bacterial activity. Numerous scholars have defined and discussed the basic ideas of biofilms, and numerous books and reviews have summarized these ideas. They usually form on the surfaces of materials. Thus, the surfaces of materials must have an impact on biofilm formation. We have attempted to demonstrate how materials impact the creation and growth of biofilms from this perspective. Bacteria, EPS (extracellular polymeric compounds), and water make up around 80% of BF. It's got that distinct sliminess. Quorum sensing, a phenomenon where bacteria stick to surfaces, grow, and release polysaccharides outside of their lipids and nucleic acids are produced in biofilms.

1.10 HEMOCOMPATIBILITY ASSAY

Hemocompatibility is a subset of biocompatibility. (blood) and tissue and cell cytocompatibility. Whether a cell is biocompatible relies on whether it survives. by the use of the material. "Cytotoxicity" refers to the series of events brought about by molecule interference with macromolecular synthesis, which unquestionably results in cellular, structural, and functional damage.

Materials used in biomedical devices such as medical membranes, catheters, and cardiovascular implants must be hemocompatibility to prevent immunological

responses and surface-initiated coagulation processes. The components of the blood may be destroyed or activated by this contact.

Blood is a complicated tissue made up of plasma and cells. Blood plasma is an electrolytic isotonic solution with a high protein concentration of about 40–60 g/L and 0.9% sodium chloride. More than half of all plasma proteins are made up of albumin, although many other low-level components are essential to the body's overall function.

1.11 CONTACT ANGLE MEASUREMENT

The contact angle is the angle created tangential to the liquid surface at the point where the three phases converge and the plane of the solid material's surface where the liquid substance is present or flows. Sessile drop instruments are typically used to measure the contact angle. While some research has shown that very hydrophilic substrates encourage the formation of biofilms, other studies have revealed that bacterial adherence is higher on hydrophobic surfaces. As stated by (Sang *et. al.*, 2010).

1.12 BIOFILM ADHESION ASSAY

It is necessary to do a biofilm adhesion assessment that contrasts the adherence of host cells and bacteria to implant surfaces. In this work, the adhesion difference between osteoblast-like cells and bacterial biofilm on surfaces simulating implants is measured using the laser spallation technique. The Adhesion Index is designed to provide a quantitative measure for medical implant surface biocompatibility evaluation. According to Smith *et.al.*, (2009), the first phase of medical device implantation is the most susceptible to the emergence of bacterial infections. This study primarily focuses on early colonization and first-cell adhesion. The baseline adhesion for both the host cell model and the bacterial biofilm model is tested using established growth techniques.

The bacterial biofilm is a single-species biofilm of *Pseudomonas aeruginosa*. A significant etiological agent of dental caries in humans is the Gram-positive bacterium *S. mutans*, which colonizes the oral cavity and forms bacterial biofilms (Skalniak *et al.*, 2014). Furthermore, *S. mutans* has been employed in earlier

investigations on oral biofilm adhesion and has been demonstrated to promote the proliferation and adhesion of harmful bacteria.

CHAPTER 2

LITERATURE SURVEY

2.1 GLASS IONOMER CEMENT

Innovation in dental restorative materials. The desire for biocompatible and natural-looking repair solutions is what drives innovation. In dentistry, glass ionomer cements (GICs) are a desirable substitute for amalgam and resin composites. Glass polyalkenoate cements, or GICs for short, are suitable for dental applications due to several advantageous features. GICs may be inserted into cavities and don't require bonding chemicals, which makes them ideal for use in reconstructive surgery and implant fixation. Their physical qualities can be the desire for biocompatible and natural-looking repair solutions is what drives adjusted to meet the needs of a wide range of applications by changing their composition, such as pediatric cements, luting, core build-up, sealing or lining cavity floors, and high fluoride releasing command sets. The excellent physical and chemical properties of glass ionomer cement are low thermal expansion and thermal compatibility. adherence to dentin, enamel, and mineralized tissue. Adhesion between base metals and glass particles. long-lasting and steady ionomer chemical structure. Quick-setting. altering the ratio of powder to liquid to change the physical qualities. designed to disperse fluoride in an anti-cariogenic manner. The main component of GICs is a composite made of silicate glass particles ranging from 15 to 50 μM and a polymeric ionomer-like polyalkenoic acid. (Davis *et.al.*, 2019).

The ionomer and the glass particles create a network structure during the curing process, and the ionomer's carboxylate groups function as a network modifier to create salt bridges and crosslinks with the metallic ions. Low fracture strength, reduced toughness, and decreased durability result from this. Specifically, class II restorations are high-stress areas where the tensile strength of current GICs is inadequate, especially in cases where the cavity wall is not providing sufficient support. These factors restrict the application of traditional ionomers to non-stress bearing repair. GICs have been formulated in new ways to enhance these characteristics and boost resistance to erosion, wear, and fracture. (Yong Zhang *et. al.*,2019).

2.2 MAGNESIUM OXIDE NANOPARTICLES

Magnesium oxide (MgO) nanoparticles have drawn attention for usage in biological applications (Manivannan *et.al.*,2012). They can be produced from economic precursors and are found to exhibit antibacterial efficacy against viruses, spores, and bacteria of both Gram-positive and Gram-negative varieties (Domb *et. al.*,2015). Recent research has shown that chemically produced magnesium oxide nanoparticles and Zein-based magnesium oxide nanoparticles have antibacterial properties and can form biofilms on a variety of oral pathogens, including *Streptococcus mutans* (Hamed *et.al.*,2018). This study investigates the antibacterial and antibiofilm properties of glass-ionomer cement modified with magnesium oxide nanoparticles against *Streptococcus mutans* and *Streptococcus sobrinus*. The high pH (alkaline pH) of MgO nanoparticles may also contribute to their antibacterial action (Sawai *et.al.*,2001). The alkaline characteristic of MgO nanoparticle suspensions may be of interest for combating the acidogenic microorganisms responsible for the development of dental caries and may also favor the surrounding environment for enamel re-mineralization (Braz *et.al.*, 2014). MgO nanoparticles also inhibit the essential enzymes of bacteria (Hunter *et. al.*,2013). GIC is extremely brittle, it cannot be used as a dental material due to its limited physical strength. As a result, numerous attempts have been made to modify GIC to improve its mechanical and physical characteristics.

2.3 HYDROXYAPATITE

Hydroxyapatite can be added to the GIC mix as one method. As an inorganic compound that resembles natural bone mineral, hydroxyapatite (HAP) is well-known for belonging to the apatite group, which is a group of mineral phosphates. HAP is chemically characterized as $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$, and it differs from other apatite compounds in that it has a hydroxyl end-member of the apatite group (Balu *et al.*,2012). With a hexagon crystal structure and a calcium-to-phosphate ratio of 1.67 ($\text{Ca/P} = 1.67$). The common biomineral hydroxyapatite (HAP, $\text{Ca}_{10} [\text{PO}_4]_6 [\text{OH}]_2$) is widely distributed in living things. It can be utilized as luminescent materials and as scaffolds for bones. (Chai *et.al.*,2010) To compare the shear bond strength and microhardness of conventional Glass Ionomer Cement (GIC) incorporated with nano-hydroxyapatite (nano-HAP), zircon-omer, and Composite, the current study was carried out with the following objectives: to evaluate the shear

bond strength and microhardness of GIC with nano-hydroxyapatite (Nano-HAP), zircon-omer, and Composite.

2.4 CONTACT ANGLE MEASUREMENT

The contact angle is the angle created tangential to the liquid surface at the point where the three phases converge and the plane of the solid material's surface where the liquid substance is present or flows. Sessile drop instruments are typically used to measure the contact angle.

By using the Young-Laplace equation to curve fit the entire drop profile (Kim *et.al.*, 2013). Greater hydrophilicity or enhanced wettability are indicated by a lower contact angle, whilst greater hydrophobicity or decreased wettability are indicated by a higher contact angle. When a biomaterial surface interacts with a liquid phase, as happens with restorative materials frequently employed as fillings, it is important to comprehend not only the surface properties under standard experimental settings but also the consequences of exposure to the fluid medium. Therefore, to identify a surface characteristic under conditions as similar to real-world scenarios as possible, tested materials should be subjected to a range of medium of interest. Determining whether or not dental materials should be used is based in part on how they behave in the oral environment.

2.5 CRYSTAL VIOLET ASSAY

Microbial colonies known as biofilms are affixed to a surface and encased in an extracellular polymeric material that offers the different bacterial species that are indwelling stability, protection, and nourishment. These communities can accumulate in a range of settings, including medical and industrial equipment, leading to harm, decreased productivity, and illness. Microbiological communities that are surface-attached and consist of embedded microbial cells in extracellular polymeric substances are known as biofilms.

Biofilms result in serious Biofilms, on the other hand, are employed in a few beneficial applications, such as the bioremediation of hazardous substances and the degradation of xenobiotic chemicals, which present challenges in both clinical and industrial settings. In both situations, the ability of microorganisms to produce biofilms must be assessed to employ them in bioremediation or to develop defense mechanisms against them (Neelis *et. al.*, 2008). Many model systems have been

tested for the in vitro study of biofilms throughout the last few decades. Often, biofilms are measured using the labor-intensive conventional culture plating approach to get colony-forming units/count in assays.

2.6 HEMOCOMPATIBILITY ASSAY

Precision medicine has been made possible by the invention of drug nanocarriers based on polymeric, lipid, and ceramic biomaterials. This allows for the delivery of active substances that are poorly soluble and the creation of customized doses. Nonetheless, it has been shown that these carriers' nanoscale nature can activate pathways of the When the same biomaterials are designed as larger-sized implants, the host response is different from that of the medications when they are given without a carrier. As a result, a unique regulatory system that can deliver solid scientific insights and safety data through credible testing of these cutting-edge nanodevices must be made available. In light of this, the current work offers a multi-step procedure for evaluating in vitro the hemocompatibility of nanocarriers of physiochemical nature.

The assessment of blood plasma protein adsorption, platelet adhesion and activation, platelet–leukocyte complex formation, and whole blood clotting was conducted to determine the hemocompatibility of these surfaces. The findings show that hydrothermally treated surfaces exhibit reduced levels of platelet activation, fibrinogen adsorption, cell adhesion, and whole blood clotting (Benjamin *et.al.*,2019).

2.7 BIOFILM ADHESION ASSAY

When bacteria colonize native host cells or polymeric implant surfaces, they produce a dense growth on the surface that eventually causes infection. A bacterium must undergo a sophisticated, regulated process that is dependent on multiple circumstances to transition from a motile planktonic to a nonmotile long chain of developing cells. There is a 65–80% chance of an infection linked to biofilms occurring.

The process of biofilm formation on natural tissues, as well as on orthopedic, dental, cardiac, and urological implants and vascular grafts, is rigorously assessed in this review. Designing biomaterials that inhibit bacterial adhesion and biofilm would be made easier by combining biochemical advances with established

microbiological techniques, using radio-labeled monoclonal antibodies in imaging techniques, and detecting these biofilms in vivo.

CHAPTER 3

AIM AND OBJECTIVES

3.1 AIM

- To synthesize magnesium oxide nanoparticles by sol-gel method and to determine the optimal method of synthesis of magnesium oxide nanoparticles by characterization studies using SEM, FTIR, XRD
- To synthesize hydroxyapatite by using (LMWH) lower molecular weight heparin and to know their elemental composition using SEM, FTIR, XRD
- Fabrication of nano-HAP incorporated GIC.
- To improve the antimicrobial activity of the GIC by incorporating MgO nanoparticles using maxing bactericidal concentration and inhibition of biofilm formation -CV assay

3.2 OBJECTIVES

- Synthesis of Hydroxyapatite and Magnesium oxide nanoparticle.
- Incorporation of hydroxyapatite and magnesium oxide.
- Characterization of implant materials.
- Biofilm formation study against pathogenic microbes.

CHAPTER 4

MATERIALS AND METHODS

4.1 MATERIALS

Calcium nitrate, Dipotassium hydrogen phosphate, Citric acid, Magnesium nitrate, were procured from HIMEDIA and used for the synthesis of MgO, and HAP Nanoparticles. Nutrient broth was used for culturing the bacteria and also used for biofilm adhesion assay. Goniometers were used to measure a contact angle. A microplate reader(synergy) was used to take a bacterial culture reading. A magnetic stirrer was used for the Homogenous separation of Nanoparticles.



Fig 4.1: Magnetic stirrer

4.2 METHODOLOGY

4.2.1 Preparation of magnesium oxide nanoparticle by sol-gel Method

0.04 moles of magnesium nitrate and an equimolar concentration of citric acid were dissolved separately in 40ml distilled water. The above two solutions were mixed vigorously to form homogeneous solutions. They were heated to 80°C under stirring conditions until they turned into a slimy form using a magnetic stirrer. The solution is then oven dried at 150°C for 2 hours. The crushed white mass is then calcined at 600°C for 2 hours in muffle furnace. The white powder obtained is the magnesium oxide nanoparticles

4.3 CHARACTERISATION OF SYNTHESIZED MAGNESIUM OXIDE NANOPARTICLES BY SOL-GEL METHOD

4.3.1 Fourier transform infrared spectroscopy

FTIR is an analytical method that can be used to distinguish between organic, polymeric, and occasionally inorganic materials. Infrared light is used in the FTIR analysis procedure to scan test materials and observe chemical characteristics. The most commonly used in the identification of unknown materials and confirmation of production materials.

4.3.2 XRD-X-ray powder diffraction method

A non-destructive technique for analyzing the structure of crystalline materials is X-ray diffraction. It may be used to determine which crystalline phases are present in a material and thus its chemical composition. The sample's crystal structure was determined using an X-ray powder diffractometer (XRD, Philips/X'Pert PRO, Netherlands)

4.4 PREPARATION OF HYDROXYAPATITE BY HYDROTHERMAL METHOD USING LOW MOLECULAR WEIGHT HEPARIN

Calcium nitrate was weighed at 1.67 grams and dipotassium hydrogen phosphate was weighed at 1 gram both were dissolved in 100ml de-ionized water and were stirred for 6 hours. Then the solution is oven-dried at 80°C until it turns into a dried gel. Then the dried sample is calcined at 400°C for 6 hours. The white powder obtained is hydroxyapatite. (Rajeshwari *et.al.*, 2013).

4.5 CHARACTERISATION OF SYNTHESIZED HAP

4.5.1 Fourier transform infrared spectroscopy

The functional groups that are present in the sample are identified using this technique. The FTIR spectrum of the MgO, HAP, and GIC was captured using the ATR technique using the ATR-FTIR carry 630 (Agilent) Air as background for capturing the spectrum.

4.6 INCORPORATION OF GLASS IONOMER CEMENT WITH NANOPARTICLE-HAP

For the control film, just two scoops of glass ionomer cement and one drop of mixing liquid were added, and the mixture was allowed to dry. No nano-HAP mixture was applied.

The cement needs three minutes to set. Various concentrations of the nano-HAP mixture, ranging from 5% and 10%, were added to the glass ionomer cement in the test films. The concentration of the mixing liquid was adjusted by the concentration of the nano-HAP mixture, and the film was then dried.

4.7 INCORPORATION OF GLASS IONOMER CEMENT WITH NANOPARTICLE MgO

Glass ionomer cement was prepared according to the manufacturer's instructions. Briefly, for the control, just two scoops of glass ionomer cement and one drop of mixing liquid were added, and the mixture was allowed to dry. No nano-MgO mixture was applied.

The cement needs three minutes to set. Various concentrations of the nano-MgO mixture, ranging from 5% to 10%, were added to the glass ionomer cement in the test films. The concentration of the mixing liquid was adjusted to match the concentration of the nano-MgO mixture, and the film was then dried.

4.8 CHARACTERISATION OF INCORPORATED GLASS IONOMER CEMENT WITH NPs HAP AND MgO

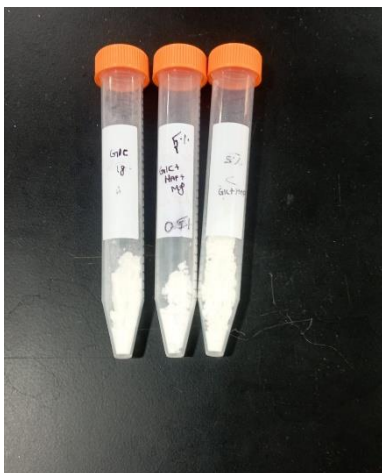


Fig4.8: Characterisation of incorporated GIC with HAP, MgO in different concentration

4.8.1 Fourier transform infrared spectroscopy

The stretching vibration mode, which is $\sim 600\text{--}850\text{ cm}^{-1}$, indicates the presence of Mg–O–Mg bonds. The unique band, which represents the surface hydroxyl group's bending vibration, is seen at wave number around 1434 cm^{-1} . It has been reported that MgO shows a broad absorption peak at $260\text{--}330\text{ nm}$. Two absorption humps can be seen in the spectrum: one at 380 nm in the range of $320\text{--}400\text{ nm}$ and another between $500\text{--}600\text{ nm}$, with a peak at 550 nm .

4.8.2 X-ray powder diffraction method

A non-destructive technique for analyzing the structure of crystalline materials is X-ray diffraction. It may be used to determine which crystalline phases are present in a material and thus its chemical composition. The sample's crystal structure was determined using an x-ray powder diffractometer (XRD, Philips/X' Pert PRO, Netherlands)

4.9 CONTACT ANGLE MEASUREMENT

The contact angle is the angle created tangential to the liquid surface at the point where the three phases converge and the plane of the solid material's surface where the liquid substance is present or flows. Sessile drop instruments often use the Young-Laplace equation to fit a curve that encompasses the entire drop profile to determine the contact angle. Greater hydrophilicity or enhanced wettability is indicated by a lower contact angle, whereas greater hydrophobicity or restricted wettability is indicated by a higher contact angle.

Ceramic disks were positioned on the Contact Angle Goniometer's tilt stage. With a 10 μl droplet volume, a deionized water droplet was dispensed using a micropipette. The contact angle measurement was recorded.

Droplet disperses over a surface and its contact angle is less than 80° , surface wetting takes place. This angle will be zero degrees and 'full soaking' will have taken place when the droplet spreads out completely. Given the direct relationship between wetness and contact angle, a contact angle goniometer is the most appropriate tool for measuring surface wetting. When there is minimal surface energy, there is little wetting, or little spreading of the liquid droplet onto the surface, as shown by a high contact angle. The water droplet spreads out further on the surface when the contact angle is low, indicating that the high surface energy is high wetting.



Fig 4.9: Goniometer

4.10 CRYSTAL VIOLET ASSAY (BACTERIAL CYTOTOXICITY)

A 24-hour culture should be ready for the crystal violet test. UV light was used to disinfect ceramic disks. Put a disk in a 24-well plate and let it sit for up to 30 minutes with a 1.5 ml culture. Remove the medium from the well plate and wash it twice with a PBS pH of 7.4 after the incubation period. For 20 minutes, disks were fixed in 70% ethanol. use a pipette to dispose of the ethanol. Fill each well with 200 microliters of crystal violet, then let it sit for 20 minutes. We need to add 500 micro litter each time we wash the PBS three times. then fill each well with 500 microliters of acetic acid solution. Shake well and incubate for ten minutes. take out each wells200 micro litter eluted solution, then transfer it into a 96-well plate. OD at 570 nm to identify a biofilm mass.

4.11 HEMOCOMPATIBILITY ASSAY

To rule out any potential negative consequences that the scaffold could create, a hemocompatibility test was performed. One of the most crucial requirements for the effective usage of tested material in vivo has been suggested to be this method of evaluating the material. The evaluation of biomaterials and equipment that come into and remain near blood. The procedure started with the collection of blood in vials containing ethylene diamine tetra acetic acid (EDTA), an anticoagulant, from a healthy volunteer. To get rid of the plasma and other leftovers, the red blood cells were centrifuged for ten minutes at 4° C. They were then rinsed three times with PBS. All samples were incubated at 37° C for one hour, after which the sample was centrifuged and the lysis rate was measured at a wavelength of 540 nm. The procedure was analyzed with triplicates of all samples, and the results were compared with a negative control using a hemocompatibility assay.

4.12 BIOFILM ADHESION ASSAY

The UV-sterilized implant disks were incubated with *Pseudomonas aeruginosa* for 24 hours. The media was removed and the samples were visualized under a Scanning electron microscope.

CHAPTER-5

RESULTS AND DISCUSSION

5.1 CHARACTERIZATION OF MgO NANOPARTICLES

5.1.1 FTIR-Fourier transform infra-red spectroscopy

The FTIR analysis was used to determine the possible functional groups in the chemically synthesized MgO NPs. FTIR spectra show major peaks at 503.63, 863.45, 1453.52, and 3381.96 cm^{-1} for the sol-gel method. The band obtained at 503.63 cm^{-1} are the bond angles stretching of Mg-O linkage, and the band obtained at 863.45 cm^{-1} indicates the presence of $\text{NO}_3/\text{CO}_3^{2-}$ groups. The IR band obtained at 1453.52 cm^{-1} is indicative of the presence of H-O-H stretching and bending vibrations. The bands at 3381.96 cm^{-1} correspond to the O-H stretching (fig 5.3). The following are the band patterns obtained for the co-precipitation method. The bands at 3415.50 cm^{-1} and 3194 cm^{-1} corresponds to the O-H and H-O-H stretching respectively. The IR band obtained at 1385.55 cm^{-1} is indicative of the presence of C=O stretching vibrations. The band at 987.47 cm^{-1} indicates Mg-O-H stretching and the band at 867.23 indicates the presence of $\text{NO}_3/\text{CO}_3^{2-}$ groups. The band at 508.72 cm^{-1} is characteristic of Mg-O stretching which is in reference to Cai *et.al.*, (2016).

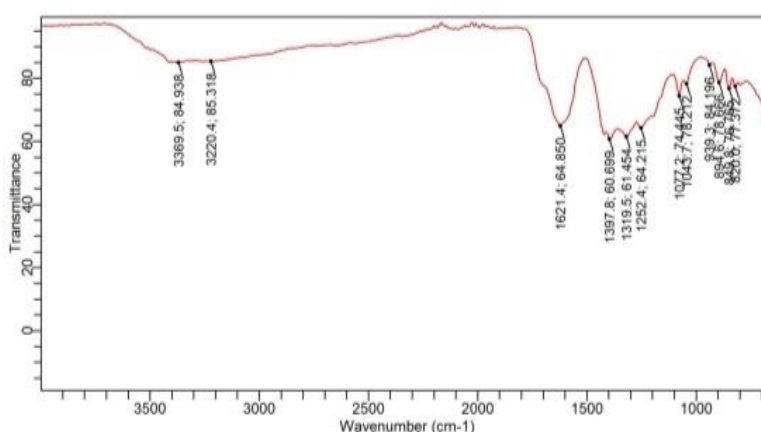


Fig5.1.1: Fourier transform infrared spectroscopy of MgO nanoparticles by Sol-gel method

Table 5.1.1: FTIR Results for MgO nanoparticles by sol-gel method

S.NO	WAVELENGTH cm^{-1}	INTENSITY
1	820.01432	77.37164
2	849.83303	76.76541
3	894.56108	78.66554
4	939.28913	84.19578
5	1043.65459	78.21217
6	1077.20063	74.44514
7	1252.38551	64.216531
8	1319.47759	61.45421
9	1397.75169	60.69871
10	1621.39196	64.85000
11	3220.41989	85.31792
12	3369.51340	84.93799

A medium peak obtained that 1454 cm^{-1} could be attributed to the presence of O-H group, A strong peak obtained that 3268 cm^{-1} could be attributed to the presence of O-H group, A broad peak obtained that $3300\text{-}3600\text{cm}^{-1}$ could be attributed to the presence of MgO A medium peak obtained that 800cm^{-1} could be attributed to the presence of Mg-O group, A medium peak obtained that $400\text{-}4000\text{cm}^{-1}$ could be attributed to the presence of Mg-O group, A medium peak obtained that 3420cm^{-1} could be attributed to the presence of N-H group, A strong peak obtained that $1099\text{-}1484\text{cm}^{-1}$ could be attributed to the presence of C-O group.

5.2 X- RAY POWDER DIFFRACTION METHOD

X-ray diffraction is a non -destructive method used to analyze the structure of crystalline material, used to identify the crystalline phases present in a material

thereby revealing chemical composition. crystal structure of the sample was characterized by an x-ray powder diffractometer.

The GIC is amorphous with no significant peaks.

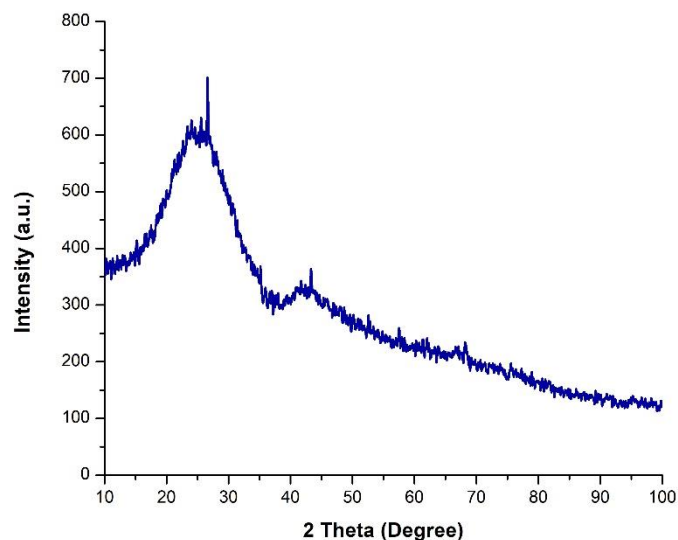


Fig 5.2.1: X-ray powder diffraction of GIC

The incorporation of HAP brings about significant changes in the amorphous GIC leading to sharp crystalline peaks at 38° and 50° indicating the incorporation of HAP into the lattice structure of GIC.

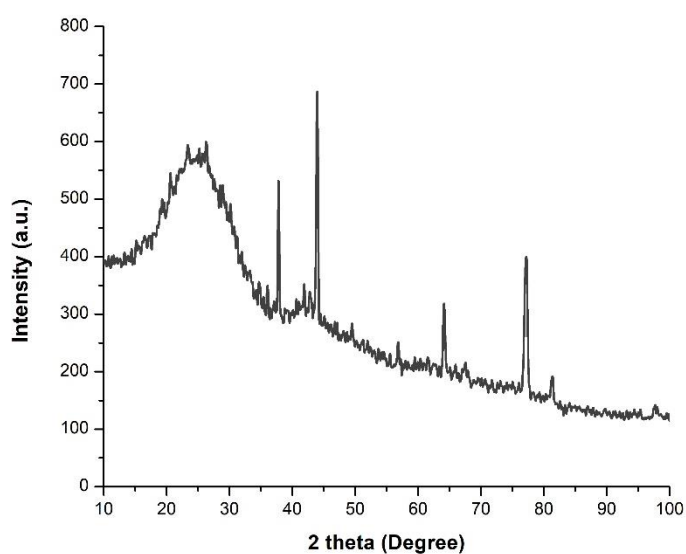


Fig 5.2.2: X-ray powder diffraction of GIC+ HAP 5 %

The MgO incorporated GIC+ HAP showed two sharp peaks at 42° and 62° at two different theta degree planes indicating the incorporation of MgO.

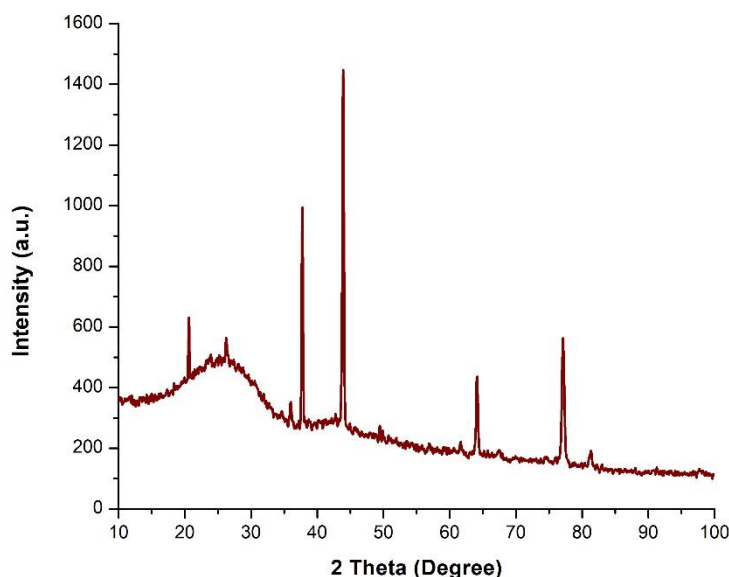


Fig 5.2.3: X-ray powder diffraction of GIC+ HAP+ MgO 5 %

5.3 CHARACTERIZATION OF HAP NANOPARTICLES

5.3.1 FTIR-Fourier transform infra-red spectroscopy

The FTIR analysis is done to identify the functional groups in synthesized HAP. The following are the bands obtained at 2994cm^{-1} is C-H stretching. The IR band obtained at 1383cm^{-1} is C-N stretching. The band obtained at 1150cm^{-1} is C-O stretching. The band obtained at 961.56cm^{-1} is C=C bending. The band obtained at 560.1 is C-C stretching (Halo compound) concerning Rajeshwari *et. al.*, (2013).

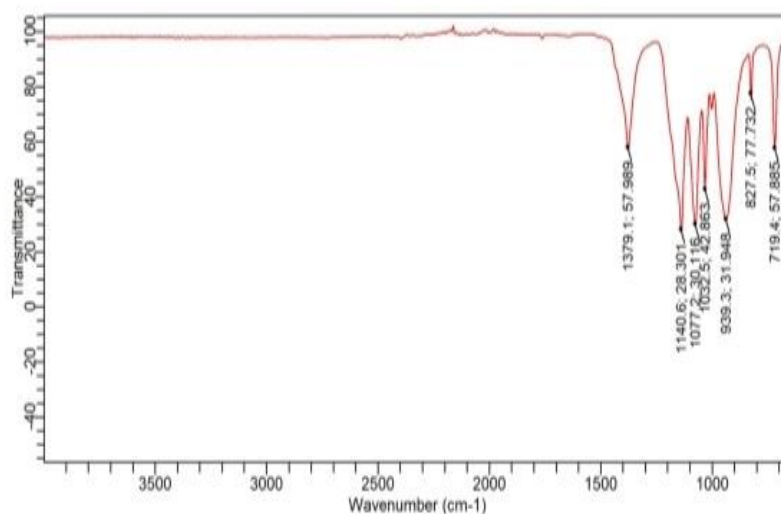


Fig5.3.1: Fourier transform infrared spectroscopy of HAP nanoparticles by LMWH method

Table5.3.1: FTIR Results for HAP nanoparticles by LMWH method

PEAK NO	WAVELENGTH cm^{-1}	INTENSITY
1	719.37620	57.88516
2	827.46900	77.73241
3	939.28913	31.94779
4	1032.47258	42.86263
5	1077.20063	30.11646
6	1140.56538	28.30129
7	1379.11500	57.98940

A medium peak obtained that 1457cm^{-1} could be attributed to the presence of CO_3^{2-} group, A medium peak obtained that 1631cm^{-1} could be attributed to the presence of NH group, A strong peak obtained that 1072cm^{-1} could be attributed to the presence of P-O group, A medium peak obtained that 1026cm^{-1} could be attributed to the presence of O-P-O group.

5.4 CHARACTERIZATION OF GLASS IONOMER CEMENT

FTIR spectra for the integration of zinc-silicate GIC were successfully obtained over a 20-minute period. Because the setting reaction was slower at room temperature, the selected collecting time was significantly longer than the actual setting time. Both the ionized PAA band at c. 1550 cm^{-1} and the typical band for free PAA (not coordinated to a metal ion) at c. 1700 cm^{-1} were found. (Valliant *et.al.*,2016).

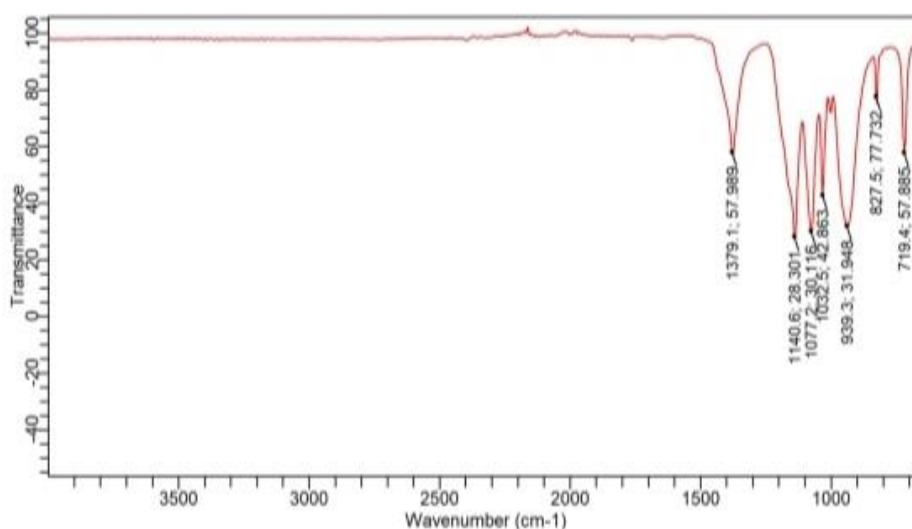


Fig5.4.1: Fourier transform infra-red spectroscopy of GIC

Table5.4.1: FTIR Results for GIC

PEAK NO	WAVELENGTH cm^{-1}	INTENSITY
1	685.83016	72.68053
2	726.83088	75.01026
3	995.19920	65.21033
4	3712.42849	98.06023

A strong peak obtained that $730\text{-}665\text{cm}^{-1}$ could be attributed to the presence of C=C group, A strong peak obtained that $690\text{-}515\text{cm}^{-1}$ could be attributed to the presence of C-Br group, A strong peak obtained that $600\text{-}500\text{cm}^{-1}$ could be

attributed to the presence of C-H group, A strong peak obtained that 995-985 cm^{-1} could be attributed to the presence of C=C group, A strong peak obtained that 980-960 cm^{-1} could be attributed to the presence of C=C group, A medium/sharp peak obtained that 3700-3584 cm^{-1} could be attributed to the presence of O-H group, A strong/ broad peak obtained that 3550-3200 cm^{-1} could be attributed to the presence of O-H group

5.5 CHARACTERIZATION OF HAP INCORPORATED WITH GIC 5%

5.5.1 FTIR-Fourier transform infra-red spectroscopy

FTIR analysis of conventional glass ionomer cement and hybrid MgO-nano HAP incorporated glass ionomer cement were obtained. The following are the bands obtained at different peaks for the hybrid GIC, at 3770 cm^{-1} O-H stretching, 2994 cm^{-1} C-H stretching, 1383 cm^{-1} C-N stretching, and 1150 cm^{-1} C-O stretching, 961.5 cm^{-1} C=C stretching, 723.2 cm^{-1} $\text{NO}_3/\text{CO}_3^{2-}$ groups are present. At 480.8 cm^{-1} Mg-O stretching is present.

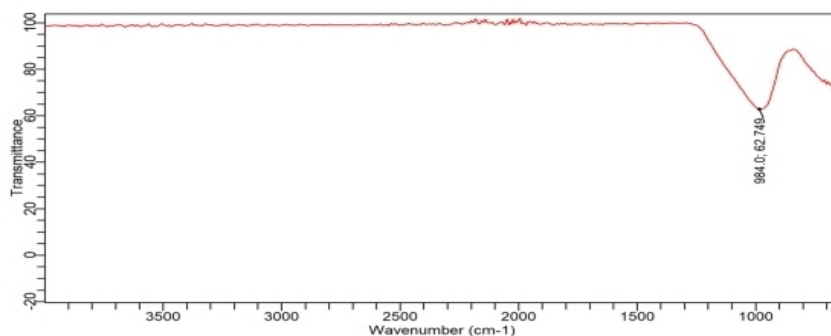


Fig 5.5.1: FTIR results of GIC+HAP 5%

Table 5.5.1: FTIR results for HAP+GIC nanoparticles by LMWH method

Peak no	Wavelength cm^{-1}	Intensity
1	984.01719	62.74941

A strong peak obtained that $995\text{-}985\text{cm}^{-1}$ could be attributed to the presence of the C=C group, A strong peak obtained that $980\text{-}960\text{cm}^{-1}$ could be attributed to the presence of the C=C group.

5.6 CONTACT ANGLE MEASUREMENT

The phase boundary is stationary, contact angles are measured. The wettability value that Theta, or contact angle, quantifies the amount of liquid that has wetted a solid. The angle that a liquid forms at the three-phase boundary where a liquid, gas, and solid intersect is the geometric definition of the contact angle. This three-phase contact point between a solid, liquid, and gas, or occasionally a solid, liquid, and liquid, is subject to three distinct forces. Three categories of contact angles exist: static, dynamic, and roughness-corrected. When the droplet is stationary and the three are measured the most frequently are static contact angles. It works best on surfaces that are homogeneous and reasonably smooth. The surface free energy of the substrate, often known as the surface tension of a solid, is also determined by static contact angles. Wettability can be measured quickly, simply, and quantitatively using the static contact angle. When there is minimal surface energy, there is little wetting, or little spreading of the liquid droplet onto the surface, as shown by a high contact angle. The water droplet spreads out further on the surface when the contact angle is low, indicating that the high surface energy is high wetting.

GIC+ NM 10% has the highest contact angle and lowest surface energy indicating low possibility of biofilm formation.

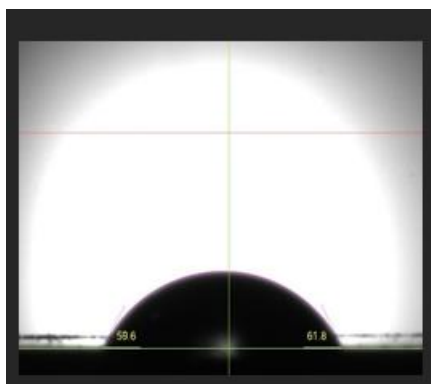


Fig5.6.1: GIC



Fig5.6.2: GIC+HAP 5%

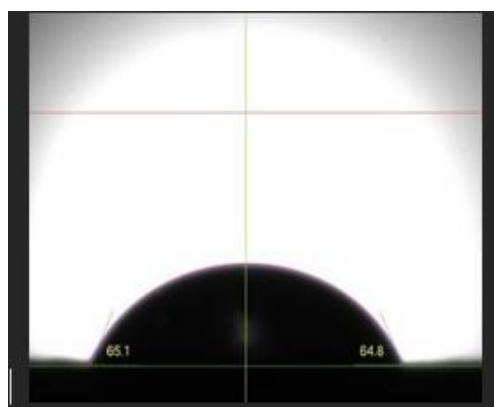


Fig5.6.3: GIC+ HAP 10%

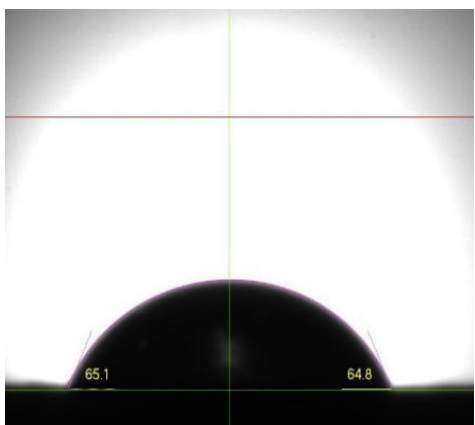


Fig5.6.4: GIC+NM 5%

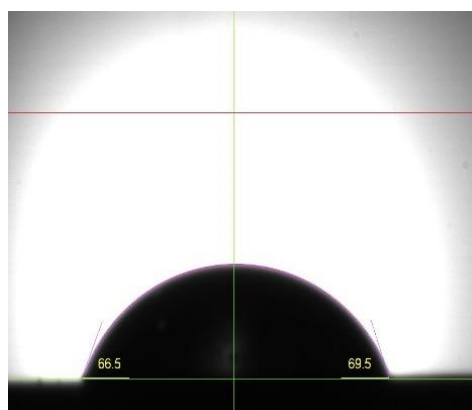


Fig5.6.5: GIC+NM 10 %

Table 5.6.1: Results for contact angle measurement using Goniometer

GIC		GIC+ HAP 5%		GIC+ HAP 10%		GIC+ HAP+ MgO5%		GIC+ HAP+ MgO10%	
Average	SD	Average	SD	Average	SD	Average	SD	Average	SD
53.42	0.02	51.17	0.01	64.35	0.09	66.67	0.02	69.07	0.02
Surface energy									

51.76	0.01	53.08	0.01	45.19	0.06	43.77	0.01	42.29	0.01
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GRAPHICAL VISUALISATION

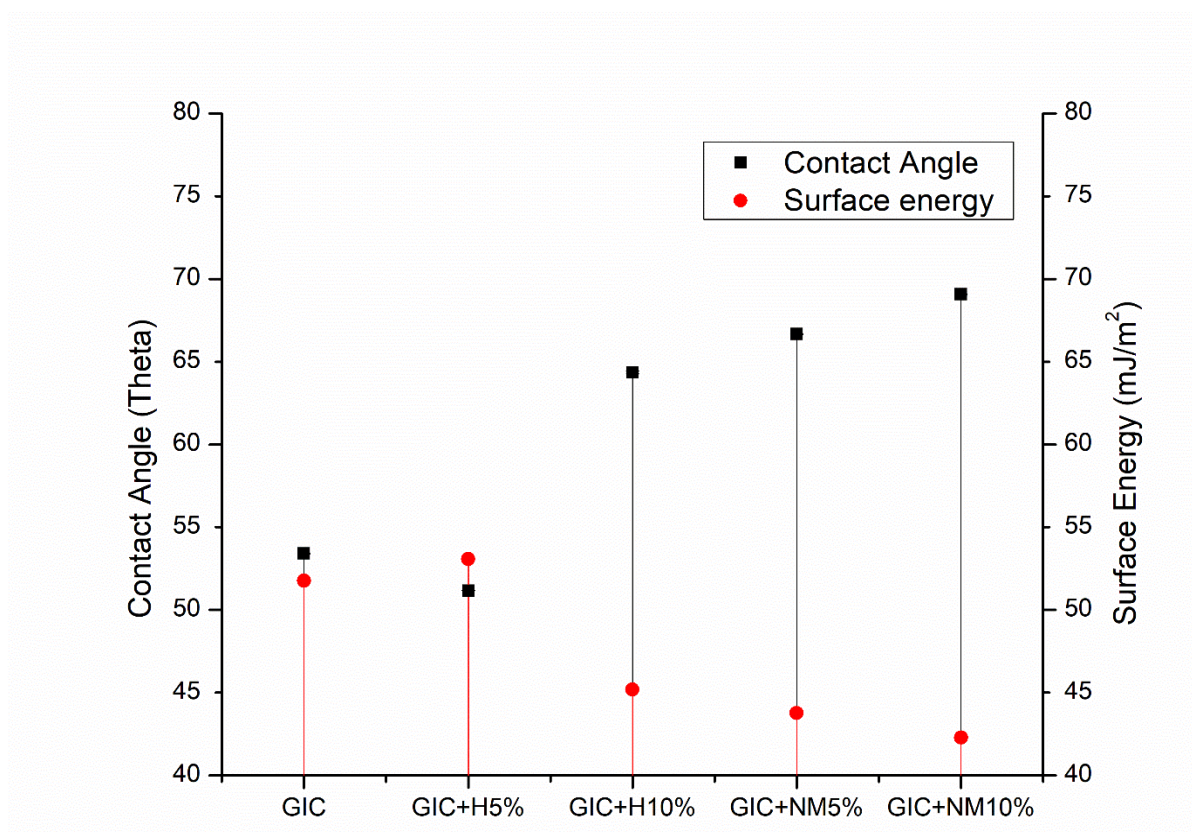


Fig5.6.6: Graphical visualization of contact angle measurement

5.7 HEMOCOMPATIBILITY ASSAY

The procedure started with the collection of blood in vials containing the anticoagulant substance ethylene diamine tetra acetic acid (EDTA) from a healthy volunteer. The red blood cells underwent three rounds of washing after being centrifuged for ten minutes at 4° C. plasma and other residues using phosphate buffer saline (PBS, pH 7.4). The red cell lysis rate in the presence of the scaffold was measured using a hemocompatibility assay, and the results were compared to a negative control. All samples were analyzed in duplicate during the procedure. For

one hour, all samples were incubated at 37° C. After centrifuging this material, the lysis rate was measured at 540 nm. Using the formula

$$[(\text{Control-sample}) / \text{Control}] \times 100$$

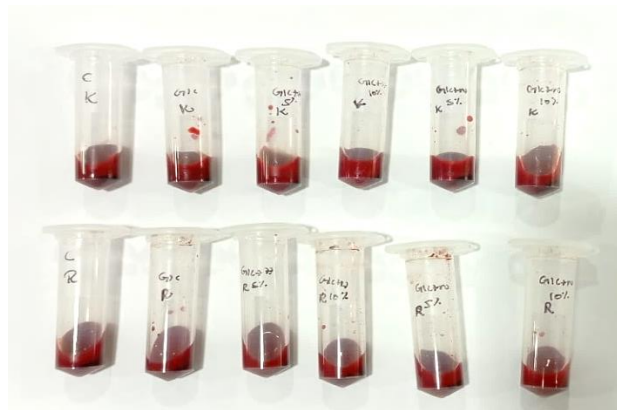


Fig5.7.1: Dental implants coated with blood

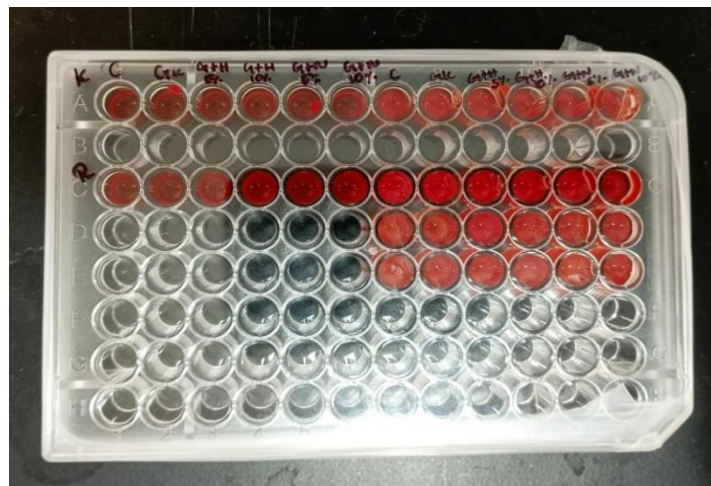


Fig5.7.2: Lysis rate was observed at OD 540nm

Table5.7.1: Lysis percentage

S.NO	GIC	GIC+HAP 5%	GIC+HAP 10%	GIC+HAP +MgO 5%	GIC+HAP +MgO 10%	CONTROL
PERSON1	2.669	3.638	2.998	2.724	2.813	3.188
Lysis percentage	19.44	16.86	7.11	17.38	14.05	
PERSON2	2.647	2.828	2.805	2.853	2.893	2.692
Lysis percentage	1.7	5.13	4.26	6.08	7.59	

GRAPHICAL VISUALISATION

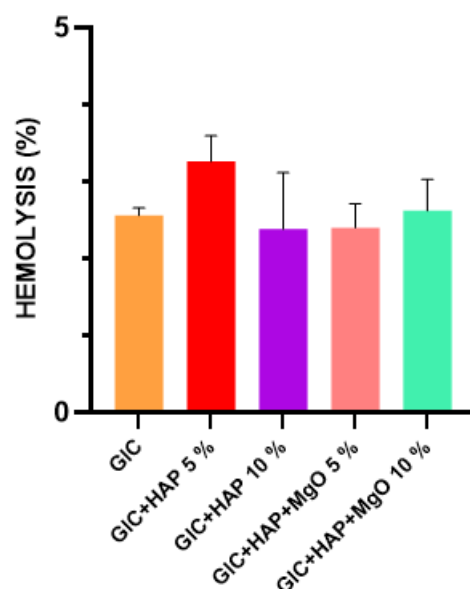


Fig5.7.3: Hemolysis percentage of blood exposed to dental implants (Person 1)

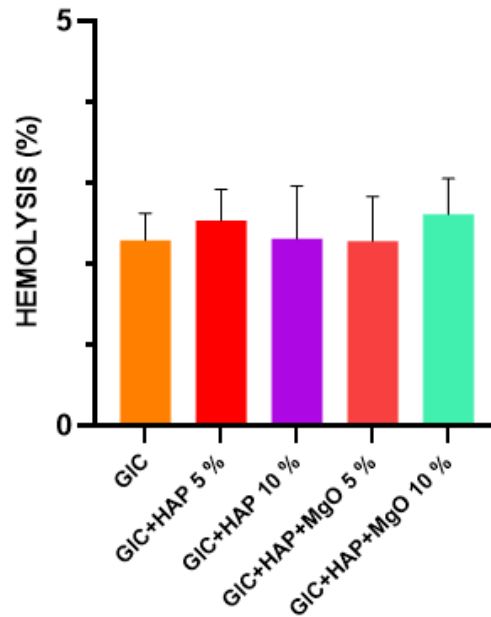


Fig5.7.4: Hemolysis percentage of blood exposed to dental implants (Person 2)

5.8 CRYSTAL VIOLET ASSAY

A 24-hour culture should be ready for the crystal violet test. UV light was used to disinfect ceramic disks. Put a disk in a 24-well plate and let it sit for up to 30 minutes with a 1.5 ml culture. Remove the medium from the well plate and wash it twice with PBS PH of 7.4 after the incubation period. For 20 minutes, disks were fixed in 70% ethanol. use a pipette to dispose of the ethanol. Fill each well with 200 microliters of crystal violet, then let it sit for 20 minutes. We need to add 500 micro litter each time we wash the PBS three times. then fill each well with 500 microliters of acetic acid solution. Shake well and incubate for ten minutes. take out each well's 200 micro litter eluted solution, then transfer it into a 96-well plate. OD at 570 nm to identify a biofilm mass.

Table5.8.1: Crystal violet assay readings OD 570nm

S	C	C	GIC	GIC	GIC+ HAP 5%	GIC+ HAP 5%	GIC+ HAP 10%	GIC+ HAP 10%	GIC+ HAP+ MgO 5%	GIC+ HAP+ MgO 5%	GIC+ HAP+ MgO 10%	GIC+ HAP+ MgO 10%
SA	0.482	0.483	2.498	1.82	2.124	2.468	3.348	1.496	4.00	4.00	3.417	4.00
PA	0.091	0.092	0.089	0.091	0.092	0.09	0.089	0.089	0.091	0.093	0.092	0.093

STAPHYLOCOCCUS AUREUS**Table5.8.2: Biomass of *Staphylococcus aureus* culture**

Sample	Mean	SD
Control	0.480667	0.001528
GIC	2.495667	0.003215
GIC+HAP 5%	2.122000	0.002646
GIC+HAP 10%	2.342000	0.009539
GIC+ HAP+ MgO 5%	3.980	0.026
GIC+ HAP+ MgO 10%	3.413667	0.004933

PSEUDOMONAS AERUGINOSA

Table5.8.3: Biomass of *Pseudomonas aeruginosa* culture

Sample	Mean	SD
Control	0.090667	0.001528
GIC	0.087000	0.002000
GIC+HAP 5%	0.091667	0.006506
GIC+HAP 10%	0.084333	0.008963
GIC+ HAP+ MgO 5%	0.088	0.004
GIC+ HAP+ MgO 10%	0.081000	0.081000



Fig5.8.1: Dental implants sample

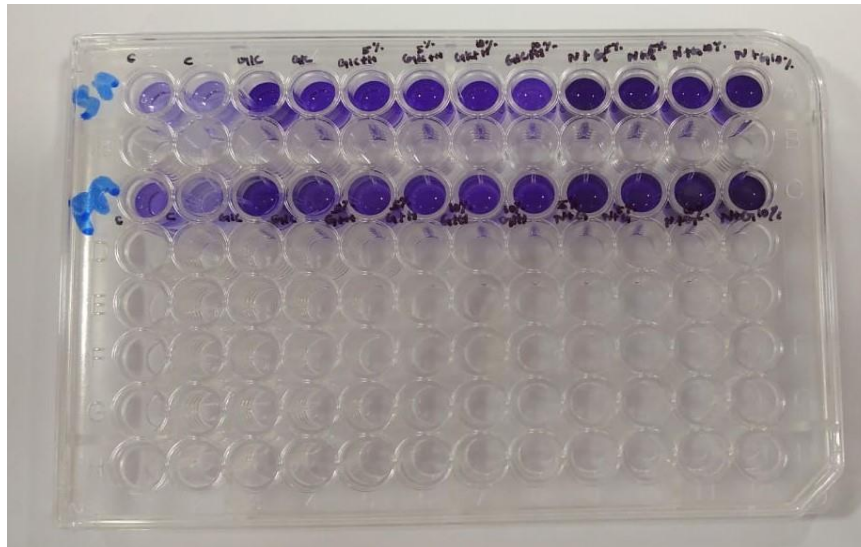


Fig5.8.2: Crystal violet assay reading using microplate reader

GRAPHICAL VISUALISATION

PSUEUDOMONAS AERUGINOSA

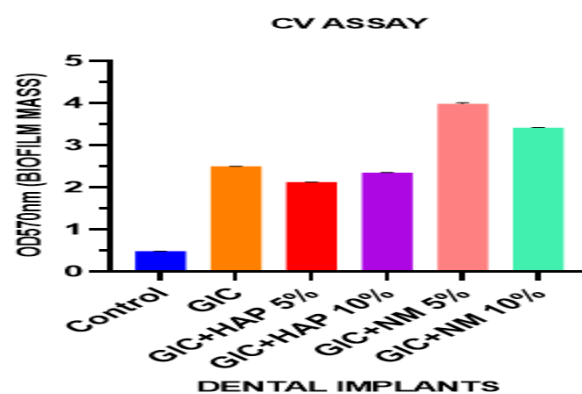


Fig5.8.3: Biofilm formation (*Pseudomonas aeruginosa*)

STAPHYLOCOCCUS AUREUS

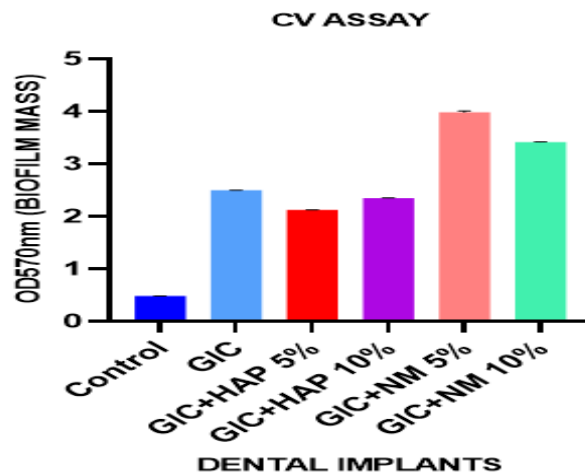


Fig5.8.4: Biofilm formation (*Staphylococcus Aureus*)

5.9 BIOFILM ADHESION ASSAY

The UV-sterilized implant disks were incubated with *Pseudomonas aeruginosa* for 3 hours. The media was removed and the samples were visualized under a scanning electron microscope.

The GIC sample showed bacterial adhesion which can be seen as attached rods on the surface.

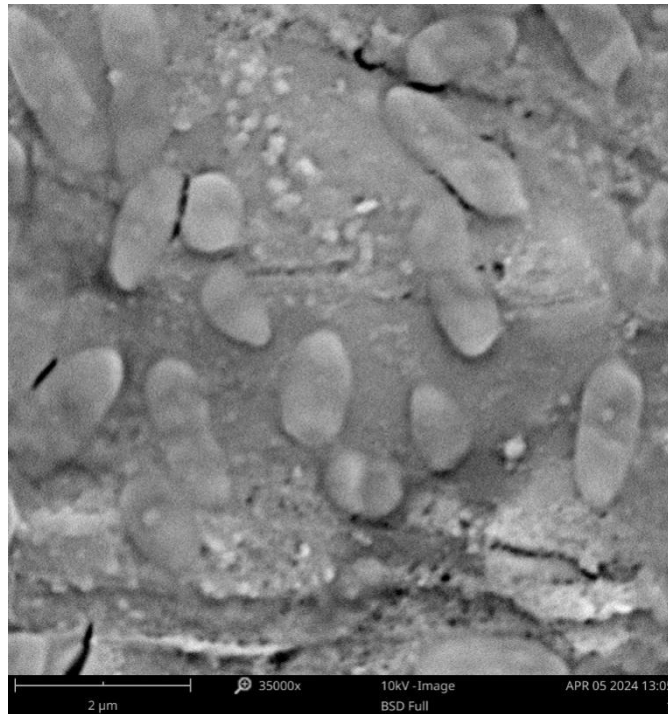


Fig 5.9.1: GIC

The GIC+ HAP 5% sample showed lesser bacterial attachment. the bacterial cells were disrupted and this disruption could be due to the addition of HAP.

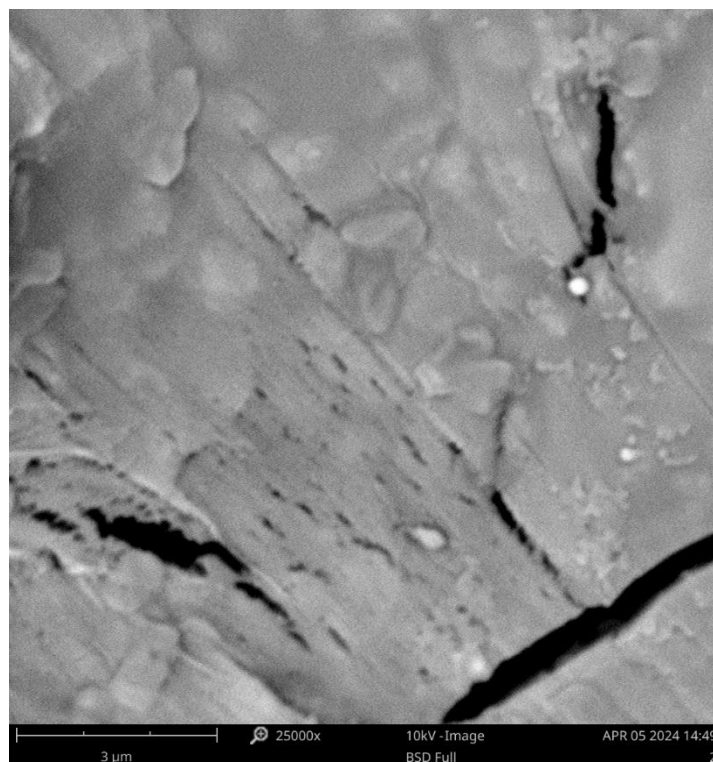


Fig 5.9.2: GIC+ HAP 5%

The incorporation of MgO in the dental implant GIC+ HAP+ MgO 5% not only retained the porosity of the implant but also prevented bacterial adhesion and biofilm formation.

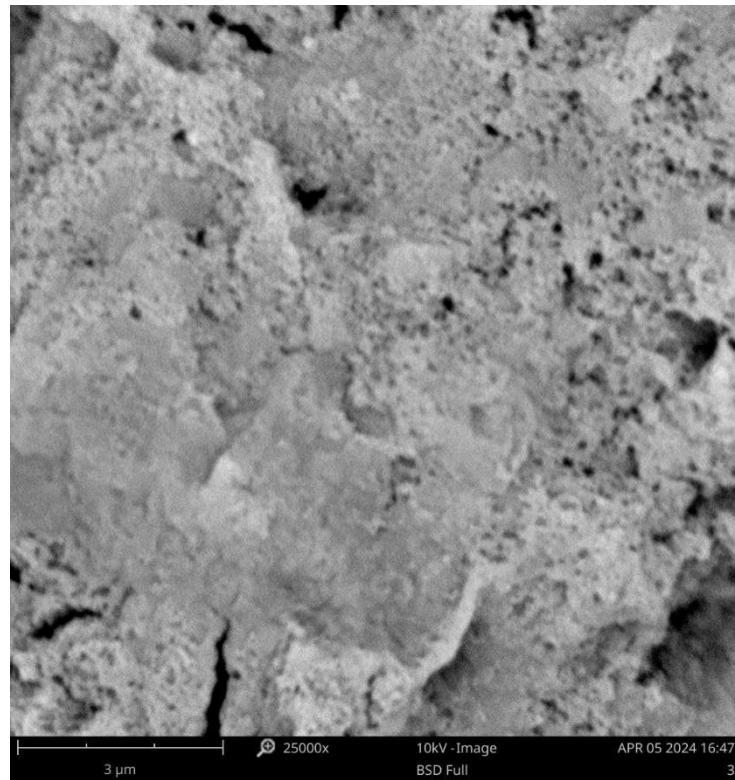


Fig5.9.3: GIC+ HAP+ MgO 5%

CHAPTER-6

SUMMARY AND CONCLUSION

Magnesium oxide nanoparticles were chemically synthesized using the sol-gel method. The synthesized magnesium oxide nanoparticles are spherical in shape and non-uniform, according to semi analysis. The existence of functional groups indicating O-H group ions at wave numbers 3381.96 and 3451.15, respectively, was further verified by FTIR analysis. The components present at specific nanometers were further revealed by energy-dispersive X-ray analysis. The highest concentration and intensity of oxygen are found here. Low molecular weight heparin was used in the chemical hydrothermal synthesis process to create hydroxyapatite. The existence of C-H group ions was indicated by FTIR analysis at 2994 wavenumber, respectively. The diameter of the produced HAP particles is 3547.3 nm on average. Zeta potential assesses the content. The glass ionomer cement with integrated MgO-nano-HAP was created. The presence of O-H group ions is verified by FTIR analysis at 3770cm^{-1} wavenumber, respectively. Two methods were used to investigate the hybrid GIC's antibacterial activity against *P. aeruginosa*, and *S. Aureus*: maximal bactericidal concentration and the biofilm formation/crystal violet assay the surfaces of materials must have an impact on biofilm formation. We have attempted to demonstrate how materials impact the creation and growth of biofilms from this perspective. Bacteria, EPS (extracellular polymeric compounds), and water make up around 80% of BF. It's got that distinct sliminess. Quorum sensing, a phenomenon where bacteria stick to surfaces, grow, and release polysaccharides outside of their lipids and nucleic acids are produced in biofilms in which the hybrid GIC's antibacterial activity is demonstrated by readings obtained using a microplate reader. The contact angle is the angle created tangential to the liquid surface at the point where the three phases converge and the plane of the solid material's surface where the liquid substance is present or flows. Sessile drop instruments often use the Young-Laplace equation to fit a curve that encompasses the entire drop profile to determine the contact angle. Greater hydrophilicity or enhanced wettability is indicated by a lower contact angle, whereas greater hydrophobicity or restricted wettability is indicated by a higher contact angle. The artificial biomaterials and blood cells exhibited good agreement in the biocompatibility test. As the biomaterial's concentration increases, the fraction of red

blood cells that lyse in surviving erythrocytes drops. It has been noted that this substance is both biocompatible and biodegradable. It is necessary to do a biofilm adhesion assessment that contrasts the adherence of host cells and bacteria to implant surfaces. In this work, the adhesion difference between osteoblast-like cells and bacterial biofilm on surfaces simulating implants is measured using the laser spallation technique

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