

PLANT BASED COATING FOR BONE IMPLANTS TO IMPROVE THE ANTI- INFLAMMATORY PROPERTIES

Submitted in partial fulfilment of the requirements for the award of
Bachelor of Technology degree in Biomedical Engineering

By

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SATHYABAMA

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

Accredited with Grade "A" by NAAC

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MAY - 2022



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BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of **ABHISHEK S (38240001), AJAY R (38240003)** who carried out the project entitle "**Plant based coating for Bone Implants to Improve the Anti-Inflammatory Properties**" under our supervision from **September 2021 to March 2022**.

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DECLARATION

We **ABHISHEK.S (38240001) AND AJAY R (38240003)** hereby declare that the Project title entitled “**Plant based coating for Bone Implants to Improve the Anti-Inflammatory Properties**” done by us under the guidance of **MS. SINDU DIVAKARAN., M.E., (PH.D.,) DEPARTMENT OF BIOMEDICAL ENGINEERING** submitted in partial fulfilment of the requirements for the award of Bachelor of Technology Degree in Biomedical engineering.



DATE: 02/05/2022

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ACKNOWLEDGEMENT

We are pleased to acknowledge our sincere thanks to **Board of Management of SATHYABAMA** for their kind encouragement in doing this project and for completing it successfully. We are grateful to them.

We convey our thanks to **Dr. T SUDHAKAR., M.Sc., Ph.D.,** Head of the Department, Dept. of Biomedical Engineering for providing us necessary support and details at the right time during the progressive reviews.

We would like to express our sincere and deep sense of gratitude to our Project Guide **Ms. SINDU DIVAKARAN., M.E., (PH.D.,)** for her valuable guidance, suggestions and constant encouragement paved way for the successful completion of our project work.

We would like to express our thanks to all **Teaching and Non-teaching staff** members of the Department of Biomedical Engineering who were helpful in many ways for the completion of the project.

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ABSTRACT

Tissue engineering helps to regenerate, restore and improve the damaged tissues. Bone Implant coating need to satisfy few criteria to allow osseointegration, Osseo conductivity and Osseo inductivity. The choice of materials is crucial. Anything external, inserted into the body can cause inflammations and infections. Plants extract for implant coating have been studied for its natural healing properties and some study shows that the plants stimulate bone repair, the rate of calcification, and the formation & mineralization of bone callus, accelerating the process of new bone formation on the fracture region. Our aim is to synthesis and characterized the plant extract for bone implant which can be used as anti-inflammation coating. Bone implants are commonly made of metal coated in a similar material to bone, i.e., synthetic hydroxyapatite, to improve the bio compatibility and to aid bonding between the natural bone and the implant. Fourier Transform Infrared Spectroscopy (FTIR) identifies chemical bonds in a molecule by producing an infrared absorption spectrum. A scanning electron microscope (SEM) scans a focused electron beam over a surface to create an image. The electrons in the beam interact with the sample, producing various signals that can be used to obtain information about the surface topography and composition.

Keywords: Bone implant coating, Inflammations, plant extract, anti-inflammation coating, hydroxyapatite, bio compatibility.

LIST OF ABBREVIATIONS

Beta TCP – Beta Tri Calcium Phosphate

COX-Cyclooxygenase

CQ- Cissus quadrangularis

FTIR - Fourier Transform Infrared Spectroscopy

HA- Hydroxy Apatite

NC – Non-Coated

SEM - Scanning Electron Microscope

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CHAPTER 1

INTRODUCTION

Tissue engineering helps to regenerate, restore, and improve the damaged tissues. Bone Implant coating need to satisfy few criteria to allow osseointegration, Osseo conductivity and Osseo inductivity. The choice of materials is crucial. Anything external, inserted into the body can cause inflammations and infections. Plants extract for implant coating have been studied for its natural healing properties and some study shows that the plants stimulate bone repair, the rate of calcification, and the formation & mineralization of bone callus, accelerating the process of new bone formation on the fracture region. Our aim is to synthesis and characterized the plant extract for bone implant which can be used as anti-inflammation coating.

1.1 CISSUS QUADRANGULARIS (CQ)



Fig: 1.1: *Cissus Quadrangularis*

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Vitale

Family: Vitaceae

Genus: Cissus

Species: quadrangularis

CQ (Fig 1.1) is a perennial plant of the grape family native to tropical Asia, the Middle East, and much of Africa (Piyush S. Bafnaa Payal et al., 2019). It is believed to promote healing of broken bone and injured joint (Hemal R. Brahmshatriya, et al., 2015). A scientific report states that when a human cell exposed to CQ it shows increased osteoblast production (cells responsible for new bone formation). CQ Linn. has potent fracture healing property and antimicrobial, antiulcer, antioxidative, antiosteoporotic, gastroprotective, cholinergic activity as well as beneficial effects on cardiovascular diseases. Aerial parts of CQ are dried in shade and blended into powder and kept in clean sterile plastic bags until the extraction process.

1.2 ANTI-INFLAMMATORY ACTIVITY:

CQ exerted inhibitory effect on the edema formation in this animal model. It has been observed that flavonoids, several flavones, flavanols, flavanols and flavanones are inhibitors of lipoxygenase, especially luteolin which is one of the compounds found in CQ. Furthermore, the anti-inflammatory activity of β sitosterol was also demonstrated which is another active constituent of CQ also possess anti-inflammatory activity on a mg per mg basis comparable to aspirin or ibuprofen (Sadiya Zaki, et al., 2020). CQ constitutes one of the ingredients of an Ayurvedic preparation, 'Laksha Gogglu', which has been proved to be highly effective in relieving pain, reduction of swelling and promoting the process of healing of the simple fractures as

well as in curing the allied disorders associated with fractures. The anti-inflammatory activity of the extract was expressed as the percentage of inhibition of prostaglandin synthesis using a COX-1 assay.

1.3 IMPLANT

Bone needs stimulation in order to grow and stay healthy. The implant is placed directly in the bone and after the bone has fused to the implant, the action of chewing and biting acts on the post as it did on the root of the natural tooth. The stimulation keeps the bone healthy (Xing Gao, et al.,2019). If the implant fails to show bio compatibility, it cause inflammation. To show biocompatibility an implant needs to be coated with some polymer or other substances

1.4 INFLAMMATION

Think of inflammation as the body's natural response to protect itself against harm Inflammation is a reaction initiated by our white blood cells when something external enters into the body

Inflammation types

- Acute - Acute inflammation goes away within hours or days
- Chronic-Chronic inflammation can last months or years, even after the first trigger is gone.

1.5 IMPLANT COATING

An implant is usually coated with materials like **porous titanium and hydroxyapatite (HA)** to show biocompatibility property and encourage bone integration to sustain in the body. Usually, implants are made with stainless steel, titanium, ceramics or resins. If these materials are kept inside the body without coating, they do not show biocompatibility property and causes inflammation in the site. Implant coating makes the implant compatible with the body (Anna Mieszkowska et al.,2018)

1.6 BONE FORMATION

When you break your bone, the healing process begins. As long as the break in your bone is not too large, your bone cells can repair it. Sometimes, though, a fracture results in a large loss of bone, like when a large chunk of the bone crumbles away. In these cases, your bone might not fully heal without a bone graft or an implant may be needed.

CHAPTER 2

LITERATURE SURVEY

Hemal R. Brahmshatriya, Kruti A. Shah, and Mansi H. Brahmshatriya conducts Clinical evaluation of cissus quadrangularis as osteogenic agent in maxillofacial fracture CQ helps in reducing pain, swelling, and fracture mobility and accelerate the healing of fracture jaw bones.

Sadiya zaki, R Malathi, V Latha and Sibi aims to tells us about Review on efficacy of cissus quadrangularis in pharmacological mechanisms. In treating various ailments. The major pharmacological activities of the plant include antimicrobial, anti-diabetic, anti-inflammatory, anti-obesity, anti-oxidant, bone turnover, cardiovascular and hepatoprotective.

Anna Mieszkowska, Justyna Folkert, Bernard burke, Owen Addison, Katarzyna Gurzawska describes about Pectin coating of titanium and polystyrene surfaces modulates the macrophage inflammatory response. Bacterial colonization of implants leads to prolonged immune cell activation and bone resorption. A new strategy to improve implant biocompatibility and prevent peri-implantitis is to develop pectin surface nano coatings. The plant-derived polysaccharides are promising candidates for surface nano coatings of titanium implants due to their osteogenic and anti-inflammatory properties.

Piyush S. Bafnaa Payal, H.Patilasaurabh K and Marubakesh E.Muthaa gives us a review on Cissus quadrangularis a comprehensive multidisciplinary review. This is a need of time as the conventional system of medicine is associated with various side effects. CQ is a climbing herb commonly known as hadjod and asthisamadhani in india and traditionally used in the management of many illnesses.

Xing gao and Manon Fraulob describes about Biomechanical behaviours of the bone–implant interface. The article is to provide more insight on the current state of the art concerning the evolution of the biomechanical properties of the bone implant interface as a function of the implant's environment

Amir, Dehghan Ghadikola and Behzad Fotovvati tells us the various Coating techniques for functional enhancement of metal implants for bone replacement. It has been observed that these metallic biomaterials undergo a series of corrosion reactions in human body fluid, which are toxic in high dosages. To resolve this issue, researchers have proposed depositing coatings, on implant substrates in order to enhance bone/implant interaction while covering the substrate from corrosion

Narges Shayesteh Moghaddam, Mohsen, Taheri Andani , Amirhesam Amerinatanzi, Christoph Haberland, Scott Huff and Michael Milller suggest the suitable Metals for bone implants: safety, design and efficacy. This paper reviews the safety, design, and efficacy of metal implants in general and specifically for the repair of mandibular segmental defects. These problems include implant incorporation, implant failure mechanisms corrosion and toxicity, infection, and muscle re-attachment

Rahul Mahajan, Sanjeev Gupta, Nitin Choudhary, Neeraj Mahajan, Vishal R Tandon, Jyotsna Suri and Ghanshyam Dev describes about effect of cissus quadrangularis on fracture healing. The study groups received oral cissus quadrangularis in solution. Fracture healing of study group was compared with the control group radiologically and histo pathologically to establish the role of cissus quadrangularis on fracture healing,

Callum Stewart and Behnam Akhavana gives us outline in review of biomimetic surface functionalization for bone integrating orthopaedic implants: mechanisms, current approaches, and future directions. The surface properties that govern protein-surface interactions are subsequently explained. Progress in biomolecule functionalization of orthopaedic surfaces performed via adsorption, chemical covalent immobilization, and physical covalent immobilization are discussed and reviewed.

Marc Bohner, Bastien Le Gars, Santoni and Nicola Döbelin describes about β -tricalcium phosphate for bone substitution: synthesis and properties. The present review has documented the richness, breadth, and interest of the research devoted to β -tcp. β -tcp is synthetic, osteo conductive, osteo inductive, and its resorption is

cell-mediated, thus making it one of, or if not, the most potent bone graft substitutes. B-tcp is widely used in the clinics.

CHAPTER 3

AIM AND SCOPE

3.1. AIM AND SCOPE OF THE PRESENT INVESTIGATION:

We are going to create anti inflammation coating for bone implants to reduce inflammation, with plant extract and some other bio polymers.

3.2. OBJECTIVE

Plants extract for implant coating have been studied for its natural healing properties and some study shows that the plants stimulate bone repair. The rate of calcification and the formation and mineralization of bone callus, accelerating the process of new bone formation on the fracture region. Bone Implant coating need to satisfy few criteria. Anything external, inserted into the body can cause inflammations and infections. Our priority is to create plant extract which reduces inflammation.

CHAPTER 4

MATERIALS & METHODS

4.1. PREPARATION OF EXTRACT

Infusion method: An infusion is a dilute solution of easily soluble constituents of the raw drug. Is suitable for the aromatic drugs, to prevent volatile oils evaporate at other temperatures. The infusion is carried out by immersing the plants parts to use in an amount of boiling water, allowed to stand 15 minutes and then filtered through a *filter or filter paper*.

Materials required: Distilled water, ethanol, Cissus Quadrangularis dried powder. gelatin, pectin, beta tri calcium phosphate

4.2 PREPARATION OF COATING SAMPLE

Wet Chemical Method: Wet chemistry is a form of analytical chemistry that uses classical methods such as observation to analyse materials. It is called wet chemistry since most analysing is done in the liquid phase.

4.3 PLASMA SPRAY COATING

Plasma spraying is a thermal process which entails creating a coating by spraying particles softened by heat onto a substrate. The particles sprayed are normally in the form of powder which is injected into the plasma flame to be heated to high temperatures. Plasma spraying is also known as plasma arc spraying. (Amir et al.,.2019)

The plasma spraying process involves the generation of a plasma jet, the injection and treatment of particles within the plasma jet and finally the formation of the coating. To generate the plasma jet, a working gas such as an argon/hydrogen mixture is passed through a powerful electric arc discharge formed in the gap between a cathode and anode. Rapid expansion occurs, lifting the speed of the jet, giving it a very high nozzle speed. The coating material in a fine powder form is then

injected into the plasma jet. Molten droplets form, which are propelled at high speed towards the object to be coated (substrate) as shown in fig 4.1.

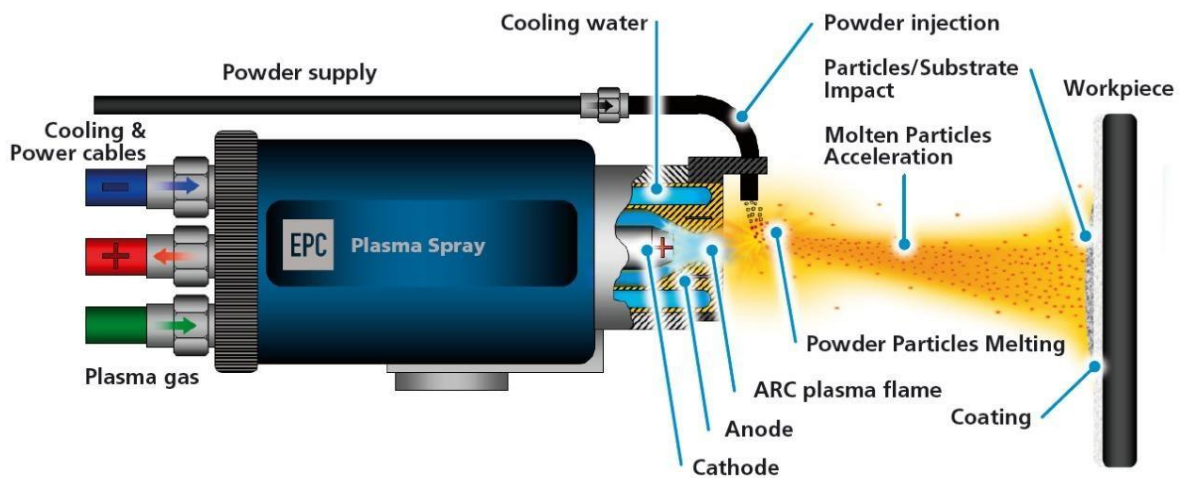


Fig: 4.1: Plasma Spray Working

4.4 FTIR (FOURIER TRANSFORM INFRA-RED SPECTROSCOPY)

In FTIR analyses, Infrared light from the light source passes through a Michelson interferometer along the optical path. The Michelson interferometer comprises a beam splitter, moving mirror, and fixed mirror. The light beam split into two by the beam splitter is reflected from the moving mirror and fixed mirror, before being recombined by the beam splitter. As the moving mirror makes reciprocating movements, the optical path difference to the fixed mirror changes, such that the phase difference changes with time. The light beams are recombined in the Michelson interferometer to produce interference light. The intensity of the interference light is recorded in an interferogram, with the optical path difference recorded along the horizontal axis. (Dr. Amir Dehghanghadikolaei et.al., 2019)

Parts: A common FTIR spectrometer consists of a source, interferometer, sample compartment, detector, amplifier, A/D convertor, and a computer. The source generates radiation which passes the sample through the interferometer and reaches the detector. Then the signal is amplified and converted to digital signal by the amplifier and analog-to-digital converter, respectively.

Working: FTIR analysis measures the range of wavelengths in the infrared region that are absorbed by a material. This is accomplished through the application of

infrared radiation (IR) to samples of a material. The sample's ability to absorb the infrared light's energy at various wavelengths is measured to determine the material's molecular composition and structure as shown in fig 4.2.

Unknown materials are identified by searching the spectrum against a database of reference spectra. Materials can be quantified using the FTIR materials characterization technique as long as a standard curve of known concentrations of the component of interest can be created. FTIR Analysis can be used to identify unknown materials, additives within polymers, surface contamination on a material, and more.

The results of the tests can pinpoint a sample's molecular composition and structure. A simple device called an interferometer is used to identify samples by producing an optical signal with all the IR frequencies encoded into it. The signal can be measured quickly. Then, the signal is decoded by applying a mathematical technique known as Fourier transformation.

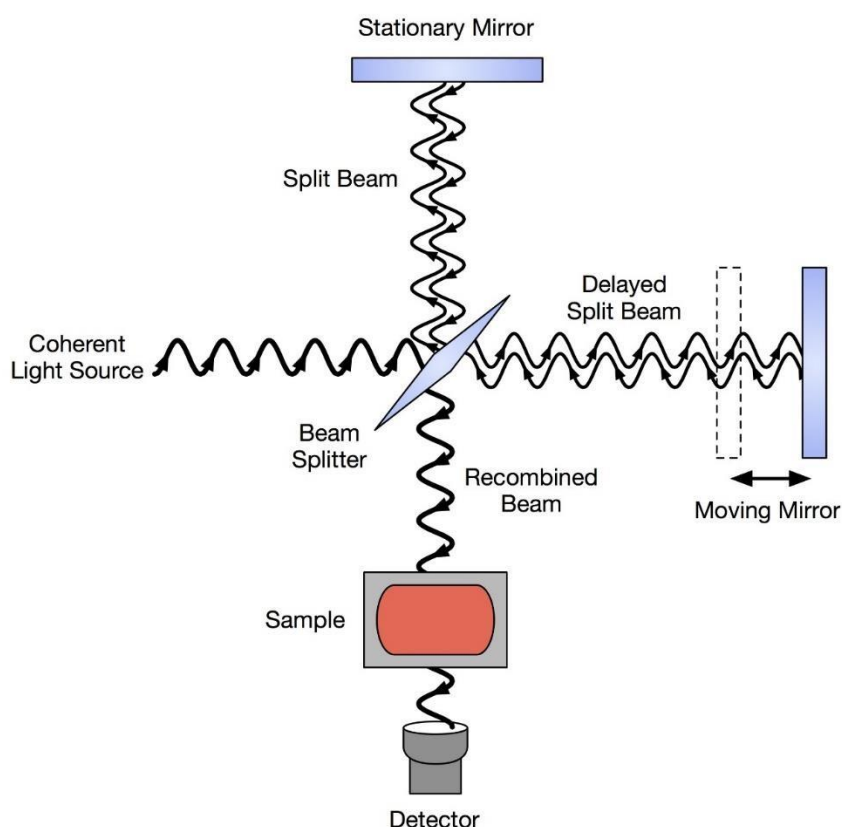


Fig: 4.2: FTIR Working

This computer-generated process then produces a mapping of the spectral information. The resulting graph is the spectrum which is then searched against reference libraries for identification. With the microscope attachment, samples as small as 20 microns can be analyzed. This allows quick and cost-effective identification of unknown particles, residues, films or fibers. FTIR testing can also measure levels of oxidation in some polymers or degrees of cure in other polymers as well as quantifying contaminants or additives in materials.

4.5 SEM (SCANNING ELECTRON MICROSCOPY)

The Scanning electron microscope works on the principle of applying kinetic energy to produce signals on the interaction of the electrons. These electrons are secondary electrons, backscattered electrons, and diffracted backscattered electrons which are used to view crystallized elements and photons. Secondary and backscattered electrons are used to produce an image. The secondary electrons are emitted from the specimen play the primary role of detecting the morphology and topography of the specimen while the backscattered electrons show contrast in the composition of the elements of the specimen.

Parts: Electron Source – This is where electrons are produced under thermal heat at a voltage of 1-40kV. the electrons condense into a beam that is used for the creation of an image and analysis. There are three types of electron sources that can be used i. e Tungsten filament, Lanthanum hexaboride, and Field emission gun (FEG)

Lenses – It has several condenser lenses that focus the beam of electrons from the source through the column forming a narrow beam of electrons that form a spot called a spot size.

Scanning Coil – They are used to deflect the beam over the specimen surface.

Detector – It's made up of several detectors that are able to differentiate the secondary electrons, backscattered electrons, and diffracted backscattered electrons. The functioning of the detectors highly depends on the voltage speed, the density of the specimen.

Power supply

Vacuum system

Working:

The source of the electrons and the electromagnetic lenses are from tungsten filament lamps that are placed at the top of the column and it is similar to those of the transmission electron Microscope. The electrons are emitted after thermal energy is applied to the electron source and allowed to move in a fast motion to the anode, which has a positive charge. The beam of electrons activates the emission of primary scattered (Primary) electrons at high energy levels and secondary electrons at lowenergy levels from the specimen surface. The beam of electrons interacts with the specimen to produce signals that give information about the surface topography and composition of the specimen.

The specimen does not need special treatment for visualization under the SEM, even air-dried samples can be examined directly. However, microbial specimens need fixation, dehydration, and drying in order to maintain the structural features of the cells and to prevent collapsing of the cells when exposed to the high vacuum of the microscope. The samples are mounted and coated with thin layer of heavy metal elements to allow spatial scattering of electric charges on the surface of the specimen allowing better image production, with high clarity.

Scanning by this microscope is attained by tapering a beam of electrons back and forth over a thin section of the microscope. When the electrons reach the specimen, the surface releases a tiny straw of electrons known as secondary electrons which are then trapped by a special detector apparatus. When the secondary electrons reach and enter the detector, they strike a scintillator (a luminescence material that fluoresces when struck by a charged particle or high-energy photon). This emits flashes of light which get converted into an electric current by a photomultiplier, sending a signal to the cathode ray tube. This produces an image that looks like a television picture that can be viewed and photographed.

The quantity of secondary electrons that enter the detector is highly defined by the nature of the specimen i.e., raised surfaces to receive high quantities of electrons, entering the detector while depressed surfaces have fewer electrons reaching the surface and hence fewer electrons enter the detector as shown in fig 4.3.

Scanning Electron Microscopy (SEM)

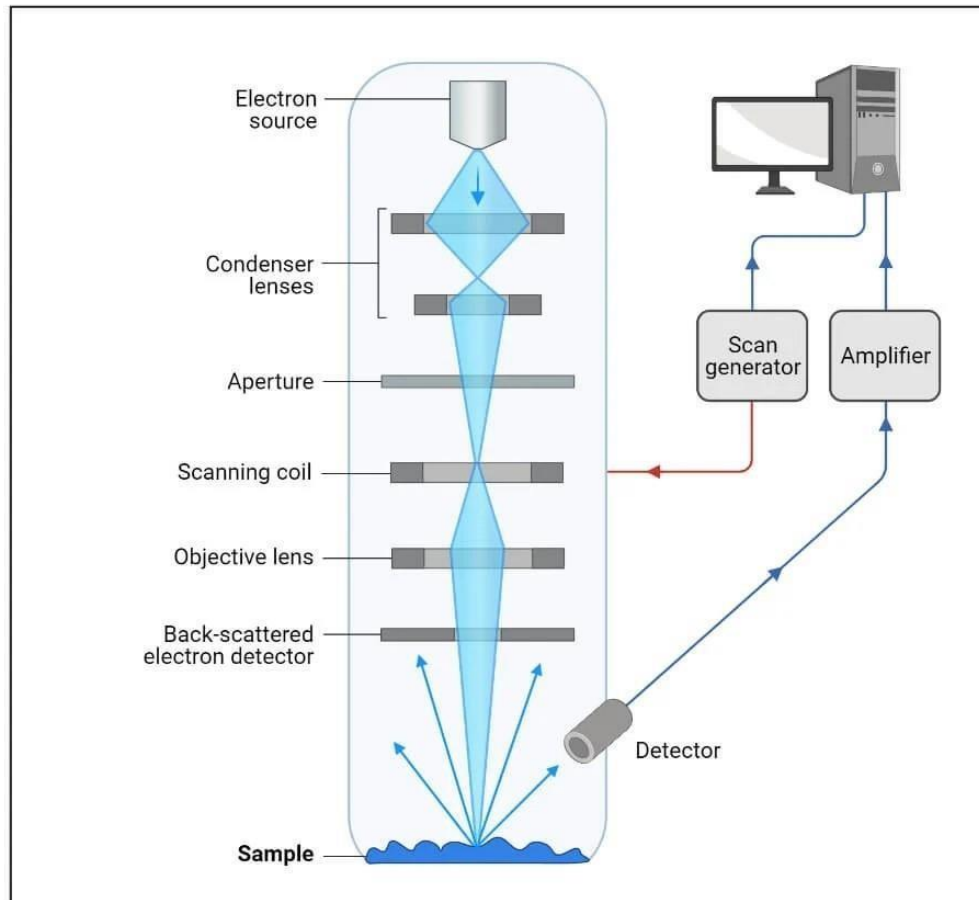


Fig: 4.3: SEM Working

4.6 ANTIBACTERIAL TESTING

An antibiotic sensitivity test can help find out which antibiotic will be most effective in treating your infection. Antibiotics are medicines used to fight bacterial infections. There are different types of antibiotics. Each type is only effective against certain bacteria. The test can also be helpful in finding a treatment for antibiotic-resistant infections. Antibiotic resistance happens when standard antibiotics become less effective or ineffective against certain bacteria. Antibiotic resistance can turn once easily treatable diseases into serious, even life-threatening illnesses.

CHAPTER 5

RESULTS AND DISCUSSION

5.1 SAMPLE PREPARATION RATIOS:

5.1.1 Trial 1

- 40ml of water and 10 ml of ethanol is heated to 60 ° to 70 ° C for 1 hour.
- 1g of powdered *Cissus Quadrangularis* is mixed with the above solution and heated for 1 hour.
- And the solution with the plant is heated for every 24 hours for 2 days.
- After two days the solution is filtered and the extract is obtained.
- The sample is obtained as shown in fig 5.1.



Fig: 5.1: Prepared Sample Of CQ Trial 2

5.1.2 Trial 2

- 50ml of water is heated to 60 ° to 70 ° C for 1 hour.
- 1g of powdered Cissus Quadrangularis is mixed with the above solution and heated for 1 hour.
- And the solution with the plant is heated for every 24 hours for 2 days.
- After two days the solution is filtered and the extract is obtained.
- The sample is obtained as shown in fig 5.2.

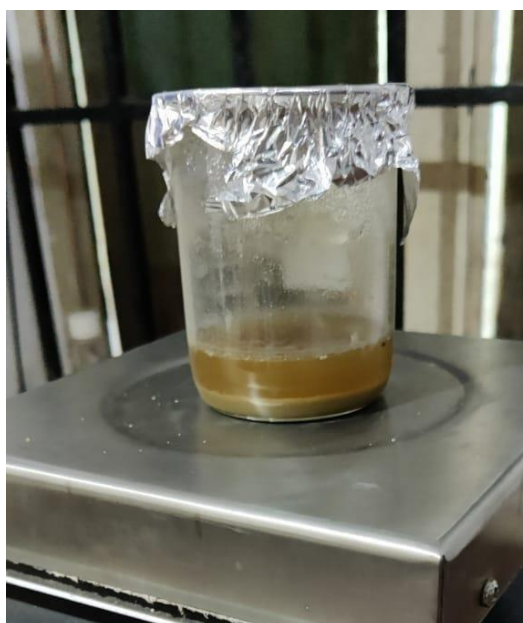


Fig: 5.2: Prepared Sample Of CQ Trial 2

5.1.3 Trial 3

- 50ml of ethanol is taken.
- 1g of powdered Cissus Quadrangularis is mixed with the above solution and heated for 1 hour.
- And the solution with the plant is heated for every 24 hours for 2 days.
- After two days the solution is filtered and the extract is obtained.
- The sample is obtained as shown in fig 5.3.



Fig: 5.3: Prepared Sample Of CQ Trial 3

5.2 WET CHEMICAL METHOD

5.2.1. Trial 1

- The obtained plant extract is heated for 15 minutes. 0.56 grams of gelatin is added slowly to the extract solution.
- 0.56 grams of pectin is added slowly to the extract solution. 0.3 grams of beta tri calcium phosphate are added
- The solution is heated for 1 hour and the solution is left to cool down at room temperature.
- A semi solid substance is obtained as shown in fig 5.4.



Fig: 5.4: Prepared Coating Sample of Trial 1

5.2.2 Trial 2

- The obtained plant extract is heated for 15 minutes. 0.56 grams of gelatin is added slowly to the extract solution.
- 0.56 grams of pectin is added slowly to the extract solution. 0.3 grams of beta tri calcium phosphate are added.
- The solution is heated for 1 hour and the solution is left to cool down at room temperature.
- A semi solid substance is obtained as shown in fig 5.5.



Fig: 5.5: Prepared Coating Sample of Trial 2

5.2.3 Trial 3

- The obtained plant extract is heated for 15 minutes. 0.56 grams of gelatin is added slowly to the extract solution.
- 0.56 grams of pectin is added slowly to the extract solution. 0.3 grams of beta tri calcium phosphate are added.
- The solution is heated for 1 hour and the solution is left to cool down at room temperature.

5.3 ANALYTICAL INTERPRETATION

5.3.1 FTIR graph of *Cissus quadrangularis* plant extract

The extracted sample of the plant are being sent for FTIR analysis which shows varying vibration bands at different wavenumbers range from 500 to 4000. After the FTIR test wavenumber and intensity were taken from the sample in which extracted plant samples of *Cissus quadrangularis* the graphs were plotted from the FTIR point data for the sample. At 1371.6 wavenumber chemical bond such as C-F, O-H, S=O And chemical groups such as Fluro compound, phenol, sulfonate are seen. At 1736.93 wavenumber chemical bond such as C=O And chemical groups such esters, gammalactone, aldehyde are seen. At 2847 wavenumber chemical bond such as C-H And chemical groups such alkanes, carboxylic acid, alcohol, amine salt are seen. At 2914 wavenumber chemical bond such as C-H And chemical groups such Alkane, carboxylic acid, alcohol, amine salt are seen.

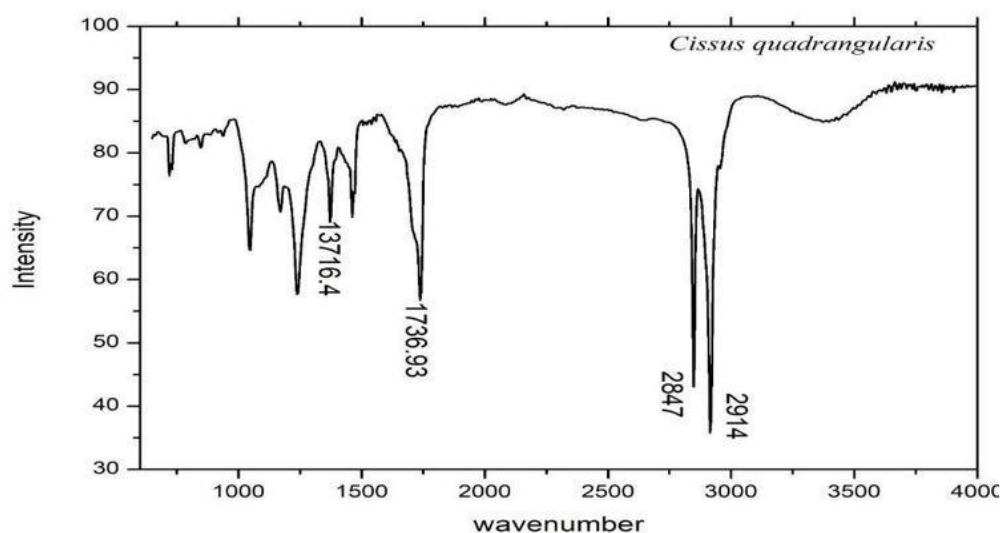


Fig: 5.6: FTIR Of CQ Plant Extract

From the peak points in the graph vibrational bands were found out and based on that, the presence of certain chemical bonds was indicated which give us an idea that certain functional groups were present in extract that are said to be

present in the phytochemicals required for bone regeneration. The possible phytochemicals that are said to be present are Flavonoids, Tannins, Anthocyanins, Ascorbic acids, Beta- carotenoids, Poly Lactic Acids.

Table: 5.1: Phytochemicals And Their Property Other Than Anti oxidance

Possible Phytochemical	Properties
Flavonoids	Anti-Inflammatory, antibiotic, osteogenesis, anticarcinogenic
Beta-carotenoids	anticancer, cure skin, vision and cognitive function.
Tannins	anti-inflammatory, antitumor
Ascorbic acids	antibacterial, collagen formation, bone & tissue regeneration,
Anthocyanins	antimicrobial, anti-inflammatory, anticancer

5.3.2 FTIR Beta TCP & OTHER

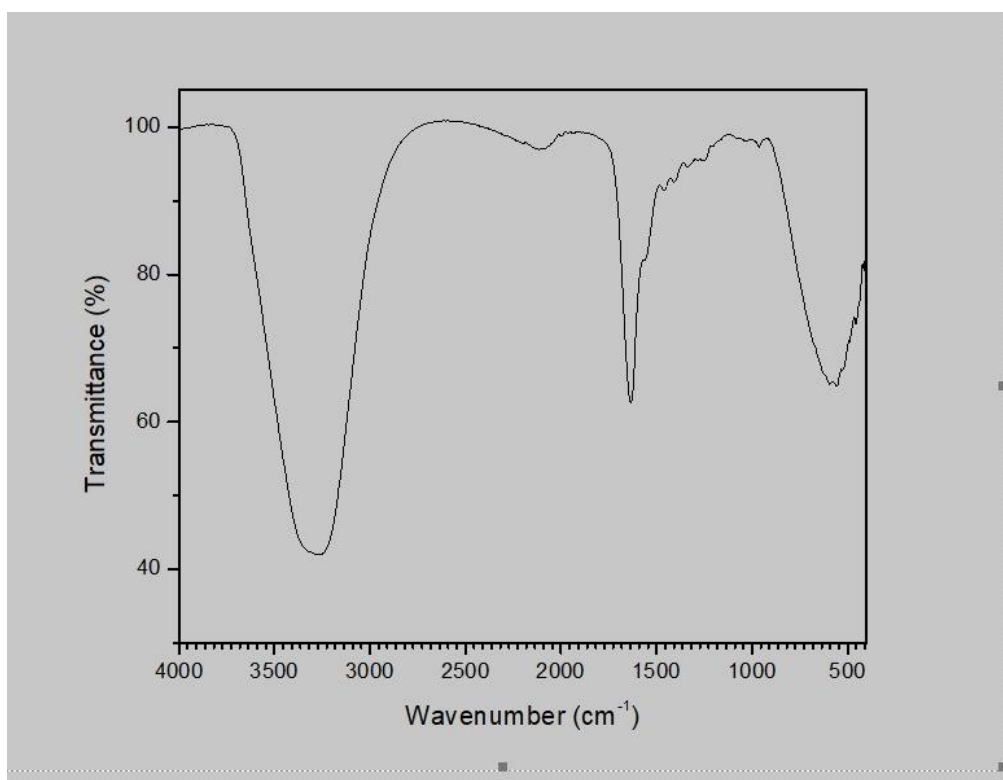


Fig: 5.7: FTIR of Beta TCP and others

At 3500- 3000 wavelength chemical bonds such as N–H stretch, O–H stretch, free hydroxyl and functional groups such as 1°, 2° amines, amides, alcohols, phenols are seen. At 1700-1500 wavelength chemical bonds such as C=O stretch, C=O stretch and functional groups such as carboxylic acids, α , β –unsaturated aldehydes, ketones are seen. At 1200-1000 wavelength chemical bonds such as C–N stretch, C–O stretch and functional groups such as aliphatic amines, alcohols, carboxylic acids, esters, Ethers are seen.

The FTIR absorption band of beta tri-calcium phosphate peaked at 1050cm⁻¹ and it contain calcium, phosphorous and oxygen groups. The peak corresponding to the bending mode of O-P-O bonds falls on 550cm⁻¹. At 700-500 wavelength chemical bonds such as $\text{--C}\equiv\text{C--H}$: C–H bend and functional groups such as alkynes are seen.

The bands are generally larger due to the macro and micro molecular structure of the compound containing numerous intermolecular bindings.

5.3.3. Morphological interpretation

Therefore, raised surfaces will appear brighter on the screen while depressed surfaces appear darker. Image A and B shows the implant at 100x and 3500x respectively. Many bones metallic implant materials are used in humans to facilitate patient healing. Unfortunately, metallic implants will show some deficiencies like low/high corrosion resistance, releasing toxic ions and low biocompatibility.

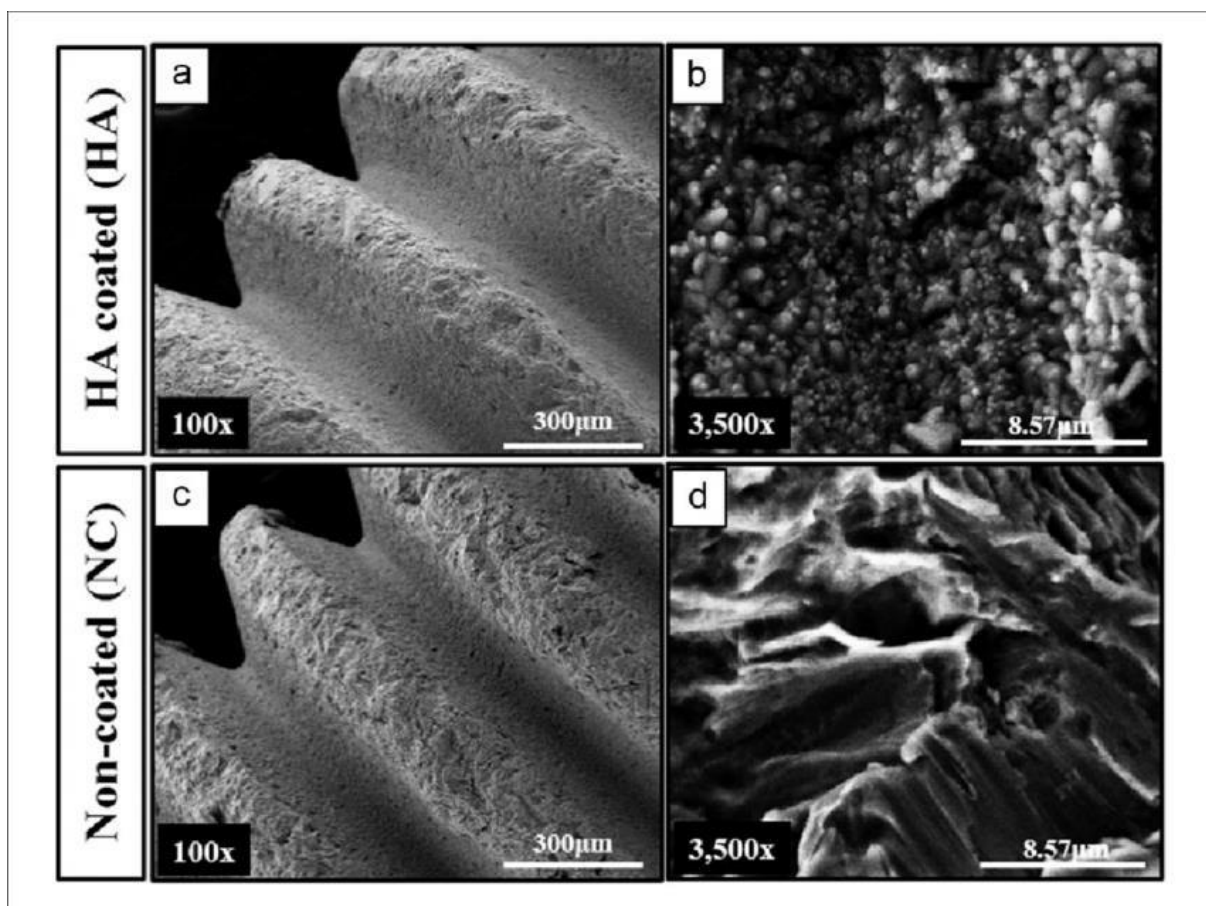


Fig: 5.8: SEM Image Of Coated Implant

To resolve these issues with many surfaces' modifications method are proposed, one of the proposed methods is coating technique. Coating method is selected based on their patient needs and applications. Here we use spray coating technique, in addition it was observed in SEM analysis. At 100x we can see the

coated particles are present. At 3500x we can clearly see the coated particles as dotted structure and it shows a tiny porous in coated sample where the pores help to cell interaction between implants and the bone cells. The implant coating surfaces could chemically improve the molecular and cellular responses and reduce the infection rates, facilitating osseointegration. Image C and D shows the non-coated implant at 100x and 3500x shows the Coating surface of the sample where we can analysis the micro-cracks in the microscopic image.

5.3.4 Antibacterial Test Interpretation

We are done the anti-bacterial testing with *Escherichia coli* and *Streptococcus* and it exhibit good inhibition zone.

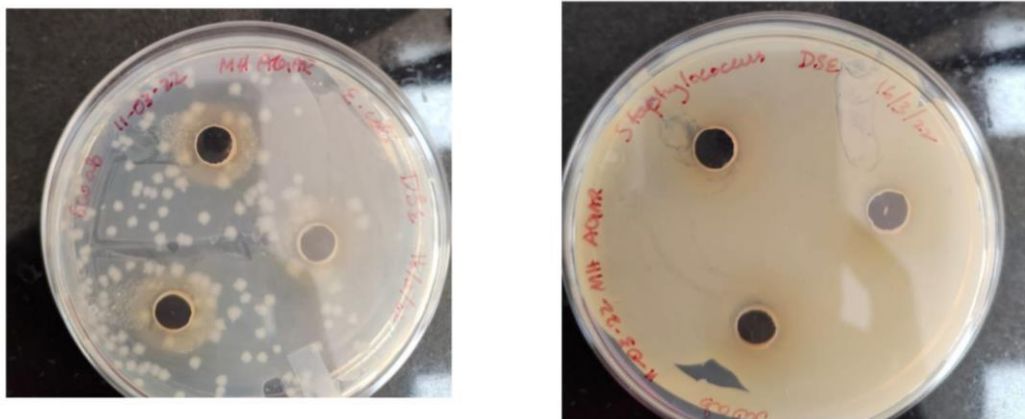


Fig: 5.9: Antibacterial Test Results

Our anti-bacterial study shows an effective against gram-positive bacteria and gram-negative bacteria, Beta-tricalcium phosphate coating material have a biological stability and confirmed its good antibacterial ability. Aseptic inflammation is likewise a fundamental part of orthopaedic implants, particularly artificial joints that should be permanently held in the human body. From one perspective, metal prostheses are immersed in body fluids for long time, while proteins, organic acids, alkali metals, and inorganic salts contained in body fluids can cause erosion of metals, resulting about the releasing of metal particles. Then again, metal

prostheses keep on going through different types of external force after implantation, which can destroy the inert oxide layer on a superficial level and produce metal particles and metal ions. Moreover, the tissue-engineered bone will deliver various kinds of chemicals during the process of degradation after implantation, that will heal the inflammation around the implants. The bio-ceramic coating has strongly inhibited a biofilm formation on the surfaces of the bone implants and reduced the number of bacteria in the surrounding tissue.

CHAPTER 6

CONCLUSION & SUMMARY

Tissue engineering helps to regenerate, restore and improve the damaged tissues. Bone Implant coating need to satisfy few criteria to allow osseointegration, Osseo conductivity and Osseo inductivity. The choice of materials is crucial. Anything external, inserted into the body can cause inflammations and infections. Plants extract for implant coating have been studied for its natural healing properties and some study shows that the plants stimulate bone repair, the rate of calcification, and the formation & mineralization of bone callus, accelerating the process of new bone formation on the fracture region. Our aim is to synthesis and characterized the plant extract for bone implant which can be used as anti-inflammation coating. Bone implants are commonly made of metal coated in a similar material to bone, i.e., synthetic hydroxyapatite, to improve the bio compatibility and to aid bonding between the natural bone and the implant.

We have done various methods such as infusion method, Wet chemical method to prepare plant extract and coating sample. An infusion method is a dilute solution of easily soluble constituents of the raw drug. Is suitable for the aromatic drugs, to prevent volatile oils evaporate at other temperatures. The infusion is carried out by immersing the plants parts to use in an amount of boiling water, allowed to stand 15 minutes and then filtered through a filter or filter paper. Wet chemical method is a form of analytical chemistry that uses classical methods such as observation to analyse materials. It is called wet chemistry since most analysing is done in the liquid phase.

We also have performed various number of trails and have selected our final output.

An implant is usually coated with materials like porous titanium and hydroxyapatite (HA) to show biocompatibility property and encourage bone integration to sustain in the body. Usually, implants are made with stainless steel, titanium, ceramics or resins. If these materials are kept inside the body without coating, they do not show biocompatibility property and causes inflammation in the site. Implant coating makes the implant compatible with the body, coating of implant is done by plasma spray coating.

Plasma spraying is a thermal process which entails creating a coating by spraying particles softened by heat onto a substrate. The particles sprayed are normally in the form of powder which is injected into the plasma flame to be heated to high temperatures. Plasma spraying is also known as plasma arc spraying

Fourier Transform Infrared Spectroscopy (FTIR) identifies chemical bonds in a molecule by producing an infrared absorption spectrum. A scanning electron microscope (SEM) scans a focused electron beam over a surface to create an image. The electrons in the beam interact with the sample, producing various signals that can be used to obtain information about the surface topography and composition.

Anti-bacterial test has shown good resistance to *Escherichia coli* and *Streptococcus* and shown good size of inhibition zone.

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