

SYNTHESIS AND CHARACTERIZATION OF NANO POROUS BANDAGE USING POVIDONE-IODINE INCORPORATED PLGA SHEET

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

By

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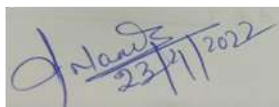
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This is to certify that this Project Report is the bonafide work of **Renuka S (Reg.no:38240028)** and **Sandra Ann R (Reg.no:38240030)** as a team. Who have carried out the project entitled " **SYNTHESIS AND CHARACTERIZATION OF NANOPOROUS BANDAGES USING POVIDONE - IODINE INCORPORATED PLGA** " under the supervision from January 2022 to April 2022

Internal Guide

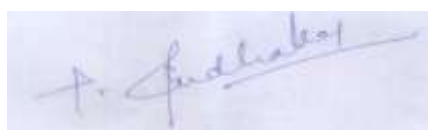


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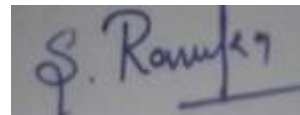
DECLARATION

We **Renuka S (38240028)** and **Sandra Ann R (38240030)** hereby declare that the Project Report entitled **SYNTHESIS AND CHARACTERIZATION OF NANOPOROUS BANDAGES USING POVIDONE-IODINE INCOPORATED PLGA SHEET** done by us under the guidance of **Dr.ANIMA NANDA.,** Ph.D.,FNRS,FAB,FIAS COORDINATOR IQAC., (Internal) and (External) **Dr.V.KARTHIK M.Tech.,Ph.D** at **CENTRE FOR OCEAN RESEARCH , COL.DR.JEPPIAAR RESEARCH PARK, SATHYABAMA INSTITUTE OF SCIENCE AND TECHNOLOGY, CHENNAI 600019** is submitted in partial fulfilment of the requirements for the award of Bachelor of Engineering / Technology degree in biomedical engineering.

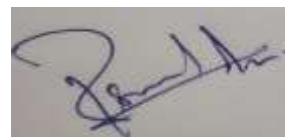
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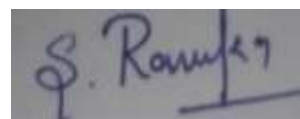
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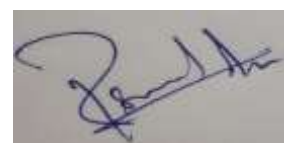
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ABSTRACT

Wound healing is a complex process that requires optimum conditions over a period of time. Any infection to the wound healing process through formation of inferior quality collagen or inducing higher collagenolytic activity. Since the cutaneous wound are more exposed to direct air, chances of infection are high. Preventing wound infection remain a key objective in healing. Research on efficient prevention is going on here the proposed study aims to synthesise nano porous PLGA sheet to prepare bandages incorporated with povidone-iodine. PLGA is one among the FDA approved and well documented biocompatible material for drug delivery and well described reproducible methods of fabrication. The nano hole and povidone-iodine perform the role of antimicrobial activity, where nano holes help to exclude the microbial attachment to the surface by electron transport through the nanohole. The atomic vacancies in nanohole facilitates electron transport and it effectively destroys microbial attachment. Surface incorporated colloidal povidone-iodine enhance the antimicrobial efficiency of nano porous PLGA. In the proposed study synthesise and characterization of nano porous PLGA sheets and its antimicrobial efficiency will be investigated.

Key Words: wound healing, nano porous ,PLGA, povidone-iodine,bandage

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ABBREVIATION

PLGA	Poly (lactic-co-glycolic acid)
PEG	PEGylation
DCM	Dichloromethane
NPs	Nanoporous
SEM	Scanning Electron Microscope
MHB	Mueller Hinton Agar
AFM	Atomic Force Microscope
FTIR	Fourier Transform Infrared Spectroscopy
W1-W3	Wound sample
S4-S6	Sputum sample
PVP-I	Povidone-Iodine
CH ₃ COOH	Acetic acid
FEG	Field Emission Gun

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CHAPTER-1

INTRODUCTION

1.1 INTRODUCTION TO POLYMERS

Biodegradable polymers have been more important in the pharmaceutical and biomedical fields over the last three decades (Huang et al., 2010). Despite significant advancements in biodegradable materials, some drug delivery vehicles have been discovered to cause acute inflammation (Kawazoe et al., 2009). Controlled release technology is a strategy and approach for optimising the dosage of a medicine released to a specific location for a certain period of time (Liu et al., 2011). Tissue engineering has received a lot of attention as a promising therapeutic option for damaged or missing tissues or organs. Injectable scaffolds, such as hydrogels (Taguchi et al., 2005), collagen (Nam et al., 1999), gelatin (A.G.Mikos et al., 1993), chitosan (T.W.King., et al., 2000), and poly(ethylene glycol) (PEG) (King et al., 2000), are commonly employed to increase structural consistency and stability. Into the field biodegradable, personalised scaffold for wound healing had been introduced and under research for diabetes patients who needs much attention in wound healing.

1.2 POLY LACTIC GLYCOLIC ACID (PLGA)

PLGA, PLG, or poly(lactic-co-glycolic acid) is a copolymer which is used as a host of Food and Drug Administration (FDA) approved therapeutic devices, owing to its biodegradability and biocompatibility (Hirenkumar et al., 2011). PLGA degrades by hydrolysis of its ester linkages in the presence of water. It has been shown that the time required for degradation of PLGA is related to the monomers' ratio used in production: the higher the content of glycolide units, the lower the time required for degradation as compared to predominantly lactide materials. An exception to this rule is the copolymer with 50:50 monomers' ratio which exhibits the faster degradation (about two months). In addition, polymers that are end-capped with esters (as opposed to the free carboxylic acid) demonstrate longer degradation half-lives (Horisawa et al., 2022). This flexibility in degradation has made it convenient for fabrication of many medical devices, such as, grafts,

sutures, implants, prosthetic devices, surgical sealant films, micro and nanoparticles.

1.3 POLYETHYLENE GLYCOL (PEG)

Polyethylene glycol (PEG) is a polyether compound derived from petroleum with many applications, from industrial manufacturing to medicine. PEG is also known as polyethylene oxide (PEO) or polyoxyethylene (POE), depending on its molecular weight. PEG is also used as an excipient in many pharmaceutical products. Polyethylene glycol has a low toxicity and is used in a variety of products. PEG is a flexible, water-soluble polymer. It also is unlikely to have specific interactions with biological chemicals. These properties make PEG one of the most useful molecules for applying osmotic pressure in biochemistry and bio-membranes experiments, gene therapy vectors (such as viruses) can be PEG-coated to shield them from inactivation by the immune system and to de-target them from organs where they may build up and have a toxic effect. The size of the PEG polymer has been shown to be important, with larger polymers achieving the best immune protection.

1.4 RECENT TRENDS OF RESEARCH USING POLYMERIC NANOPARTICLES

Polymeric nanoparticles (NPs) composed of biocompatible and biodegradable synthetic polymers, such as poly investigated biocompatible support materials and drug delivery vehicles. PLGA-based NPs systems are one of the most successful and interesting colloidal systems. One of the main advantages of PLGA NPs is their capability of encapsulating therapeutic agents, protecting them, and increasing their stability so that therapeutic agents can be released in the body as the polymeric matrix progressively degrades, improving pharmacokinetic and pharmacodynamics profiles.

Recently, the trend has been shifted towards natural polymers, such as alginates, cellulose, starch, and chitosan (CS), among others, as controllers of the surface charge and stabilizer agents of NPs. This is because of the negative surface charge of PLGA NPs, which tends to limit their interaction with the cell membranes, usually negatively charged. Guo et al prepared PLGA NPs coated with CS using the oil-in-water emulsion and solvent evaporation technique. They obtained a good correlation between PLGA-CS NPs size and adsorbed CS on the

NPs surface. Besides, zeta potential of PLGA NPs coated with CS was positive and increased with CS concentration. On the other hand, *Wang et al* modified PLGA NPs with CS through physical adsorption and chemical binding methods. They observed that after the introduction of CS, the zeta potential of PLGA NPs changed from negative charge to positive one, enhancing the affinity of drug carriers with cancer cells.

1.5 POLYMERS AND WOUND HEALING

Wound healing is a complex process of replacing cellular structures and tissue layers. Patients with diseases, elderly, genetic disease like sickle cell disease are predisposed to abnormal wound healing or leading to long term. Non healing wounds affect about 3 to 6 million people in US (Mathius et.al., 2006). Wound healing process has four phases haemostasis, inflammation, proliferation and tissue remodelling or resolution. Oxygenation is also very important for wound healing. Temporary hypoxia after injury triggers wound healing, but chronic hypoxia delays wound healing (Bishop, 2008; Rodriguez et.al., 2008). These events are driven by growth factors and cytokines, which are secreted at the wound site by inflammatory cells and other stromal cells in response to tissue injury, development of controlled-release systems that protect the recombinant growth factors from enzymatic degradation and provide sustained delivery at the wound site during healing to enhance tissue regeneration (J.Fang et al., 2014). The following materials listed which helps in wound healing are used in this study.

1.5.1 CHITOSAN

Chitosan is a linear polysaccharide composed of randomly distributed β -(1 $\rightarrow$ 4)-linked D-glucosamine (deacetylated unit) and N-acetyl-D-glucosamine (acetylated unit)(Randy Chi Faicheung et al., 2015). It is made by treating the chitin shells of shrimp and other crustaceans with an alkaline substance, such as sodium hydroxide. Chitosan is used within some wound dressings to decrease bleeding(Sherif Ashraf Fahmy et al.,2021). Upon contact with blood, the bandage becomes extremely sticky, and this adhesive-like effect seals the laceration. Chitosan-containing wound dressings received approval for medical use in the United States in 2003. Chitosan may also decrease the growth of bacteria and

fungi, and may be useful as a burn dressing. Chitosan haemostatic agents are salts made from mixing chitosan with an organic acid (such as succinic or lactic acid). The haemostatic agent works by an interaction between the cell membrane of erythrocytes (negative charge) and the protonated chitosan (positive charge) leading to involvement of platelets and rapid thrombus formation.

1.5.2 POVIDONE-IODINE (PVP-I)

Povidone-iodine (PVP-I), also known as iodopovidone, is an antiseptic used for skin disinfection before and after surgery. It may be used both to disinfect the hands of healthcare providers and the skin of the person they are caring for. It may also be used for minor wounds. It may be applied to the skin as a liquid or a powder. Povidone-iodine is a broad-spectrum antiseptic for topical application in the treatment and prevention of wound infection (Bigliarbi P.^{a,b} et al., 2017). It may be used in first aid for minor cuts, burns, abrasions and blisters. Povidone-iodine exhibits longer lasting antiseptic effects than tincture of iodine, due to its slow absorption via soft tissue, making it the choice for longer surgeries. Consequently, PVP-I has found broad application in medicine as a surgical scrub; for pre- and post-operative skin cleansing; for the treatment and prevention of infections in wounds, ulcers, cuts and burns; for the treatment of infections in decubitus ulcers and stasis ulcers; in gynaecology for vaginitis associated with candidal, trichomonal or mixed infections. For these purposes PVP-I has been formulated at concentrations of 7.5–10.0% in solution, spray, surgical scrub, ointment, and swab dosage forms; however, use of 10% povidone-iodine though recommended, is infrequently used, as it is poorly accepted by health care workers and is excessively slow to dry (Kewin Y Woo 2013).

Because of these critical indications, only sterile povidone-iodine should be used in most cases. Non-sterile product can be appropriate in limited circumstances in which people have intact, healthy skin that will not be compromised or cut. The non-sterile form of Povidone iodine has a long history of intrinsic contamination with *Burkholderia cepacia* (aka *Pseudomonas cepacia*), and other opportunistic pathogens. Its ability to harbour such microbes further underscores the importance of using sterile products in any clinical setting. Since

these bacteria are resistant to povidone iodine, statements that bacteria do not develop resistance to PVP-I, should be regarded with great caution: some bacteria are intrinsically resistant to a range of biocides including povidone-iodine. Antiseptic activity of PVP-I is because of free iodine (I₂) and PVP-I only acts as carrier of I₂ to the target cells. Most commonly used 10% PVP-I delivers about 1-3 ppm of I₂ in a compound of more than 31,600 ppm of total iodine atoms. All the toxic and staining effects of PVP-I is due to the inactive iodine only.

1.6 ANTIMICROBIAL ASSAYS

Antimicrobial assays are important tools to test and screen the inhibitory effects of myriad compounds against microorganisms before establishing their inhibitory spectra (broad vs. narrow) (Melvin Weinstein et al.,2009). Knowledge of the inhibitory spectra of antimicrobial compounds before their application in the fields of agriculture, biotechnology, and medicine is crucial. Various conventional and contemporary methods are available, but they vary in their sensitivity and efficacy. In the conventional antimicrobial bioassay, agar well diffusion, spot-on-lawn, and disc diffusion assays were performed against pathogenic *Bacillus cereus*, *Listeria monocytogenes*, *Escherichia coli* and *Salmonella enterica* (Katrina F et al.,2014). In this study, our objective was to measure and compare the sensitivity and efficacy of an agar-based diffusion bioassay (Mounyr Valouiri et al.,2016) and a fluorescence-based assay for antimicrobial activity.

Here in this investigation, the antibacterial activity and increment in wound healing activity regulated by chitosan and antimicrobial activity served by PVP-I are combined and utilised with the presence of PLGA to put in the formation of biodegradable sheets. The sheets are investigated for the antimicrobial activity and morphological parameters in the study.

CHAPTER-2

LITERATURE SURVEY

PLGA-based drug delivery system containing several wound healing medicines, as well as PLGA's own wound healing promotor are proposed by (Kiran Kumar Chereddy et al. 2016). The US FDA has approved PLA and PGA in biomedical product and devices such as destructive sutures (Pandey et al. 2015). Polyester PLGA co-polymer of poly lactic acid (PLA) and poly glycolic acid (PGA). PLA contain asymmetric α -carbon as D or L form in stereochemical terms as R and S forms. PLGA microspheres and their scaffolds are one of the most promising carriers for long-term medication delivery to wounds (Mittal et al.). Kirill T. Kalinin et al. (2021) reported that lactide copolymerization with glycolide reduces hydrophobicity and increases polymer degradation rate, allowing for better control of integrated medicinal release, structure size and morphology of PLGA particles are unaffected by drug loading. The use of PLGA nanoparticles in the nanoformulation of oxaliplatin can improve cancer therapeutic efficacy while reducing side effects. (Xingyu He et al. 2020) demonstrated that PLGA implant made of nanoporous PLGA sheet can selectively release drug molecules, keeping liposomal drug inside. This implant is biocompatible won't cause any kind of inflammation or any foreign body response when implant for 6 months. This implant shows great potential for on-demand dose-controllable drug release applications. PLGA is widely used in biomedical applications (i.e) PLGA-cisplatin nanoparticles were used to increase bioavailability, and the cytotoxicity of free cisplatin and PLGA-cisplatin were compared. This minimize toxicity and enhance medication efficacy (Saud Alarifi et al. 2021). (Maria Mir et al. 2017) proposed that Nanoporous PLGA has molecular level medications for targeting and revolutionized the in-vivo molecular imaging and therapeutic monitoring, drug delivery tool to cancer, CVDs and CNS. (Jan Setiawan et al. 2018) combined PLGA- Collagen with citrus paradise extract as antibacterial absorbable surgical suture its proven on tensile strength, swelling test, cytotoxicity test, antibacterial test and degradation test. Biocompatibility and anti-infection capacity is examined in vitro and vivo studies of nanohole enabled nanoparticle. Nanomaterials are non resistant than antibiotics. They use NBET for pathogenic bacterial infection and

drug resistance (Xuan Hou et al.2021). Microspheres of PLGA injected into rat brains were tested for biocompatibility with brain tissues, even if they remained in the brain for two months (Menei et al.,1993;Nicholas et al.,2002).

Sima Rezvantlab et al.2018 stated in his literature that comparison with natural polymer synthetic polymer(Polyethylene) is created with purity and well controlled process.PLGA-PEG copolymer used as micelles as transport for insoluble drugs (S.M.Jusu et al.).Other beneficial qualities of PEG particles have been discovered through experimental studies, including the capacity to reduce aggregation while increasing stability and biological potency (Goudarzi et al.2018).(Erica Locatelli et al.2014) demonstrated that PEG reduces blood circulation time by inhibiting quick opsonization recognition. (Hwang et.al) proposed a new Proposed a new approach for fabricating PLGA microfibers in a polydimethylsiloxane-based microfluidic chip for 3D tissue engineering scaffolds. Nayak PG et al.2020 Sesamol-PLGA nanosuspension enhances diabetic foot ulcer wound healing by restoring and altering the wound healing process in diabetic circumstances.(C.Reid et al) investigated that by preventing hydrogen bonding and membrane structure on microbial cells and discovered that it affects the structure and function of enzymes, cell proteins, and damages bacterial cell function.(Fumal et al.) demonstrated that with povidone iodine, venous ulcers is healed sooner.(S.Karger AG et al.2017) conducted some human studies and found the efficacy of povidone iodine in healing bacteria in acute and chronic wounds. Eming SA et al.J stated in his work that higher concentration of povidone iodine reduce the activity of elastase and plasmin in wound infections.Recently Iodine is widely used in biomedical applications as Eggers et al. demonstrated that povidone iodine inactivate both MERS-CoV and Severe acute respiratory syndrome coronavirus 1(SARS-COV-1).

CHAPTER-3

AIM AND SCOPE

3.1 AIM

To synthesis nonporous PLGA sheet to prepare a bandage coated with povidone iodine to prevent wound infection, to check the antimicrobial efficacy, biocompatibility and to reproduce the method of fabrication and bandages for large scale.

3.2 OBJECTIVE

- To synthesise nan porous PLGA sheet
- To prepare a bandage coated with povidone-iodine
- To check antimicrobial efficiency
- To check biocompatibility
- To prevent wound infection
- To reproduce the method of fabrication and bandage for large scale

CHAPTER-4

MATERIALS AND METHOD

4.1 MATERIALS

CHEMICAL USED

Poly-d, l-lactide-co-glycolide (PLGA) 50:50 with mw 30000–60000 da and glacial acetic acid 100% purchased from Sigma Aldrich, USA. Dichloromethane (DCM) poly (ethelene glycol) PEG mw 4000, chitosan and PVP - I, purchased from sisco research laboratory.

EQUIPMENTS USED

- Magnetic stirrer
- Centrifuge
- FTIR
- Stereo Zoom microscope
- SEM

4.2 SYNTHESIS OF NANO POROUS SHEET USING POVIODINE- IODINE INCORPORATED WITH PLGA

The NPs PLGA sheet is prepared using particulate leaching and solvent casting method. The method was carried out by dissolving 50 mg PLGA in 1 ml of dichloromethane. To the mixture, 8.3mg of PEG is added. The ratio of PEG & PLGA tested was 0.17 by weight. To the above mixture 50 mg chitosan is added to it, 1% CH₃COOH (10µm:1ml H₂O) is also added in order to disperse chitosan in the entire solution.

Now the solution is stirred up at 700-800 rpm with the help of a magnetic stirrer at kept for 24 hrs. In order to get a fine mixture of the solution the solutions undergo vortexing and is ready for casting.

4.3 CASTING OF NPS SHEET

A stainless-steel ring with inner diameter of 40 mm pasted over a glass protector. The ring is kept aside overnight. Now to the ring, prepared solution mixture is added in drops and evenly spread and kept in air dry condition for 48 hrs. After drying sheet is ready to scraped out. The sheet is removed with the help of surgical or razor blade. The scrapped samples are stored in cool and dry conditions for characterization and further analysis. The scraped sheet is soaked in deionized water overnight to check its biodegradability. Then the sheet is viewed under microscope to check its nano porosity.

4.4 MICROSCOPIC ANALYSIS

Initial microscopic analysis had been conducted using Automated stereo microscope (Leica, Germany). Further SEM analysis is utilized for detailed imaging. The high magnification, high-resolution imaging of our SEM analysis supports the determination of the number, size, and morphology of small particles. The sample was analysed using FEI Quanta FEG 200-High Resolution Scanning Electron Microscope.

4.5 ISOLATION OF MICROBES

Isolation of bacteria had been done by obtaining the isolates from an open wound infection. The wound area was rubbed using the swab and prepared a spread plate using bacterial nutrient agar. 8 g of Agar-agar and 3.9 g of Nutrient Broth is taken in 500ml of conical flask and dissolved in 300 ml of distilled water. Then it is sealed and kept for autoclave. Sterile the media and plates in Laminar Air Flow then the prepared media is poured in plates and allowed to solidify under UV for 15 minutes. On to the media the sample was allowed to grow. The bacterial plates had been incubated for 24 hours at 37°C. Later separately the bacteria from each sample were streaked over another fresh media over isolation. Plates were streaked and placed in an incubator for 24 hours to allow bacteria to proliferate. From the spread plate, distinctive colonies have been isolated and transferred to separate bacterial nutrient agar plates and prepared streak plates.

4.5.1 GRAM STAINING FOR IDENTIFYING THE ISOLATES

The bacterial culture is smeared, air dried, and heat fixed in a clean glass slide. Few drops of crystal violet stain are added to the smear for 60 seconds before being washed with distilled water to eliminate excess strain. Second, a few drops of gram's iodine is added for 60 seconds and rinsed with deionized water. The smear was then decolorized for a few seconds with 95 percent alcohol before being rinsed with deionized water. Finally, the smear is covered in safranin for 60 seconds before being cleaned and allowed to air dry. The morphology of cells is evaluated microscopically using a 40x and 100x objective on the smear.

4.6 ANTIBIOTICS SENSITIVITY TEST

The disc diffusion method is commonly used technique for determining the susceptibility of the organisms. This is highly standardized technique that allows the drug to diffuse in the surrounding medium. The concentration of the drug is higher near the disc and decrease with distance. The zone of inhibition exists the organism is sensitive to given antibiotics if there is no zone of inhibition the organisms is resistance to the given antibiotics.

5g of Mueller Hinton Broth and 7g of Agar-Agar is dissolved in 250 ml deionized water and sterilized under 121°C for 45 min in 15 lbs. The wound sample is spread over the sterilised media and a paper disc impregnated with known amount of antibiotics is placed on the surface of inoculated plates. It is kept in incubator for 24 hrs. After incubation plates were observed for zone of inhibition. The diameter of zone of inhibition were measured and compared with the standard chart and tabulated.

4.7 ANTIBACTERIAL ACTIVITY ANALYSIS

The synthesized nano porous sheet was analysed for antibacterial activity. 5g of Mueller Hinton Broth and 7g of Agar-Agar is dissolved in 250 ml deionized water and sterilized under 121°C for 45 min in 15 lbs. The plates have been prepared and 3-gram positive bacteria isolated was spread into it. The sheet has been made

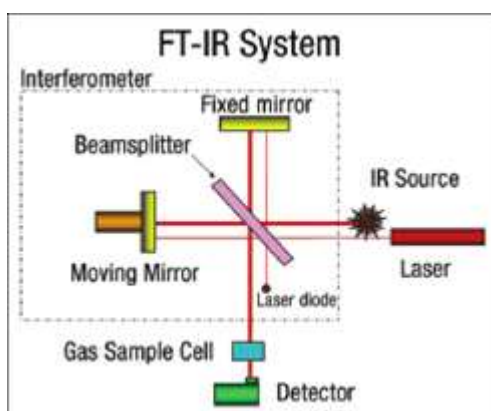
into small uniform pieces (0.5 cm x 1 cm) and immersed for 10 minutes in Povidone solution having 3 different concentrations (2 % w/v, 1 % w/v, 0.5 % w/v, and 0.25 % w/v). After drying in sterile conditions these sheets had been placed on the inoculated plates and incubated for 24 hours in 37°C. The zone of inhibition was checked after 24 hours.

4.8 CHARACTERIZATION

Generally the main parameters like size and shape can be determined by various characterization techniques due to their specificity. Characterization study can be majorly done to identify the structure and properties of the material that are less than 100nm in size. The major characterization studies include various optical radiation, microscopic studies, mechanical testing, thermal analysis, density calculation, and various spectroscopic techniques. It is the basic process in identifying the nature of the material and from that further study can be analyzed.

4.8.1 FTIR (Fourier Transform Infrared Spectroscopy)

FTIR (Fourier Transform Infrared Spectroscopy) is a measurement technique in which it measures the range of wavelengths in the infrared region that are absorbed by a material (Aging and Munajad, 2018) (Ismail, van de Voort and Sedman, 1997) . The principle of FTIR is clearly explained in Fig.



Through FTIR any kind of unknown materials, quality of the sample and also the

amount of components in a particular molecule can be determined. Any kind of samples can be determined through this method. Dry sample can give out exact results rather than other two kinds. This kind of analysis is done to identify the composition of the molecules and also its structure by analyzing the fingerprint region. The region to the right-hand side of the diagram (from 1650 to 500 cm^{-1}) usually contains a complicated series of absorption and this region will help in determining the specific functional group of the compounds. These kind of functional groups can be determined by the above table 4.3. The frequency of the infrared radiation matches the natural frequency of the bond, the amplitude of the vibration increases, and the infrared is absorbed.

4.8.2 SEM

In scanning electron microscopy the image of surface of sample is determined by scanning the surface with electron beam of accelerating voltage ≤ 50 kilo Volt. It uses a focused electron probe to extract structural and chemical information point- by-point from a region of interest in the sample. The high spatial resolution of a SEM makes it a powerful tool to characterize a wide range of specimens at the nanometer to micrometer length scales. The following category are the information that are obtained from SEM as follows:

- **Topography**
The surface features of a molecule.
- **Morphology**
The shape and size of the molecule. (Strength, shape).
- **Composition**
The elements and compounds that the molecule is composed of and the relative among them
- **Crystallography**
The arrangement of atoms in the molecule.

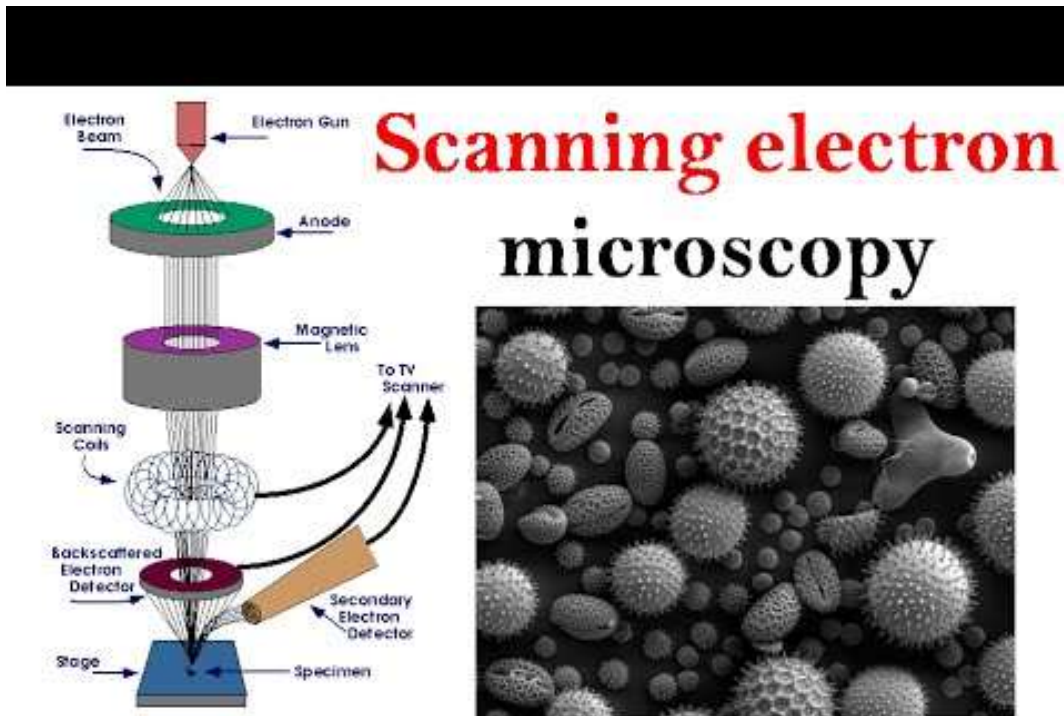


Fig: 4.8.2

Scanning electron microscopy

The signals used by a scanning electron microscope to produce an image result from interactions of the electron beam with atoms at various depths within the sample. Various types of signals are produced including secondary electrons (SE), reflected or Back-scattered electrons (BSE). These in turn determine the morphology of the molecules. Dry sample is always preferred for this kind of analysis(Mourdikoudis,2018).

CHAPTER-5

RESULT AND DISCUSSION

5.1 FOURIER TRANSFORM INFRARED SPECTROSCOPE

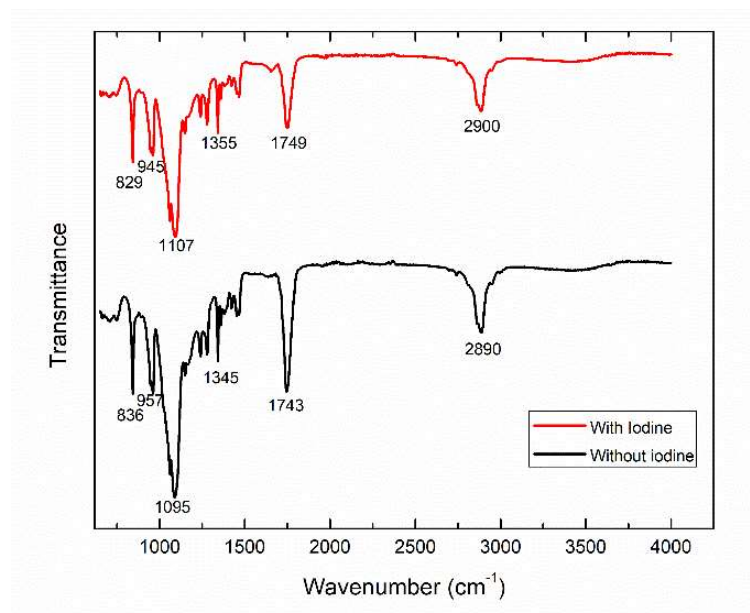


Fig:5.1.1

FTIR images showing functional group of the particular molecule

From the obtained result it is confirmed that the prepared solution was PLGA. Each and every peak in the result determine the functional group of the particular molecule. With the graphs obtained we can infer that the bonds created without iodine coat on the sheets are similarly found when the sheet is coated with iodine. There is no new bonds observed when coated with iodine, only the peak slightly varies whereas the bonds remain the same.

5.2 MICROSCOPIC ANALYSIS

Preliminary analysis of the synthesized nano-porous sheet has been performed using automated stereo microscope and the results have been recorded. The images showed minute porous structures and well-integrated sheet structure. Followed by the analysis using stereo microscope, Scanning Electron microscope is employed to get the accurate morphology of the material. The results showed successful synthesis of sheets with cavities all over the surface.

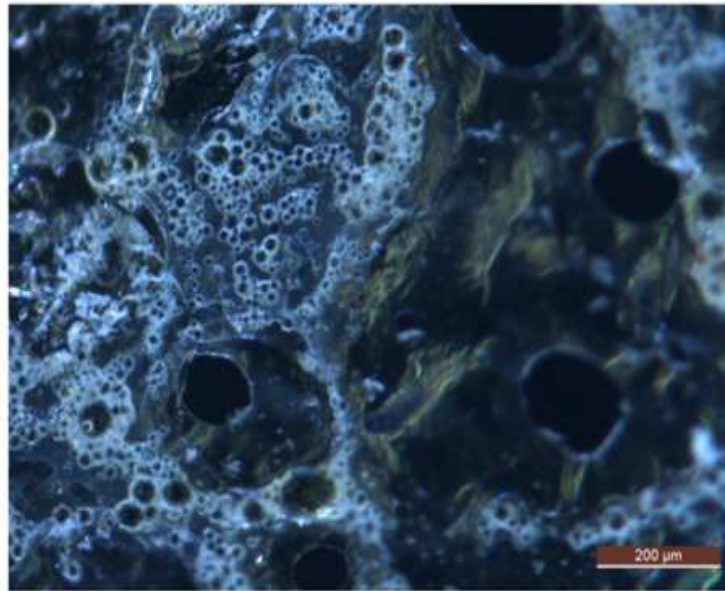


Figure 5.2.1

Automated stereo microscope images of the synthesised PLGA sheets

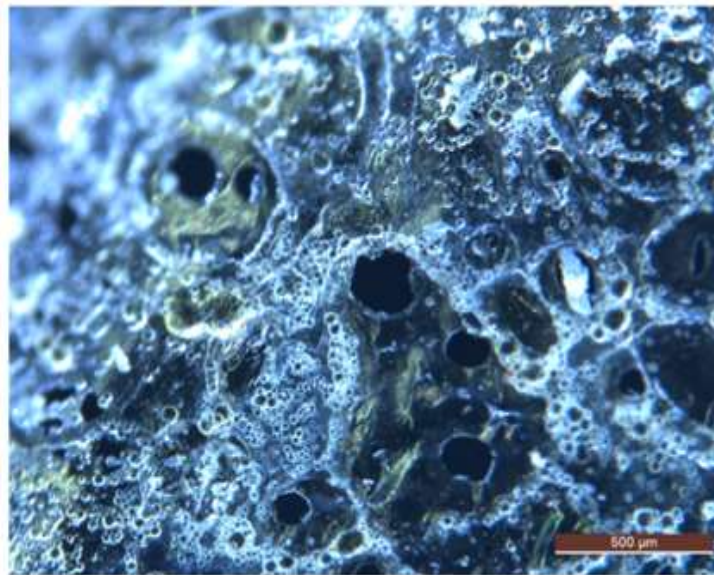


Figure 5.2.2

Automated stereo microscope images of the synthesised PLGA sheets

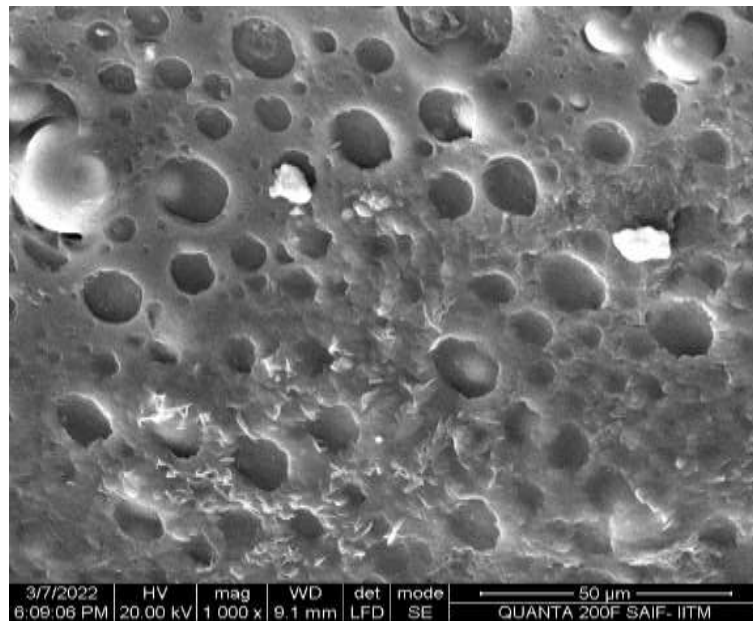


FIGURE 5.2.3

SEM images at 50μm

showing the porous nature of the PLGA sheet upon synthesis

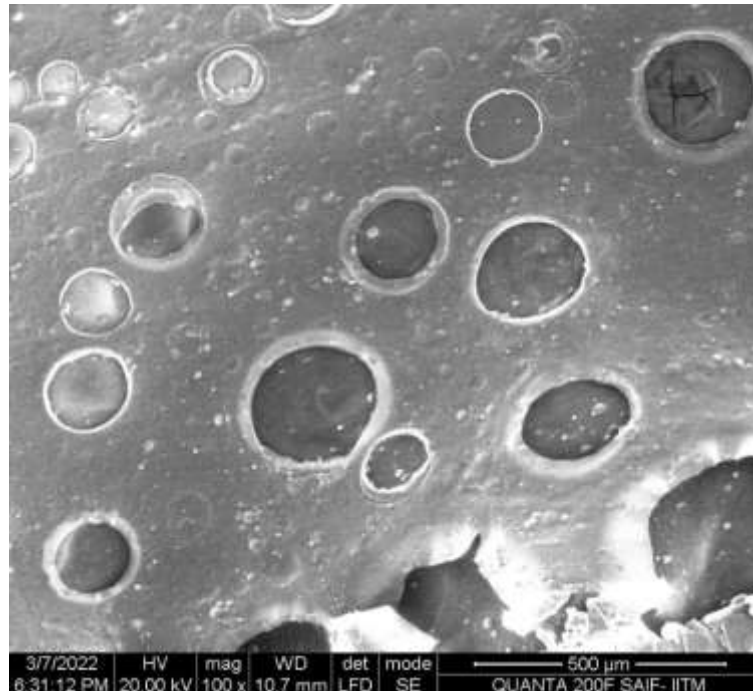


Fig:5.2.4

SEM images at 500μm

showing the porous nature of the PLGA sheet upon synthesis

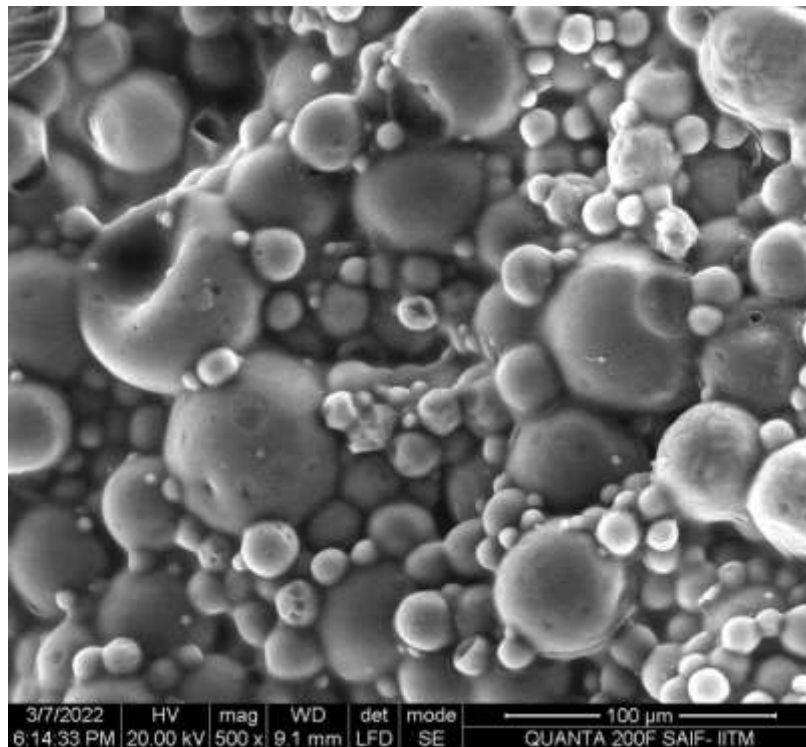


Fig:5.2.5

SEM images at 100µm showing the pores in the spheres obtained from the PLGA sheet upon synthesis

The above figures 5.2.3,5.2.4,5.2.5 shows the SEM images of PLGA at different magnification and also with clear resolution and the size is determined as spherical.

5.3 ISOLATION AND IDENTIFICATION OF BACTERIAL SAMPLES

Isolation of bacterium from wound has been conducted. Primary spread plate showed different colonies after 24 hours of incubation (**Figure 5.3.1**). According to the colony morphology, 6 distinctive colonies have been isolated. The isolated 6 strains had been transferred to nutrient plates by streaking (**Figure 5.3.2**). The isolates have been characterised using gram staining technique. On characterisation it was found that 3 bacteria (w1,w2 and w3) are cocci shaped and gram positive (**Figure 5.3.3**).



Figure 5.3.1

Spread plate showing multiple colonies of bacteria with different morphologies



Figure 5.3.2.

From the spread plate W₁ & W₂ , distinct colonies have been streaked and obtained.



Figure 5.3.3.

From the spread plate W_3 & S_5 , distinct colonies have been streaked and obtained.

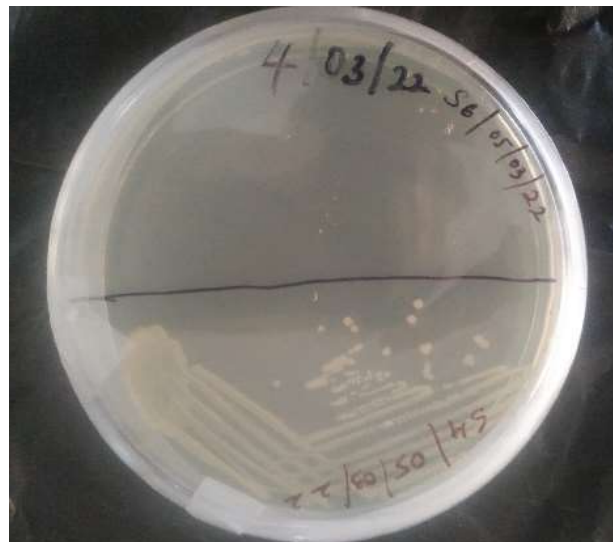


Fig:5.3.4

From the spread plate S_4 & S_6 , distinct colonies have been streaked and obtained.

5.4 ANTIBACTERIAL SENSITIVITY TEST

To analysis and identify the bacteria from the isolates, antibacterial sensitivity test had been conducted. Antibacterial sensitivity test has been conducted for narrow down the identification bacteria isolated. According to the sensitivity and resistance of the bacterial strain with the antibacterial disc, it is possible to find out the genus of the bacteria. The results are depicted below,

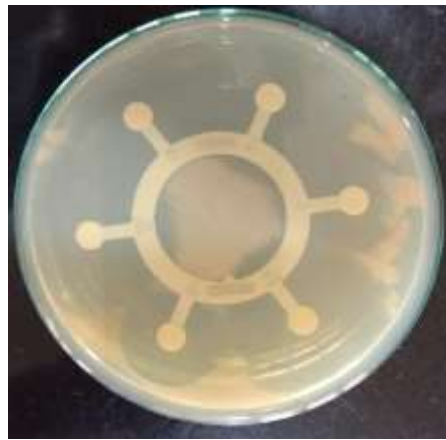


Figure 5.4.1.

Antibiotic sensitivity analysis a) sample W2



Figure 5.4.2

Antibiotic sensitivity analysis b) Sample S4



Figure 5.4.3
Antibiotic sensitivity analysis c) Sample S5

ANTIBIOTIC SENSITIVITY ANALYSIS

Sl. No	Sample	Test Antibiotic	Zone of inhibition (mm)	Resistant/Sensitive
1	W2	PENICILIN (P)	15mm	Sensitive
		OXACILIN (OX)	15mm	Sensitive
		CEPHALOTHIN (CEP)	15mm	Sensitive
		CLINDAMYCIN (CD)	9mm	Sensitive
		ERYTHROMYCIN (E)	7mm	Sensitive
		AMOXICILIN (AMC)	14mm	Sensitive
2	S4	PENICILIN (P)	6mm	Sensitive
		OXACILIN (OX)	4mm	Sensitive
		CEPHALOTHIN (CEP)	8mm	Sensitive

		CLINDAMYCIN (CD)	-	Resistant
		ERYTHROMYCIN (E)	-	Resistant
		AMOXICILIN (AMC)	6mm	Sensitive
3	S5	PENICILIN (P)	4mm	Sensitive
		OXACILIN (OX)	2mm	Sensitive
		CEPHALOTHIN (CEP)	4mm	Sensitive
		CLINDAMYCIN (CD)	-	Resistant
		ERYTHROMYCIN (E)	7mm	Sensitive
		AMOXICILIN (AMC)	5mm	Sensitive

Further the analysis and specific identification of bacteria are to be analysed and confirmed.

5.5 ANTIBACTERIAL ASSAY USING THE PLGA SHEETS

The synthesized sheets had been tested for antibacterial activity using the isolated bacteria. After 24 hours of incubation the sheets with different concentration of Povidone have been checked for the antibacterial activity. A clear inhibition zone was visible (Figure 5.4.1) after 24 hours for W2 and W3 samples and W1 bacteria invaded the PLGA sheets. All 4 concentrations of the povidone showed mild inhibition zone during the analysis.



Fig:5.5.1

Sample W1 not showing sensitivity to the PLGA sheet



Sample W2 showing mild inhibition zone of 1 mm on all 4 concentrations



Fig:5.5.3

Sample W3 showing mild inhibition zone of 1 mm on all 4 concentrations.

CHAPTER-6

SUMMARY AND CONCLUSION

CONCLUSIONS

In the present study, PLGA nano porous sheet had been synthesised and incorporated with chitosan for the preparation of healing aid on subcutaneous wounds. As an antibacterial agent, commercially available povidone is used and showed the positive result of resisting bacterial growth on the sheet. Biodegradability of the material was also tested and found that 48 hours of exposure to water degraded the material. The study is underway to find out interaction of sheets and bacteria, also the growth capability of cells onto the biomaterial and adherence to it.

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