***Asparagus racemosus* AND *Matricaria recutita* INFUSED ELECTRO-SPUN NANO-FIBERS TOWARDS WOUND HEALING APPLICATION**

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**ABSTRACT:**

In order to enhance regeneration of wound healing in human bodies, we must optimise the encapsulated poly (e-caprolactone)/poly ethylene glycol nanofiber membrane creation by electro-spinning techniques. During polymerization, plant extracts from *Matricaria recutita and Asparagus racemosus* must be introduced, and each plant extract can be changed at one of two potential PCL ratios: PEG: CHAMOMILE: CHLOROFORM=0.88:0.1:0.02:10, 0.86:0.1:0.04:10. This procedure was carried out in a rotatory evaporator to separate the ethanol content in the sample preparation solution and reach the right concentration. The concentrated sample was then electro-spun in order to create membranes. Fourier Transform Infrared, Scanning Electron Microscope, Contact angle, and X-ray diffraction spectroscopy have all been used to examine the membrane creation.The physiological and morphological properties of PCL components in raw samples have significantly varied using this instrumentation technique when compared to the extraction mixed compound. According to recent research, zebra fish share between 74% and 90% of the genes in the human genome and can be used to study toxicity, external appearance, and experimental development tolerance. Cell lines were used in *in-vitro* studies to examine bacterial pathogenesis and antimicrobial defense.

**Keywords:** polycaprolactone, electro-spinning, *Asparagus racemosus*, *Matricaria recutita,* membrane formation, toxicity, and in-vitro studies

**INTRODUCTION:**

Wound healing is a process of regeneration, development, and rapid growth of cells by cell elongation, cell proliferation, and cell adhesion in the affected place of the body. In anciperiodsriod, the wounds were tailored with herbal medicinal value plants and their extracts prevent infections. These are the plants that possess antioxidant, anti-inflammatory, anti-diabetic and, biocompatible with the infected body. The advancement of tailored wounds by the patches of nanofiber membrane through electrospinning technique **[1].**

The membrane patches depend on their flexibility, biodegradability, complexity, porosity, and size of their nature.Wound-healing membrane patches were made up of many compounds of antibiotics, nanomaterials, and plant extract incorporated into the polymer solution for the regeneration of wounds. Mostly nanomaterials like TiO2, silica, gold, synthetic amorphous silica, Fe2O3, etc. The gold particles (AuNPs) were mixed with the chitosan then only possess high antibacterial and antioxidant properties in a high ratio **[2]**.Another technology was a 3D bioprinting patch which can be used to fabricate the individual patches depending on their size and shape of characterized damaged tissue. In the bioprinting technique, gelatine methacrylate (GelMA) and hyaluronic acid methacryloyl (HAMA) possessed better biocompatibility and bio-degradable in nature and could be to produce bio-printed patches **[3].**

Many achievements have been done in the past years using polymers for the production of nanofiber membranes through the electro-spun technique. The membrane-formed polymers are PCL, PEG, polystyrene, polyurethane, chitosan/P(LLA-CL) nanofibers **[4]**, and chitosan/PLA blend micro/nanofibers **[5]**. Poly (lactic acid) (PLA) **[6]**, cellulose/chitosan hybrid nanofibers **[7]**, PET/chitosan nanofibrous mats **[8]**, poly (vinyl alcohol)/chitosan blend nanofibers **[9].**

These are the polymers that have been widely used for nanofiber membrane formation to heal wounds in the body with a high rate of regeneration. Moreover, PCL and PEG-6000 polymers are the two synthetic polymers of commercially available in markets and polycaprolactone is an aliphatic biodegradable polymer and drug delivery biomaterial in mentioned part of the body. PCL polymers are owned by the capability of fabrication of a variety of structures, forms, and high thermal stability at relatively low cost. Due to its enormous character, it could be easy to form a filament with a three-dimensional structure of scaffolds by spinning method while membrane sized with nanometre to the millimeter with detailed studied **[10].** The PEG-6000 polymer is a white color and available in the market as a form of solid flakes or powder and the melting range in between 58-63℃. The PEG-6000 polymer owned biocompatibility, bioconjugation, and drug delivery the biomedical applications. By the compliance of PEG with plant extract it can be directly conjugated to the drugs or with the surface of the nanofiber membrane **[11].** Furthermore, PEG used for the nanomaterials coating, is usually for improving the effectiveness of drugs and also drug delivery to target cells and tissues**.**

Nanofiber membrane was prepared by several methods of bubbfil spinning, centrifugal spinning, freeze drying and electro spinning. Recently, the reason for its simplicity, scalability, cost-effectiveness, and versatility, electrospinning is the most widely used technique for creating nanofibers. These electros spun nanofibers have a wide range of uses, including filtration, wound-dressing materials, protective fabrics, tissue scaffolds, and biomedical devices. They outperform conventional antimicrobial materials in terms of antimicrobial effectiveness **[12].** The physicochemical properties of the nanofibers might be improved by choosing appropriate basic materials to increase porosity and endurance. Nanofibers' low crystallinity, irregular alignment, and orientation contribute to their relatively low mechanical strength. The construction of a nonwoven or membrane structure as a result of layering and thickness increments improves the functions of the nanofibers **[13].**

Chamomile is locally known as Samanthi, and its botanical name is *Matricaria recutita.* Chamomile plants have several medical and therapeutic applications wound healing process **[14].** Terpenoids, flavonoids, and lactones, among other biomolecular components, are demonstrated in the chamomile plant. Compounds called apigenin and matricin are anti-bacterial, anti-inflammatory, antioxidant, and neuroprotective, and they support chamomile's activity in the process of healing wounds **[15]**. *Asparagus racemosus* commonly known as Shatavari is a plant that has been used in traditional natural medicine for its various health benefits **[16]**. Many phytochemical substances, including steroid saponin, carbohydrates, flavonoids, is flavones, and furan compounds, as well as anti-microbial, anti-diabetic, anti-cancerous, and anti-depressant qualities, make up this plant. Health-promoting substances such saponins, polyphenols, ascorbic acid, and flavonoids will become more prevalent **[17].**

From the information, most of the polymers are incorporated with nanomaterials, antibiotic drugs with the addition of chitosan for increase the ability of antimicrobial wound-healing patches. So that, in these studies to compare and characterize the nanofibers formed by incorporating a plant extract of *M. chamomile* and *A. racemosus* for developing a wound-healing process with antimicrobial activity.

**2. MATERIALS AND METHODS:**

**2.1 MATERIALS**

The nanofiber membrane was prepared by electro spinning technique and the precursors used were ethanol (C2H6O, MERCK), chloroform (CHCl3, Sigma-Aldrich), poly ethylene glycol (PEG)-6000 (MW: 5000-7000, Sisco Research Laboratories Pvt. Ltd), Polycaprolactone (PCL) ((C6H10O2) n, MW: 80000, Sigma-Aldrich))

**2.2 PREPARATION OF PLANT EXTRACT**

The *Matricaria recutita* and *Asparagus racemosus* sampleswere commercially bought in the local areas shop. The *M. recutita* and *A. racemosus* were made into a dried and fine powder separately. The powders were stored at 4℃ until use. The 10 gm of the ground powder of each plant was treated with 50 ml of ethanol separately and stirred for 2 h at 400 rpm. The crude extracts were filtered using Whatman filter paper. The filtered extract was further transferred to rotary vacuum evaporator (Evator) and the solvent was evaporated. The slurry of the plant extracts was collected and stored at 4 ℃ **[18].**

**2.3 PREPARATION OF ELECTROSPINNING SOLUTION**

Preparation of the solutions

The herbal extracts were individually loaded in PCL and PEG solutions in 10ml of chloroform stirred for 2 h as mentioned in the Table 1. The PCL and PEG solution without herbal extract acted as the control nanofibers **[19]**. The codes for the prepare nanofibers were nomenclated and mentioned in the Table 1.

**2.3 FABRICATION OF NANOFIBER MEMBRANES**

The nanofiber membrane was obtained for each concentrated polymer solution by using electro spun (HOLMARC OPTO-MECHATRONICS LTD, MODEL NO: HO-NFES-040B) technique and maintained a desired flow rate, applied voltage, the distance between the mandrel collector and the syringe loaded by the polymer solution **[20].**

The control nanofiber membrane was fabricated by loaded membrane solution in syringe then make a distance between the syringe and the mandrel up to 11.5cms. The electric voltage was slowly raised while the polymer solution was completely attracted towards the mandrel. Remarkably, the flow rate was kept at 0.03µl/min and voltage maintained at 10.2V and programmed for 1hour.The higher concentration of *M. recutita* polymer solution was loaded in a syringe and applied electric current as similar as procedure to the initially established nanofiber scaffolds. But it could be varied at applied voltage of 10.4V, distance maintained up to 11cms, duration of program could be 1hr and flow rate kept at 0.03ml/min. The lower concentration of *M. recutita* polymer solution was loaded in a syringe and applied electric current as similar as procedure to the initially established nanofiber scaffolds. But it could be varied at applied voltage of 12.4V, distance maintained up to 11cms, duration of program could be 1.15hrs and flow rate kept at 0.5µl/min. The higher concentration of *A. racemosus* polymer solution was loaded in a syringe and applied electric current as similar as procedure to the initially established nanofiber scaffolds. But it could be varied at applied voltage of 11.8V, distance maintained up to 11cms, duration of program could be 1hr and flow rate kept at 0.04µl/min. The lower concentration of *A. racemosus* polymer solution was loaded in a syringe and applied electric current as similar as procedure to the initially established nanofiber scaffolds. But it could be varied at applied voltage of 12.8V, distance maintained up to 11cms, duration of program could be 1hr and flow rate kept at 0.6µl/min **[21].**

**Characterization of scaffolds:**

**3.1 FTIR (FOURIER TRANSFORM INFRARED)**

The bond formation between the chemical, polymers and plant extract could be determined through the FTIR (ALPHA II-FTIR, BRUKER) spectrometer in the range of 500cm-1 to 4000cm-1 declared the extract was completely blend with the polymer solution. In this FTIR 64 scans was preformed to get the clear peak level **[22].**

**3.2 XRD ANALYSIS**

The prepared composite could be crystallinity in nature was confirmed by X-ray diffraction (D8 ADVANCE, BRUKER). A copper or aluminium filter is added and this is used to declare the extract contains the identification of materials based on their crystallinity of the membrane and their amorphous nature **[23].**

**3.3 SEM (scanning electron microscope)**

The morphology of the nanofiber mats (or) membrane was observed using scanning electron microscope (SEM) It can be determined by SEM (Jeol JSM IT 800) a thin membrane in the basis of SEM images the average diameter of the membranes was detected through on it and the membrane structures were obtained **[24].**

**3.4 Contact angle analysis**

Contact angle can be measured using the water droplets that can be presented in the membrane the liquid droplet that wet the surface of the membrane for the analysis and it can be determined by contact analyser (Ossila Goniometer) is used to determine the membrane gets hydrophilic or hydrophobic nature are analysed **[25].**

**4. BIOCOMPATIBILITY**

**4.1 CELL LINES STUDY:**

The cell lines study was performed in the cancer cells for the development of cell in the membrane. The cells were maintained KB, Cancer cell line was used to determine the cell Cytotoxicity activity obtained from National Centre for Cell Science, Pune, India (NCSS). The cells were maintained in Minimal Essential Media supplemented with 10% FBS, penicillin (100 U/ml), and streptomycin (100µg/ml) in a 5% CO2 at 370 c. Cells were seeded at the different membranes and incubated for 24h. After that, the medium was removed and washed with the phosphate saline solution. Then the sample was placed in a new medium containing 50ul of MTT solution (5mg/ml), to each well incubated for 4h. After the incubation, DMSO was added. The viable cells were determined by the absorbance at 570nm by a microplate reader. Doxorubicin was used as a control **[26].**

**4.2 HEMOCOMPATIBILITY:**

Blood compatibility assessment was estimated to understand the compatibility of the nano-fibers membrane. Collected blood was retained it using EDTA (ethylene di amine tetra acetic acid) to avoid coagulation. Erythrocytes was centrifuged for 10 min (4 °C), then washed 2 to 3 times using phosphate buffer saline (PBS at pH 7.4) to procure pure RBCs with extra blood components. Blood compatibility assay was performed to estimate the lytic behaviour of cells in the presence of nano-fibers compared to negative and positive controls, respectively. All the test samples were analyzed with their triplicates (n = 3). The assessment was performed with the incubated duration of 1 h (37 °C). Lastly, the samples were centrifuged and the absorbance was note at 540 nm **[27,28]** to quantify the hemolysis (%) formula mentioned below.

Sample reference-Negative control

Hemolysis (%) = X 100 **[29]**.

Positive control -Negative control

**4.3 ANTIBACTERIAL ACTIVITY:**

Plant extract that has been synthesized and a nanofiber membrane that incorporates plant extract have been performed for the antibacterial action. By using BHI media (Brain Heart Infusion Agar) and MHA (Muller Hinton Agar) plates by the agar well-diffusion technique, these membranes and extracts were tested against Gram-positive bacteria of Streptococcus mutans (SM) with antibiotic streptomycin and Gram-negative bacteria of Escherichia coli (E.coli) with antibiotic amikacin. Both MHA and BHI were produced separately in double distilled water (pH 7.0) and sterilized in an autoclave at 121 C for 15 min. The sterilized MHA and BHI were then poured into the per plate and allowed to harden at room temperature in laminar flow sterile cotton swab dampened with the suspension of the appropriate microbial culture was used to apply an inoculum comprising 106 cfu/mL of the freshly made bacterial culture to the MHA and BHI plates. With the aid of a micropipette, four wells (9 mm in diameter) were then drilled into the MHA and BHI medium for the plant extract, and they were then filled with various concentrations (100µl, 50µl, and 25µl) of the plant extract compound. For the nanofiber membrane, three pieces (22 mm in diameter) were then placed on the plate and left at room temperature for 4 hours to allow the extracted compound to diffuse into the medium. The culture plates were then incubated for 24 hours at 37 °C. Following incubation, each plate's zone of inhibition's diameter (mm) was noted. The results were expressed as mean values with standard deviation (SD) of a triplicate experiment **[30].**

**4.4 TOXICITY**

*M. recutita* and *A. racemosus* nanofiber membranes were used to test the adult zebrafish embryos for in-vitro toxicity. To determine the death rate in zebrafish over a defined period of time, different concentrations of membranes were treated and compared to untreated embryos (control). According to OECD-203 criteria, 25 distinct zebrafish embryos were examined for toxicity at various membrane concentrations (25, 50, 75, 100, and 200 g/ml). The eggs were then moved so that they would separate clearly so that the development of the head, tail, and eyes could be seen at a 40X magnification every 24 hours. The temperature of the water was maintained constant during the assessment. The dead and live fishes were recorded for every 24 h time interval to avoid contamination in the solution (SAHIB). The death rate of hatched embryo was evaluated for 24 h **[31,32]**

**CONCLUSION:**

The plant extracts of *Asparagus racemosus* and *Matricaria recutita* infused with the PCL/PEG 6000 polymer solution successfully prepared the nanofiber membrane through an electrospinning technique. This experimental data showed that the prepared nanofiber membrane has a high porosity, stability, biocompatibility, and degradability in nature. XRD and FTIR described that the membrane had a crystallinity and mostly strong bond formation with each nanofiber so that the strength of membrane rigidity. The hemocompatibility of the membrane was increased by the plant extract and also extract was equally mixed on the membrane without forming a bead formation. By the help of antioxidant activity radical present in the sample could help to accelerate the flavonoid compounds for healing wounds. From the data, the polymer and plant extract revealed enhanced properties on the antibacterial and biocompatibility on wound healing mechanism.

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