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Review Article

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Nano-biopolymeric fibers in wound healing

Anjali, Devegowda Vishakante Gowda* and Atul Srivastava

Dept. of Pharmaceutics, JSS University, JSS College of Pharmacy, SS Nagara, Mysore, Karnataka, India

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ABSTRACT

The wound healing is one of a dynamic process, which aims to re-establish the damage tissues by the process known as cytokines, macrophages and fibroblasts. The use of various bio polymeric fibers such as chitin and chitosan, alginic acid, hyaluronic acid, cellulose etc. are used for their application in soft tissue engineering, vascular tissue engineering and neural engineering. These biopolymeric fibers are worth considered as it results in a completely biodegradable composite and ensure minimum disturbance of the wound to avoid further tissue damage. These fibers are used for wound healing because of their properties like good fluid uptake for absorption of excess of exudate and blood at wound site, provide moist wound environment, better gaseous exchange and prevent from infection from microorganisms and can modify to mimic the skin environment, conditions and structure. The biopolymeric fibers enhanced wound healing by modulation of various cells, cytokines and growth factors during different phases of healing process like inflammatory, proliferative and remodeling phase. Various fabrication methods used for the preparation of fibers includes electrospinning, phase separation, self-assembly, wet spinning and template synthesis method. This review outlines the process involved in the preparation of fibers, its characterization, process involved in skin regeneration and care required for wound treatment.

Keywords: Biopolymeric fibers, wound healing, tissue engineering**.**

INTRODUCTION

Wound may be defined as the loss of continuity of epithelium tissue, along with loss of connective tissue [1]. Injury can be arise from any physical trauma which result in damage of skin layer. The skin shows an innate ability to restore cell integrity in the affected area. The healing process occur by three process they are generally inflammatory, proliferative and remodeling phase [2]. In order to heal the wound many dosage form are used namely cotton gauze, films, wafer, fibers, hydrocolloids, hydrogels, among this the fibers used commonly because of if its lots of advantages over other dosage form for healing the wound. These fibers are made up of fibers and hence called as biopolymeric fibers. Biopolymeric fibers are the class of materials which are generally continuous filaments or they are in discrete elongated piece which is similar like the thread. These fibers show great importance in holding tissue together which in turn help in wound management i.e for both acute as well as chronic. Wound healing is one of the multifunctional, complicated process. A variety of polymeric fibres such as neutral (cellulose), basic (chitin & chitosan), acidic(alginic acid & hyaluronic acid)and sulfated polysaccharides(heparin, chondroitin, dermatan and keratin sulfate) are used for wound care application [3].

In recent days nanofibers are emerging for their wound healing properties.Nanofibers are having diameter less than 1µm, due to their small scale in diameter , nanofibrous materials have a very high surface volume ratio, which makes them very good candidates for the delivery of bioactive agents as well as the tissue engineering

matrices.Nanofibrous materials promotes cells attachment , migration, proliferation and differentiation of the damaged tissue. These nanofibers are having wide application in scaffolding, biomedical application and in drug delivery system [6, 13, 17, 19, 33]. The use of these fibers for wound healing is because of its properties to degrade itself and they are non-toxic in nature. Trauma can be protected by this fibers because these are having low adherence properties [4].There is an emerging need for developing biopolymer based composites capable of promoting cellular proliferation and reconstituting the extra cellular matrix [5].

Rationale for selecting fibers as wound healing

These fibers are used to fabricate both dry as well as moist wound. These fibers are hemostatic, having good absorbent properties and are suitable for full thickness wound. The frequency for changing dressing is less and provides gelation and moist healing which is suitable for any type of wound whether it is resulting acute or chronic conditions. These biopolymeric fibers generate *in situ* moist healing environment and the consequent high absorbency and de-bridgement of wound site [3]. Due to its low adherent property on to the skin surface for any type of tissue, it provides better gaseous exchange which is preventing from bacterial infections. The advantage of nanofibers used in wound healing, as it absorb excess of exudate and blood at wound site. Moreover, they behave as thermal insulator.

VARIOUS METHODS USED IN THE SYNTHESIS OF NANO-FIBERS

Electrospinning method – This is one of the important method used for manufacturing of fibers. This is to prepare nanofibers. Fibers whose diameter is in the nanoscale (<1000nm) or microscale (>1 um) range can be prepared .This method need;-A syringe pump, a high voltage source and a collector. In this method a polymer solution or melt is charged with an electric field of high voltage. This produces charge carrier with the same polarity as that of electric field is applied. The electrostatic repulsion occur because of accumulation of similar charges which pull the polymer solution or melt forward in a cone – like shape. When the electric field voltage is high enough to overcome the surface tension of the polymer, a fiber like structure occurs at a collector plate. The collector screen of opposite charge collects these fibers [figure 1]. The electrospinnability of a polymer solution is depend on various of parameters like viscosity, conductivity, and solvents used, as well as the physical and chemical structure and molecular weight of the polymer used. Many of the polymers is not spinnable because of their solubility problem in a suitable solvent for electrospinning, having proper polar characteristics [26, 27]. To hindering this problem, an unspinnable polymer can be used for preparation of nanofibers by technique co-spinning with a spinnable polymer solution, Different structures of nanofiber, such as coreshell, bicomponent, hollow and porous, could be produced by using different spinnerets. Ideal nanofibers can be produced by controlling certain parameters like nanofiber body size, mass of the fibers and content of the polymeric solution.

Coreshell nanofibers: In these kinds of nanofiber, the exterior layer may include active agents for imparting functional properties, such as shells holding immobilized/migratable specific enzymes [36]. [Figure 2]

Hallow nanofibers: Hollow nanofibers are applicable for innovative and very specific usages, such as forNano fluidics and hydrogen storage purpose. Usually, two different methods are used such as Chemical Vapor Deposition (CVD) method and direct co-axial spinning method, for the preparation of nanofibers [35, 36].The nanofibers produced by CVD method include the following, initially the polymer is transformed into nanofiber or a “template” by a conventional electrospinning method. These are coated with suitable polymers or metals. Finally, hollow fibers are prepared by dissolving the template material and drying them with centrifugal rotation dryers or by calcining in furnaces.[Figure 3].

Porous nanofibers: The application for porous nanofibers are more than the hallow nanofibers, because of these porous nanofibers are having large and effective surface area, they are used for many purpose like filtration, fuel cell membrane, for tissue engineering , as catalysis and as a carriers for drug delivering[72] . Porous nanofibers can be prepared by special topology by selecting suitable solvents or solvent mixtures, or polymer mixtures under proper environmental medium. [Figure 4].

Wet spinning process- This is the other method which is used for manufacturing fibers. In this process the fibres can be made by extruding the water soluble solution such as sodium alginate solution. Generally alginate fibers are prepared by this method [7]. [Figure 5].

Extrusion method- In this method the solution such as sodium alginate (%5w/w)was extruded under pressure through a spinneret (5000 holes,75cm diameter) into a cross linking solution of 0.136M cations. The fibres was then washed with deionized water to remove unbound cations before drying. These fibers obtained was stored in a desiccator for at least 48 hrs before use [8].

Phase separation- This method involve various step like, polymer dissolution, gelation, phase separation, solvent removal, and drying [37, 40].It involve first dissolving the polymer in a solution at room temperature or an elevated temp. The homogenous solution is kept at the gelation temp, which depend on various factor such as:-

- Polymer type.
- Solvent
- Polymer concentration.

At gelation temp. these polymer solution will form gel and the phase separate to form nanofibers matrix. The excess of solvent is removed and it is dried to get nanofibers [figure 6,7].

Self-assembly- In self-assembly [30] process the nanofibers are produced by holding small molecules together via intermolecular interactions. Most commonly used mechanism is formation of hydrogels, which contain two phases like solid phase and the liquid phase. The liquid phase is typically a water and solid phase is network of nanofibers formed by the self-assembly by hydrogelator molecules [Figure 8].

Template synthesis method- In this method, the nanofibers are produced in hollow channels of porous ceramic or polymer templates. Its method is describe in following [figure 9].

AVAILABLE DOSAGE FORM FOR WOUND HEALING:-

Traditional wound dressings- These are used most widely such as cotton wool, natural and synthetic bandages and gauze were used as absorbent wound dressing material to absorb exudates and provide physical properties [4].[table.1]

Lyophilized wafers- They are very useful for delivering the drug to suppurating wounds, because of their direct application on the wound surface.

Hydrocolloids- They are gel forming in nature made up of elastomers, gelatin, pectin and adhesive. They are used for both moist and dry sites and provides optimal wound environment [10].

Hydrogels- They are swellable and hydrophilic in nature and they are used in primary dressing. The polymers used for this is synthetics and natural polymers e.g. PVP and methacrylate.

Wound healing films and semi – permeable films- They are generally used for both primary as well as for secondary dressing. They help to provide gaseous exchange and a moist wound environment.

Wound healing foams- They are used for controlled drug delivery and for cell growth.

Multi-layered dressings- They provide therapeutics properties simultaneously such as adhesion, absorption, mechanical support and strength and a moist wound environment [4].

Electro- spun nanofibers and scaffolds- They are used for both primary and secondary dressings for wound as well as for both dry and moist wound. These fibers mimic the properties of skin and hence act as pseudo skin.

CHARACTERIZATION AND EVALUATION

The morphology of the nanofibers was analyzed using scanning electron microscopy(SEM)and transmission electron microscopy (TEM). This is essential for knowing the structure of nanofibers. As reported earlier by Kim et al., 2010, the core shell structure was obtained by coaxial electrospinning method[25]. [Figure 10].Fourier transform infrared spectroscopy was used to identify the change in molecular structure of polymers [30].Moisture vapour transmission rate(MVTR) and porosity. This is done by measuring(MVTR) across the material with in the diameter of 35mm, the materials were mounted on the mouth of cylindrical beakers containing 10ml water with the negligible

water vapour transmittance. The material was fastened using a Teflon tape across the edges to prevent any water vapour loss and whole assembly was kept at 37°c and 35 RH, in anincubator [4].

Antibacterial activity- This can be determined by using disc diffusion method. 35mm diameter of nanofibers was taken and nutrient broth was prepared by dissolving in distilled water and then placed in conical flask and this flask is sterilized and then these are kept inside the incubator for 24 hrs at 37°C [5].The fibres was placed in petri plate and its antibacterial activity was analyzed from the inhibition zone.

Fibres strength- The bunch of fibers was taken and it was cut to pre-determined length. The minimum of 10 samples were selected randomly and their individual weight was measured. The fibers strength of each sample was measured by a tensile tester with an initial grip separation of 50mm and test speed of 500mm/min. the readings were determined for each type of fibers and the average reported [8].

Determination of fluid uptake and degradation of fibers- The fibers whose fluid uptake has to be determined they are cut into pieces of 7 cm, after cutting this the, fibers was stored in dessicator for at least 48 hrs before testing. Now, each sample was weighed (w) and kept in woven nylon pouch and then they are put into covered tubes containing few ml of test medium. These tubes was placed in water bath and maintained at 37±1°C the fiber was removed from media and placed between two pieces of filter paper, a specified load was then placed on the top to exert a constant pressure to remove surface fluid and the fiber weighed again (W1) then these fibers are kept in the desiccator and again weighed (W2) [10].

The fluid uptake: - $\frac{W1-W2 *100}{W2}$

The extent of degradation: - $\frac{W -W2 *100}{W}$

In vivo wound healing
For evaluation of in vivo wound healing properties, it is was done by using two rat models. The nanofibers were applied on the upper side of organ on the wound and it is compared with control one [8, 16, 74].

Mechanism of wound healing
The fibers are responsible for increasing TGFβ1,VEGF and IF-10.The TGFβ1 increases fibroblast proliferation, collagen synthesis, and angiogenesis and vascullogenesis. The IL-10 decreased the TNF-α which result in reduced inflammatory cells. All these factors like TNFβ1, VEGE,IL-10 will result in well-formed granulated tissue which in turn result in better epithelialization and better wound contraction, and this result in better wound healing[11,78 , 79].

Biomedical uses of fibers
Many biopolymeric fibers are used for treating many diseases like alginate fibers which are used for the treatment of foot ulcer in diabetic person.[7, 23,80] Collagen- alginate fibers in combined form used for the management of foot ulcer in many patients ,Chitin and chitosan fibers in combination are used for various soft tissue engineering.[7, 14, 15, 24] . Poly (ε-caprolactone) fiber with gelatin and Ca phosphate used as biomimetic for bone tissue engineering [34].They are used as neural tissue engineering of poly (l-lactic acid) (PLLA)-based electro spun Nano fibrous scaffolds for the purpose of nervous tissue engineering. Their study help in knowing the influence of the Nano fibrous scaffolds on neural stem cells (NSCs). This result help in determining that these Nano-fibers (150–350 nm) not only supported neural stem cell adhesion but also promoted NSC differentiation and act as a carrier for the controlled drug delivery system, for proteins delivery and DNA targeting [28].Used for various skin tissue engineering [20].engineered skin tissue would be a better alternatives, which is used to stimulate the repairing of the dermis and for healing the wound. Along with collagen fibers, various natural and synthetic polymers have been explored for skin tissue engineering now a days, nanofibers are limited in use for fabrication by electrospinning method for skin tissue engineering , vascular tissue engineering, [18, 82,.82].In Articular cartilage tissue has a limited capacity for healing wound due to the reduced availability of chondrocytes and complete absence of progenitor cells in the vicinity of the wound to mediate the repair process. The chondrocytes cells available for repair are embedded in the dense extracellular matrix of the articular surface which restricts their mobility and hence limits their contribution to the wound healing process .Used in ligament tissue engineering. Nanofibers increase the

cell response and hence were explored as scaffolds for ligament tissue engineering, for skeletal muscle tissue engineering of various part of body. Generally the techniques for skeletal muscle regeneration have explored the use of electro spun microfibers made from degradable polyester urethane (PEU) as scaffolds for skeletal muscle tissue engineering. Used for blood vessels tissue engineering. The Nano-sized fibers mimic the nature of natural ECM, provide a suitable properties comparable to human coronary artery, and form a well-defined structure for smooth muscle cell adhesion and proliferation.

Radioactive isotopes of fibers and related diseases

The radioactive properties of fibers are seen by emerging of ionizing radiation from these isotopes which are held within these fibers. Mainly Sulphur -35 isotopes whose emission is soft beta rays and phosphorus -32 isotopes whose emission is hard beta rays and these isotopes are bonded by the help of covalent bonding. These fibers in the form of oscillators can be used in the radiotherapy of non-malignant and malignant treatment, intracavitary or interstitial localization, including inoperable tumors of vital organs and tissue [12].They can also be applied as an immunological barrier to surpass the incompatibility of genetically foreign tissues and organs during transplantation. These fibres are responsible for local immunosuppressive action by the mechanism of emitting radiation and then these radiation suppress foreign body reaction.

DNA plasmid studies

Now a day's use of fibers for gene therapy for cutaneous wound healing is of great interest. These involves the insertion of a gene into the recipient skin cells that's act as carrier. The gene therapy is performed by culturing and harvesting of fibroblast and keratinocytes cells [4]. There is a need to standardize for each gene using cDNA from granulated tissues which are responsible for healing by polymerase chain reaction. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as the gene [11, 79]. The incorporation and expression of a gene product and permanent insertion of genetic material such as DNA at the target site [31]. Luu et al studied PLGA and PLA-PEG block copolymer based Nano fibrous scaffolds for plasmid DNA delivery (Fang and Reneker 1997; Luu et al 2003).The electro spun nanofibrous scaffolds delivered the gene in a controlled manner at the targeted site and consequently caused cell transfection and desired healing activity. . This approach showed higher transfection efficiency when compared with naked DNA added directly to the culture medium. With maximum amount of DNA during scaffold fabrication increased transfection efficiency of the nanofiber DNA system. Jia et al (2002) used alpha-chymotrypsin attached to electrospun polystyrene nanofibers (120 nm) as a catalytic system and examined its catalytic efficiency in biotransformations.

Anti- oxidant activities (Biosensors)

The anti-oxidant agents are incorporated inside the wound dressings. The use of anti- oxidants such as alkaloids, triterpenes and flavonoids as an application to prevent oxidative stress that may result in further pathogenesis due to lipid peroxidation and free radical scavenging[4,75].

Toxicity

The effect of shape, size and surface properties of carbon nanomaterials can directly impact cellular toxicity. Health and growth of human lung tumor cells can be correlated with increasing conc. of nanofibers [41].Many toxicity problem like they show slow wound healing, they result in cell toxicity and entrapment of cellular matrix and in turn result in damage of cell which causes carcinogenic effect to skin.

Table.1: Different type of dosage form available for wound healing

Products for wound care	Properties	References
Collagen dressings, hydrogels', hydro foams hydrocolloids.	Enhances the epithelization	51,71.
Anti microbials like silver impregnated dressings, mupirocin, and retapamulin.	Prevent infection.	48,60,61,77,81.
Maggots, debridace, enzymatic agents.	Desloughing and debriding agents	66,68.
Hydrocolloids, hydrogels alginates , collagen granules ,VAC.	Enhance granulation and tissue formation.	72,76.

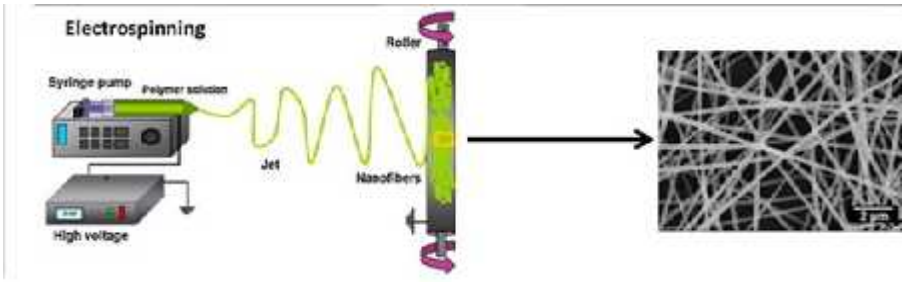

Fig.1: Preparation of nanofibers by electrospinning method

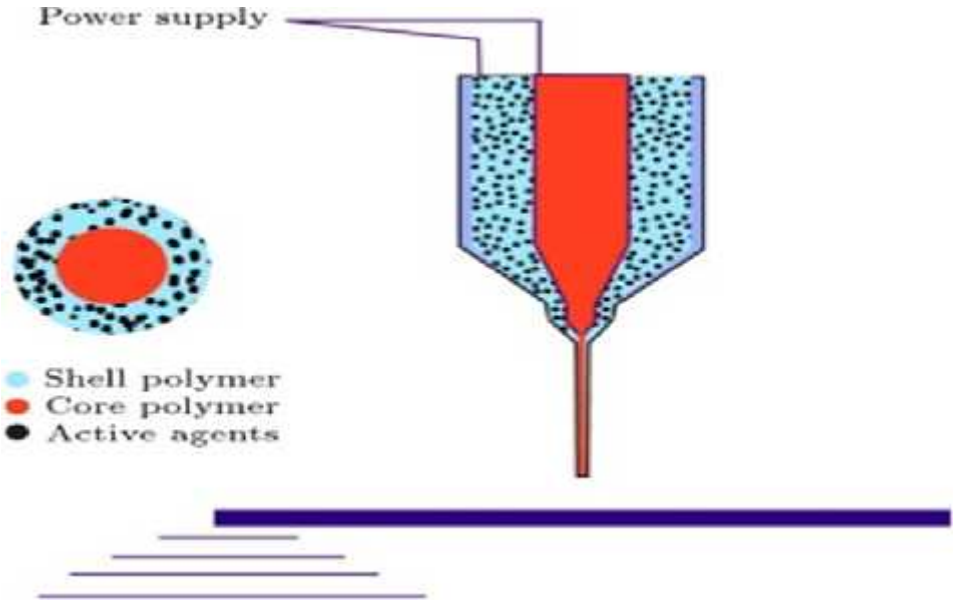

Fig.2: Preparation of core shell fibers by electrospinning.

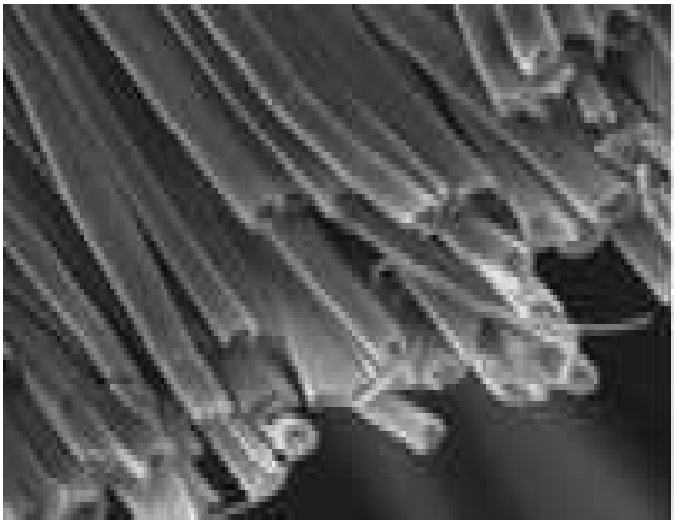

Fig.3: SEM image of TiO2/PVP poly hollow nanofiber

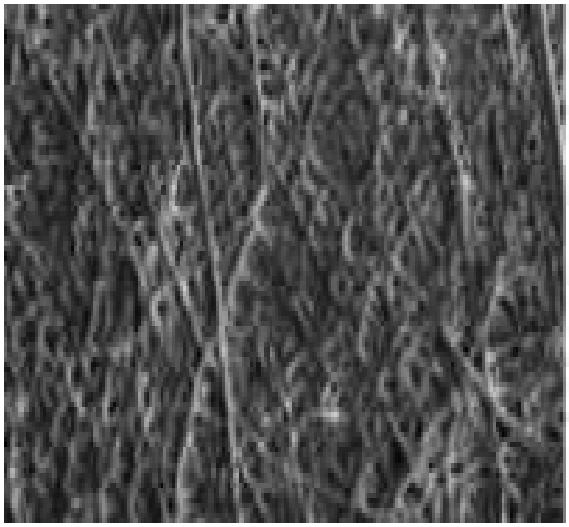

Fig.4: SEM images of porous hollow fibers collected in ethanol/acetone

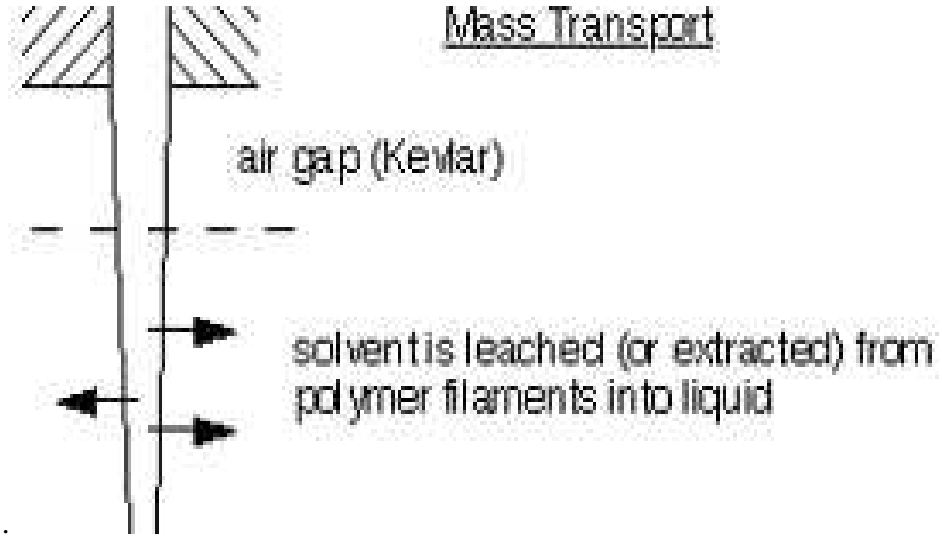

Fig.5: Wet spinning by solvent extraction method

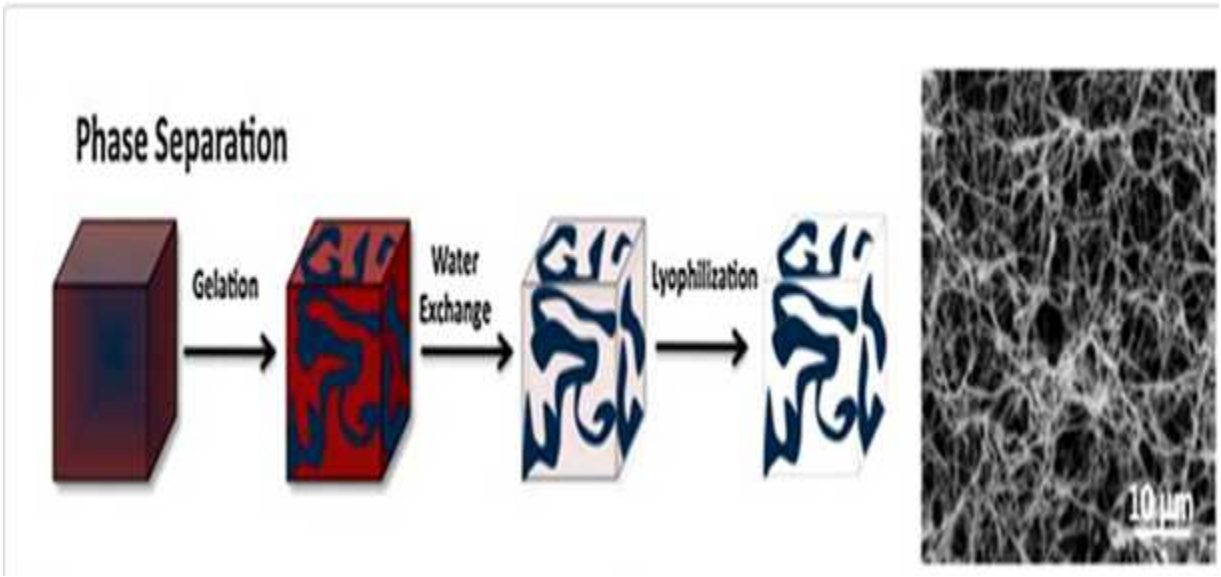

Fig.6: Preparation of nanofibers by phase separation method

Fig. 7: SEM of poly (L- lactic acid) fibers using phase separation method

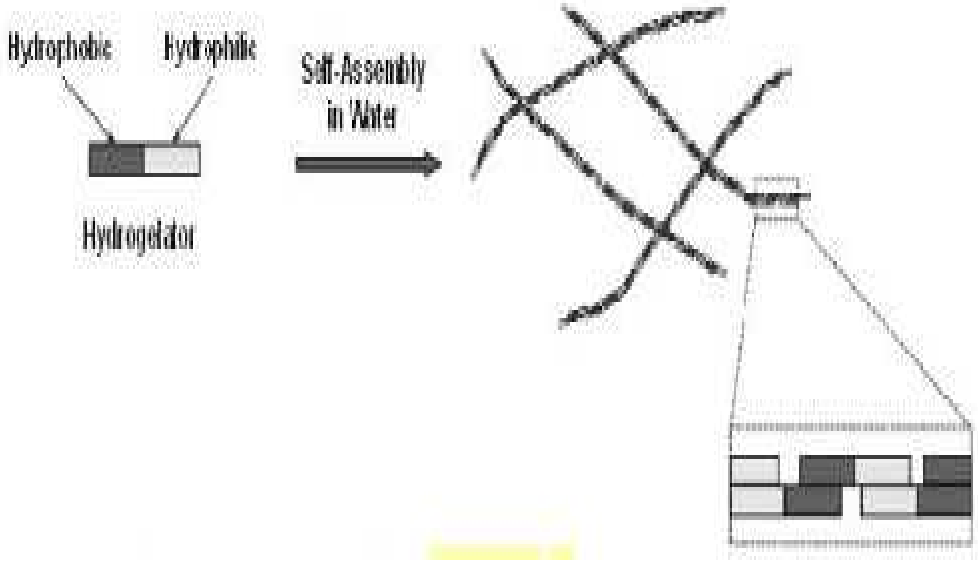

Fig. 8: Preparation of nanofibers by self- assembly method

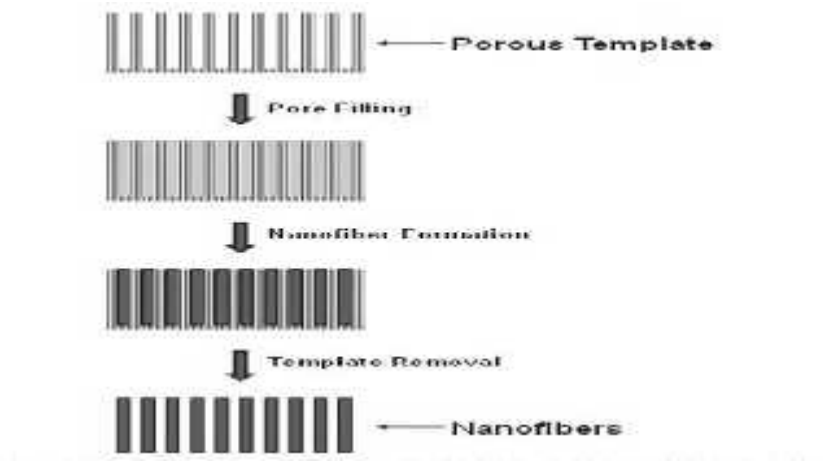

Fig. 9: Preparation of PAN nanofibers by template method

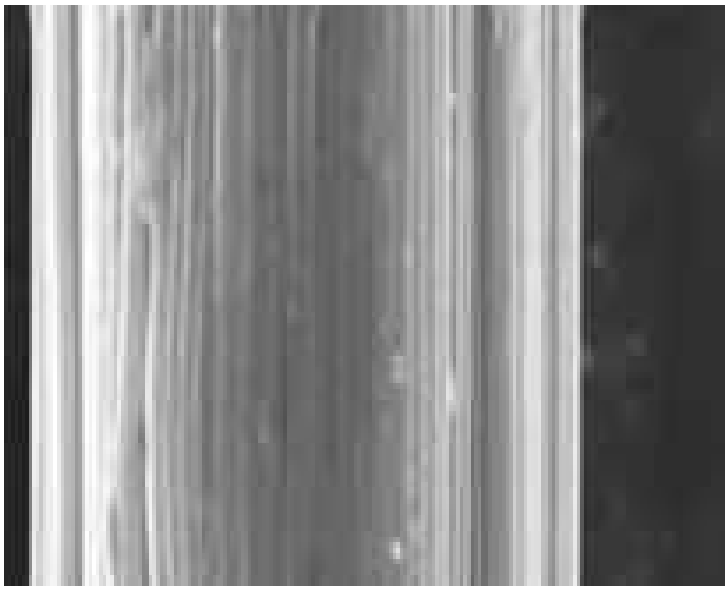

Fig.10: SEM of nanofibers

CONCLUSION

With the advancement and emergence of tissue engineering technologies individual therapy options can be explored. For targeting specific wound types and for its better and effective treatment. The use of biopolymeric fibers which can modified to mimic the skin environment, conditions and structure. The incorporation of active agents, growth factors and drugs can stimulate wound healing responses. The designing and developing a wound healing devices and techniques such as electrospinning, phase separation, self-assembly can be employed to ensure this. These fibers have shown good compatibility with other tissues when used as scaffolds and matrices. The fibers made of natural materials show most promising compatibility. This review aimed at providing an insight to the development of an idealized wound healing device which is having capacity to overcome all the current challenges.

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