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Chitosan Films Coated with Gelatin Nanofibres for Biomedical Application

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Abstract: *In this study, Gelatin, nanofibres were prepared using formic/water solvent by electro-spinning process. Different process parameters, such as electric field, spinning distance and concentration of dope solution were optimized. The gelatin nanofibres produces have mean size of 70-120 nm. The morphology and the diameter of the nanofibres was analyzed using Scanning Electron microscopy (SEM). These fibres were deposited on chitosan film. Mechanical and Morphological properties of the chitosan and gelatin nanofibres coated films were studied. It was observed gelatin nanofibres coated chitosan films showed enhanced tensile strength (200%) and elongation at break (190%) as compared to chitosan films. The nanofibre coated chitosan films have potential to be use in biomedical application.*

Keywords: *Gelatin; Electro-spinning, Nanofibre, Chitosan*

1. INTRODUCTION

Gelatin is defined as a product obtained by the partial hydrolysis of collagen derived from the skin, white connective tissue and bones of animals. Gelatin is an important material, finding application in the food, pharmaceutical and photographic industries as well as diverse technical uses. It is nearly tasteless and odorless. It is a vitreous, brittle solid faintly yellow in color. When gelatin granules are soaked in cold water they hydrate intodiscrete, swollen particles. On being warmed, these swollen particles dissolve to form a solution.Gelatin fiber mats with sub-micron and nanometer scale can simulate ECM structure of the human tissues and organs and can be used widely in the tissue engineering field because of its excellent bio-affinity, biological origin, biocompatibility, non-immunogenicity, biodegradabilityand commercial availability [1].

Electro spinning process is one of the most convenient and effective methods for preparation of nanoscale gelatin fibers, which can produce a highly porous nonwoven mat with a high surface-to-volume ratio and porosity. Electro spun gelatin nanofiber matrices in various forms including thick nanofiber sheets, tubular structures, and as a coating material have been used in a variety of biomedical applications[2].Electrostatic spinning or electro-spinning is a process by which ultrafine fibers with diameters in nanometer to sub-micrometer range can be fabricated. This process deals with the application of an intense electrostatic field to a polymer liquid (i.e., solution or melt) across a finite distance.Electro-spinning depends on working parameters such as solution concentration, viscosity, molecular weight, distance between needle tip and collector, collector type etc.

Chitosan is a linear polysaccharide composed of randomly distributed β-(1-4)-linked D-glucosamine (deacetylated unit) and N-acetyl-D-glucosamine (acetylated unit). It is made by treating shrimp and other crustacean shells with the alkali sodium hydroxide .It is a cationic biopolymer obtained by a full or partial N-deacetylation of chitin, which is known to be the second most abundant biopolymer in nature and is the major component of the exoskeleton of crustaceans. It is biodegradable and non toxic. It is not soluble in pure water or organic solvents but is soluble in aqueous solutions of organic or mineral acids under specific conditions. Chitosan films can create a semipermeable barrier capable of reducing respiration, microbial growth retardation, maintains firmness and color in fruits and vegetables.

2. DESCRIPTION OF ELECTROSPINNING PROCESS

Electrostatic spinning or electro-spinning is a process by which ultrafine fibers with diameters in nanometer to sub-micrometer range can be fabricated.This process deals with the application of an intense electrostatic field to a polymer liquid (i.e., solution or melt) across a finite distance. When a high electrostatic field is applied, charges accumulate on the surface of a droplet of the polymer liquid at the tip of the nozzle. With increasing electrostatic field strength, the Coulombic repulsion forces destabilize the partially-hemispherical shape of the droplet into a conical shape when a critical electrostatic field strength is reached. Further increasing the electrostatic field strength causes a charged stream of the polymer liquid (i.e., the charged jet) to be ejected from the apex of the cone. The charged jet travels in a straight trajectory for a short distance before undergoing a bending instability, which is thought to be additional

mechanism responsible for the further thinning of the jet during itsflight to a grounded collector [3].

Due to the unique characteristics of electro spun fibers, e.g., high surface area to volume or mass ratio and high density of pores in sub-micrometer length scale of the obtained as-spun non-woven mat, these fibers are excellent candidates for various biomedical applications (e.g., tissue scaffolds, wound dressing pads, vascular grafts, and carriers for drug delivery).

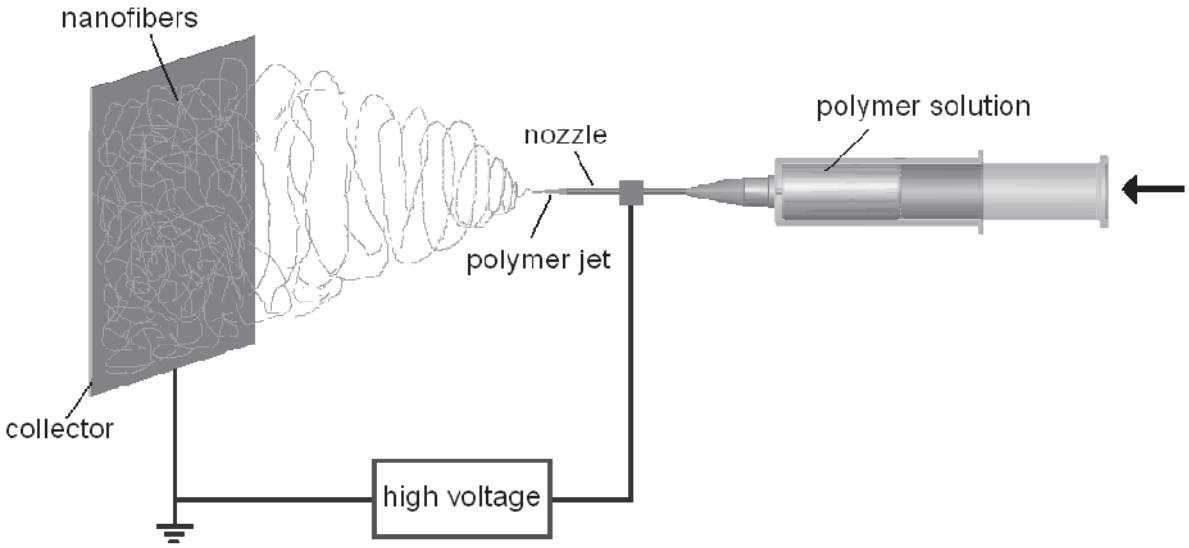

Fig. 1.Schematic for the single needle type electrospinning

3. MATERIALS AND METHODS

Formic acid solution was prepared by mixing distilled water and formic acid. Formic acid solution was taken and gelatin was added to the solution. The prepared solution was loaded in the syringe and electrospun to obtain gelatinnanofibres. The process was optimised at 6.25gmgelatin dissolved in 25% formic acid solution. Stock solution of 2% acetic acid in water was prepared. 2% (w/v) chitosan was dissolved in 50ml of the stock solution. Solution was put in oven to remove bubbles and to homogenise it. Chitosan solution was poured on a tray to form chitosan films. Solution was left to dry in air. Optimizedgelatin solution (consisting of 25% formic acid and 25wt% gelatin, kept for one day) was loaded in the syringe. Chitosan films were deposited on the collector to deposit gelatine nanofibres on the film. Electro-spinning parameters were as follows:

TABLE 1: Process parameter for electro-spinning of gelatinnanofibres

S. No	Process Parameter	Range
1	Voltage	16-20 KV
2	Flow Rate	0.5 mL/h
3	Distance between needle tip and collector	4-7.5 cm

4. CHARACTERIZATION TECHNIQUES

Samples were then characterised using Scanning Electron Microscopy (SEM) and Universal Testing Machine (UTM).

4.1 SCANNING ELECTRON MICROSCOPY

The surface morphology of gelatinnanofibers coated chitosan as well as pure chitosan films was observed with a scanning electron microscope (SEM) (Hitachi S-3700N) at a voltage of 15 kV. Specimens were sputter coated with gold before mounting on SEM machine.

4.2 MECHANICAL TESTING

The tensile strength and elongation at break properties of the films were determined using Universal Testing Machine (Instron 3369) according to ASTM D 882-02. The preconditioned test samples (60×10 mm) were mounted between grips of the machine with span length 40 mm and pulled at cross head speed of 50 mm/min. Average tensile properties of three specimens were measured.

5. RESULTS

5.1 SEM ANALYSIS OF NANO FIBERS

The morphology of electrospunfibers deposited on chitosan films is shown in the Figure 2. Figure 2(a) shows the beaded morphology of electrospungelatinfibers spun at 16 KV voltage and 4 cm distance. It is observed that with increase in voltage and distance smooth fibers morphology is obtained (Figure 2b). The formation of beaded and smooth fibers can be explained based on the interplay among the three major forces that are Coulombic force, viscoelastic force, and surface tension. At low solution concentrations, the viscoelastic force, in comparison with both the electrostatic and theCoulombic stretching forces, is

not enough to prevent either partial or total breakup of the charged jet and, as a result of the surface tension, either discrete beads or beaded fibers are formed. With increasing concentration of the solutions, the viscoelastic force is high enough to counter both the electrostatic and the Coulombic stretching forces, preventing both the partial and the total breakup of the charged jet. The monotonous increase in the viscoelastic force in comparison with both the electrostatic and the Coulombic stretching forces is also responsible for the monotonous increase in the fiber diameters with increasing solution concentration.

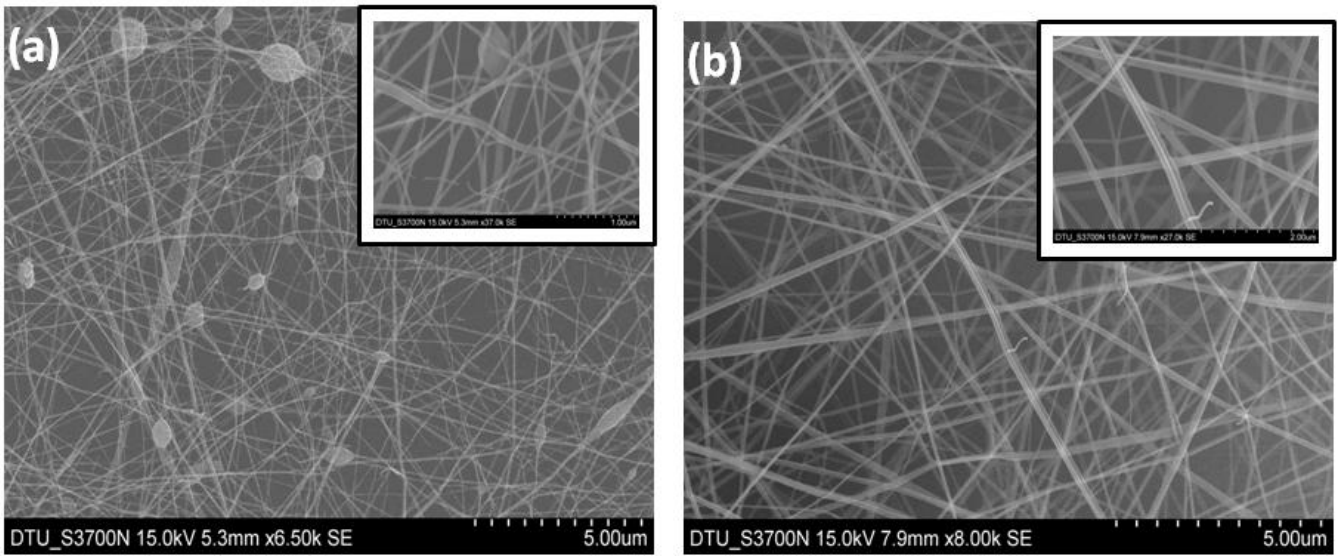

Fig. 2. Morphology of nano-fibers obtained from 25% (w/v) gelatin solution with electrospinning parameters of (a): distance 4 cm, voltage 16 KV and flow rate 0.5 mL/h (b) distance 7.5 cm, voltage 20 KV and flow rate 0.5 mL/h

5.2 TENSILE PROPERTIES

The tensile properties of pure chitosan films and films coated with gelatine nano-fibersare studied. Figure 3 (a & b) shows the stress strain curves of pure chitosan film and films coated with nano-fibers. The mechanical properties of pure chitosan film and gelatinnano-fiberscoated film aregiven in Table 2.

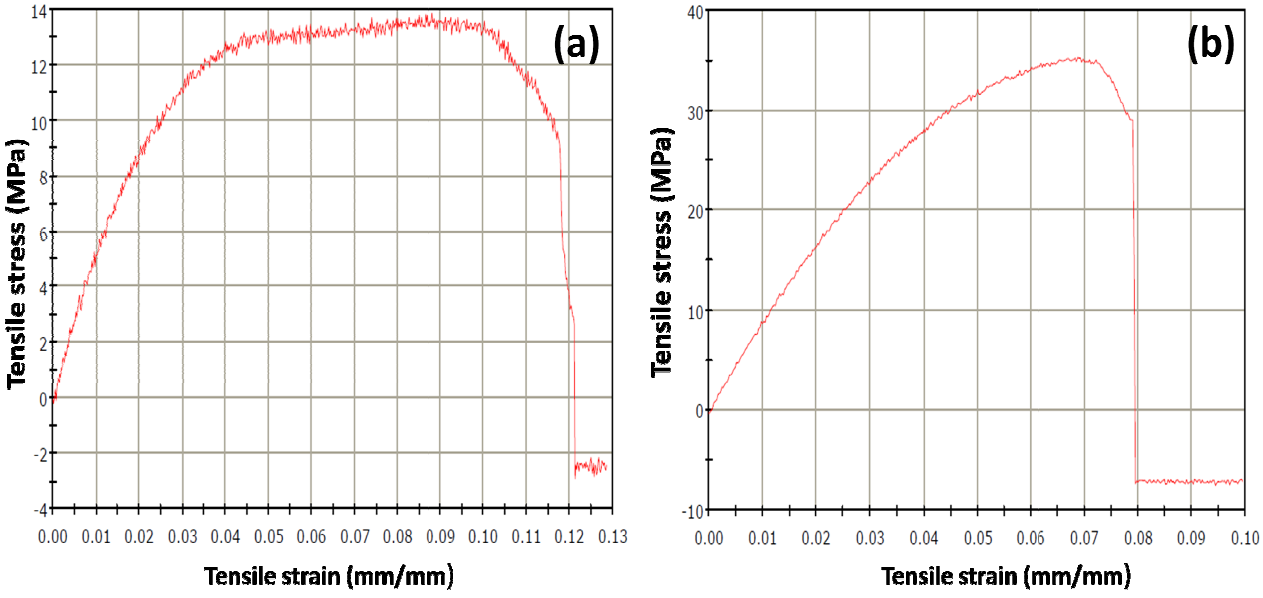

Fig. 3.Tensile stress-strain curves of (a): Pure chitosan film and (b): chitosan film coated with gelatinnano-fibers

TABLE 2: Mechanical Properties of Pure Chitosan Film and Gelatinnano-fiberscoated Chitosan Film

Properties	Pure chitosan Film	Gelatin coated chitosan film
Tensile strength (MPa)	13.85	35
Elongation at Max Load(mm)	3.99221	7.17169
Tensile Modulus (MPa)	522	671

It is observed from Figure 3, gelatinnano-fibers coated chitosan film shows higher tensile strength as compared to pure chitosan films.It was observed gelatin nanofibres coated chitosan films showed enhanced tensile strength (200%) and elongation at break (190%) as compared to chitosan films.

6. CONCLUSIONS

Gelatinnano-fibers were prepared using electrospinning machine and coated on chitosan film. The process parameter of the electrospinning machine was optimized in the term of voltage (20 KV) and distance (7.5 cm). The gelatinnano-fibers coated films show enhanced mechanical properties. These films can be promising materials for wound dressing and tissue engineering.

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