Development of Keratin based Nanofibers for Tissue Engineering Application

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Abstract:- **Keratin is one of the most copious protein which can be used in a variety of biological applications due to its physical and mechanical properties. The focus here is on keratin, one of the most unexploited non-food natural protein used for tissue engineering and for the production of affinity based Nanofibers because of its biodegradability and biocompatibility. Extraction of keratin from human hair waste using Shindai method and quantifying the protein using SDS-PAGE. Transforming keratin, gentamycin (antibiotic drug) and PVA polymer (synthetic polymer) into nanofibers using electrospinning techniques which combines the physical and mechanical properties of keratin with parameters such as high surface to volume ratio, three dimensional features and high porosity for nanostructured nonwoven fibers. The electrospun nanofibers will analyze by scanning electron microscopy (SEM) and Transmission Electron microscopy (TEM) for characterizing its morphology and structure. These qualities will develop the keratin based nanofibers for tissue engineering applications such as wound healing and inflammatory responses.**

Keywords: Hair keratin, Shindai method, Tissue engineering, electrospun nanofibers.

I) INTRODUCTION

One of the primary goals of tissue engineering research is the development of a fiber matrix or scaffolding system that mimics the structure and function of native tissue. Many scientists may explore the use of natural macromolecules due to their intrinsic ability to perform very specific biochemical, mechanical and structural roles. Especially, protein-based biomaterials have a potential benefit for many biomedical and biotechnological applications as a synthetic extracellular matrix between cell-cell and cell-matrix interactions.

Natural polymers, such as proteins and polysaccharides, are good potential materials for tissue regeneration and also abilities to improve cell–material interactions. Examples of natural biodegradable polymers based on nonanimal-derived polysaccharides are starch and chitosan. Starch is promising owing to its abundance and biodegradability but its brittleness poses a challenge in processing. Chitosan is one of the most widely used natural polymers, next to collagen, due to its biocompatibility and antibacterial properties but its rapid biodegradability, severe shrinkage and deformation have limited its use in certain aspects of tissue engineering applications. Other natural materials that have been investigated as potential tissue regenerative matrices include collagen, fibrin, elastin and gelatin. However, most of these materials are of animal origin and can hence lead to issues of immunological rejection and risk of pathogen transfer.

To circumvent these issues, keratin emerges as an attractive protein of human origin to be used as a template for tissue regeneration because it can be easily extracted from the unlimited supply of human hair, making it abundant and readily available. Keratin-based materials have shown promise for revolutionizing the natural derived protein substance due to their intrinsic biocompatibility, biodegradability, mechanical durability, and natural abundance.

II) MATERIALS AND METHODS

A) EXTRACTION OF KERATIN FROM HUMAN HAIR WASTE (Shindai Method)

Human hair collected from a local barbershop, was washed with ethanol and water several times in order to remove any dirt from the surface of the hair. The clean hair was put in a chloroform (Sigma-Aldrich) and methanol solution (2:1) v/v for 24hrs in order to remove any fat from the surface of the hair. The delipidized hair was washed with RO-water and kept in open air overnight to evaporate chloroform and methanol. From the table 1,The keratin protein extraction buffer solution was prepared by adding 0.3025g of Tris (hydroxymethyl) aminomethane or Tris-base (Fisher), which used as a buffer solution, 19.79g of thiourea (Sigma-Aldrich), 30g of urea (Fisher) and 5ml of 2-mercaptoethanol (Sigma-Aldrich) in 100ml of deionized water. The pH was then adjusted with 8M Hcl (Sigma-Aldrich) to 8.5. The Tris-HCl was used to keep the keratin extraction buffer solution at a pH of 8.5. Thiourea and urea were used to break down the non-covalent bonds found between polypeptide chains of amino acids. Additionally, 2-Mercaptoethanol was used in order to reduce the disulfide bond found between cysteine.

Compounds	Molar mass (g/mol)	60g in 1L (distilled water)	6g in 100ml (distilled water)
Tris base Hcl	121.14 g/mol	25mM	0.325g
Urea	60.06g/mol	5M	30g
ThioUrea	76.12g/mol	2.6M	19.79g
2-Mercaptoethanol	78.13g/mol	5%	5ml

Table 1: Preparation of Buffer solution and its quantity

B) PROTEIN SOLUTION FILTRATION AND EXTRACTION

A delipidized hair (6g) was cut in to smaller pieces with average length around 1mm, followed by mixing it with the 100ml buffer solution described above. The solution was juddered by hand for 3min, and kept inside a preheated oven at 500ºc for 3 days; the solution was juddered by hand every twelve hours.

A 2µm size filter paper (Whatman) was used to separate the solution containing protein from the cuticle-cortex residue for twelve days. Then, filtrate was centrifuged at 15,000 g for 20min at room temperature using 50ml centrifuging vial (Flacon), in order to remove small fragments of hair residues. The obtained supernatant was dialyzed against deionized water using Snakeskin dialysis tubing (Thermo scientific, with a molecular weight cutoff of about 3.5kDa and diameter 16mm).

During dialysis the outer water was replaced with deionized water twice a day for four days. Furthermore, the solution that contains proteins started to change its color to milkish because proteins start to aggregate and polymerize. Then dialyzed protein solution having a milkish color was kept in -80ºC refrigerator for 48hrs. Finally, the frozen protein solution was kept in a lyophilizer at pressure (3.5pa) and temperature (-48ºC) operating conditions for 48hrs.

C) QUANTIFICATION OF PROTEIN BY SDS-PAGE

The extracted keratin will identify by using Sodium dodecyl sulphate-Polyacrylamide Gel Electrophoresis. The aqueous solution of reduced keratin was subjected to Electrophoresis unit for 15ºC using a 10%– 15% gradient gel at 250 V and 10 mA. The gel was stained using Coomassie brilliant blue R-250. After staining and destaining the gel compare the molecular weight of the samples with that of the protein marker.

D) PREPARATION OF ELECTROSPUN KERATIN BASED NANOFIBERS

A previously prepared solution of keratin/Drug and polymer solution will individually fed into the syringe of 10 ml and then placed into a syringe pump in Electrospinning apparatus. The syringe pump will set to a flow rate of 1–2 ml/h for the keratin/Drug and polymer solution. The positive lead from the high voltage power supply will fix to a 21-gauge needle, and an 11-kV voltage will apply, which charged the polymer solution. Aluminum foil will wrap around the collector for collecting the fiber. The tip to collector distance will be maintaining at 10 cm. The fibers formed will deposit onto a rotating grounded collector. Nanofiber samples will collect for 2–6 h.

E) CHARACTERIZATION OF DRUG LOADED FIBROUS SYSTEM

The morphology and Structure of Nanofibrous system will observe by using Scanning Electron microscopy (SEM), XRD and DSC.The samples will observe at an accelerating voltage of 10 kV and a 5-µA current.

III) APPLICATIONS OF NANOFIBERS

Electrospun micro and / or nanofibers are morphologically similar to the extracellular matrix (ECM) of natural tissues and its nanoscale fibrous structure; it can be explored in diverse areas of tissue engineering. Natural and synthetic fibers have been widely used as promising scaffolds for tissue repair, these fibrous scaffolds are mechanically stable and capable of functioning biologically in the implant site.

Generally, Nanofibers in Tissue engineered Scaffolds are adjust with suitable biodegradation rate, adhere to cell and growth in controlled direction.

IV) CONCLUSION

This Study prominence for preparation, Characterization and application of keratin based Nanofibrous materials which helps in wound healing and tissue regeneration when it applicable with suitable synthetic polymer.

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