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Development of Clindamycin Phosphate Loaded Antibacterial Adhesive Patch Using Alpha-Keratin Extracted from Human Hair for Tissue Regeneration

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Abstract

Wound healing is a process of replacing the damage tissue caused by various factors, which involves a complex series of event. A fibrous protein called keratin is found in the top layer of skin, hair, and nails. It has an impact on wound healing process. The ability of keratin to aid in cell adhesion, proliferation, and tissue regeneration results in the provision of a biocompatible matrix for the regrowth and regeneration of damaged tissue. In this project work the main aim is to isolate the keratin from human hair and incorporate in a chitosan-based biofilm which is loaded with an antibacterial agent called clindamycin. Keratin provides a good stability against enzymatic degradation of body cell and clindamycin has an anti-microbial effect that together make the research work a best way to deliver this formulation for biomedical applications. This experimental study manages the isolated amount of by successfully incorporate it into the chitosan-based biofilm. This article manages about the properties, categorization, and structure of keratin by FTIR analysis and SEM. The extraction of keratin is carried out by using chemical precipitation method and on the other hand, the chitosan-based biofilm is made by solvent evaporation method.

Keywords

Biofilm, Chitosan, Clindamycin, FTIR analysis, Keratin Nano particle, NDDS.

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1.INTRODUCTION

Wound healing has historically been divided into three wonderful phases: inflammation, proliferation, and remodeling (1). Keratin is amongst the most precious proteins located in the reptiles, birds, and mammals. It is a structural constituent of nail, wool, feathers, and hoofs which gives energy to physique and muscles. Nowadays, the keratin-rich waste biomass produced from chicken and meat enterprise imposes danger to surroundings and residing beings. We want to discover a number of strategies and techniques for the extractions and use of keratin from waste biomass. From the industrial factor of view, keratin is a beneficial product in the medical, pharmaceutical, cosmetic, and biotechnological industries. Materials acquired from keratin may

additionally be transformed into porous foam of exceptional sponges, shapes, coatings, mats, microfibers, gels, and substances of excessive molecular weight (2).

Keratin is in two forms α- and β-keratins. α-keratins are determined in the tender tissues such as sheep wool, hair, and skin. β-keratins are current in difficult tissue protein of nails, fish scales, fowl feathers, and others (3). Keratins have excessive strength, stiffness, and insolubility in polar as properly as no polar solvents. The stabilization is the result of intermolecular and intermolecular disulfide crosslink's, hydrogen bonding, and its crystalline. It is insoluble in aqueous salt solution and soluble in solution containing denaturing agents, such as urea (4,5).

Table.1 Sources of keratin in nature

Types of keratins	Source organ
α-Keratin	Wool, quills, hair, horns, fingernails, hooves. stratum corneum
β-Keratin	Feathers, avian beaks and claws, reptilian claws and scales
	α- and β-keratin Reptilia epidermis, pangolin scales

Keratin-based substances showcase wonderful mechanical durability, are extraordinarily biocompatible and are without difficulty biodegradable (6,7). These awesome homes have prompted a revolution in the field of contemporary biomaterials. These substances are easily transformed into complicated 3D scaffolds, sponges, films, and hydro gels for quite a number biomedical application. Keratin-based hydro gels have proven promising results as biomaterials in more than a few biomedical applications. Keratin motion pictures are used to produce new biodegradable and biocompatible substances for wound restore and tissue engineering (8, 9).

Wounds classification is very essential from the factor of management, diagnosis, selecting the right treatment, needed time for wound healing, and awaiting the risks and infections that may additionally take place all through the wound healing process (10). To date, there is no well-known and usual classification in this area. This is broadly speaking due to the fact of different causes, complexity, and massive wide variety of wounds, and overlapping of number subclasses of wound types. However, a series of sporadic classifications in this subject are furnished in books, journals, scientific articles, and related websites (11, 12). These classifications have been proposed to classify a few numbers of wounds based totally on fantastic parameters. These parameters consist of cause, color, location, etiology, wound depth, contamination; length of the wound healing, and so on eight in one embodiment, and wound can be categorized with the aid of the integrity of skin (13).

Wound healing is a complex process that occurs in response to injury, and involves the coordinated activity of multiple cell types, signaling molecules, and extracellular matrix components. The process of wound healing can be broadly divided into four overlapping stages (14,15):

Homeostasis: This stage involves the formation of a blood clot to prevent further bleeding from the wound. Platelets, which are small blood cells, aggregate at the site of the injury and release various signaling molecules that activate the clotting cascade (16).

Inflammation: The inflammation stage begins immediately after homeostasis and involves the recruitment of immune cells to the site of the wound (17, 18). These cells release cytokines and growth factors that stimulate the proliferation of new cells and the formation of new blood vessels.

Proliferation: During the proliferation stage, new tissue is formed to replace the damaged tissue. Fibroblasts produce collagen, a structural protein that provides strength to the healing tissue. Endothelial cells form new blood vessels to supply nutrients and oxygen to the growing tissue (19).

Remodeling: The final stage of wound healing involves the remodeling of the newly formed tissue to improve its strength and function. During this stage, excess collagen is broken down and replaced with stronger collagen fibers (20, 21).

2.MATERIALS AND METHODS

2.1 Chemicals requirements

Human hair is collected from local saloon situated in Suri, Birbhum, West Bengal. Chitosan is collected from Nice Laboratory, Kolkata. Clindamycin is collected from central drug laboratory, Kolkata. and other required excipients are collected from Birbhum Pharmacy School, Dubrajpur.

2.2 Extraction of keratin of human hair

Human hair was washed with 0.5% SLS solution. Here the amount of hair we take is 25gm approximately and dissolved in 500ml solution of SLS. The hair is dipped for minimum 30mins for at least 3times for better washing and removal of any impurities. After that the hair is dried in a ventilated oven at 40°C for 72 h. After that the dry hair is dipped in 70% v/v ethanol solution. And stay it for 6hours at room temperature. After 6 hours again the hair is rinsed and dried the hair completely at room temperature. Then prepared 2:1 chloroform and methanol solution. This is the process to defatted the hair for better extraction of keratin. We prepare 250ml of total solution hence the amount of chloroform needed is 183.3ml and 83.3 ml methanol. The dried hair is dipped into this solution and stay it for again 24 hours. Again, rinse the solution and dry up the hair for total drying. The hair dissolved by mixing 25 g of the chopped hair into Na2S (0.125M in1 L) as optimized and used by previous studies digested at 50 °C using hot air oven with mechanical stirrer for 6 h. The prepared mixture was filtered twice by using Whitman filter paper of 110 mm diameter.

Then prepare 0.025 M HCL solution pH 2-3. Take 10 ml of pre prepared sodium sulfide keratin solution and add slowly the HCL from the top and stirred gently. Maximum 3 proportion of HCL is need for better precipitation. Precipitation keratin takes place in the desiccators to removal the moisture to get pure keratin.

2.3 Preparation of chitosan biofilm

Prepare 50ml 1% acetic acid solution by using distilled water and acetic acid. Weight 1.25gm of chitosan in a beaker. Mix the acidic solution with chitosan by using magnetic stirrer at 50^0C temperature. After mixing the chitosan, add 0.5ml glycerol in this solution with continuous stirring.

After mixing well, put it in a Petri dish and keep it into hot air oven at 50^0C for 48 hours. Now the film is ready. Move it from the dish.

2.4 Preformulation Studies of Clindamycin phosphate

Preformulation study is the initial step in the process of a active molecules. A preformulation study is an important mechanism for determination of physical and chemical properties of the drug before including it in formulation advancement. In this project we checked the solubility and melting point of clindamycin by required apparatus and techniques.

2.5 Preparation of chitosan-based biofilm with keratin extract and Clindamycin phosphate

Prepare 50ml 1.5% w/v chitosan solution by using 1% acetic acid. Prepare 5 ml Clindamycin(300mg) solution by using distilled water and one 300mg clindamycin capsule. Prepare 5ml keratin solution by using Sodium Sulfide (Na_2S) solution. Mix the above three prepared solutions well by using magnetic stirrer. After mixing, add 0.5ml glycerol with this solution with continuous stirring. After mixing well, put it in a Petri dish and keep it into hot air oven at 50ºC for 48 hours. Now the film is ready. Move it from the dish.

3. RESULT& DISCUSSION

After reviewing all the literatures, we performed all the required procedure about extraction of keratin from human hair and preparation of chitosan biofilm. After that we prepare a suitable biofilm by using chitosan and incorporate a specific amount of keratin

and clindamycin drug having antibacterial, anti-inflammatory, and wound healing properties.
Percentage of Yield= 31.9%

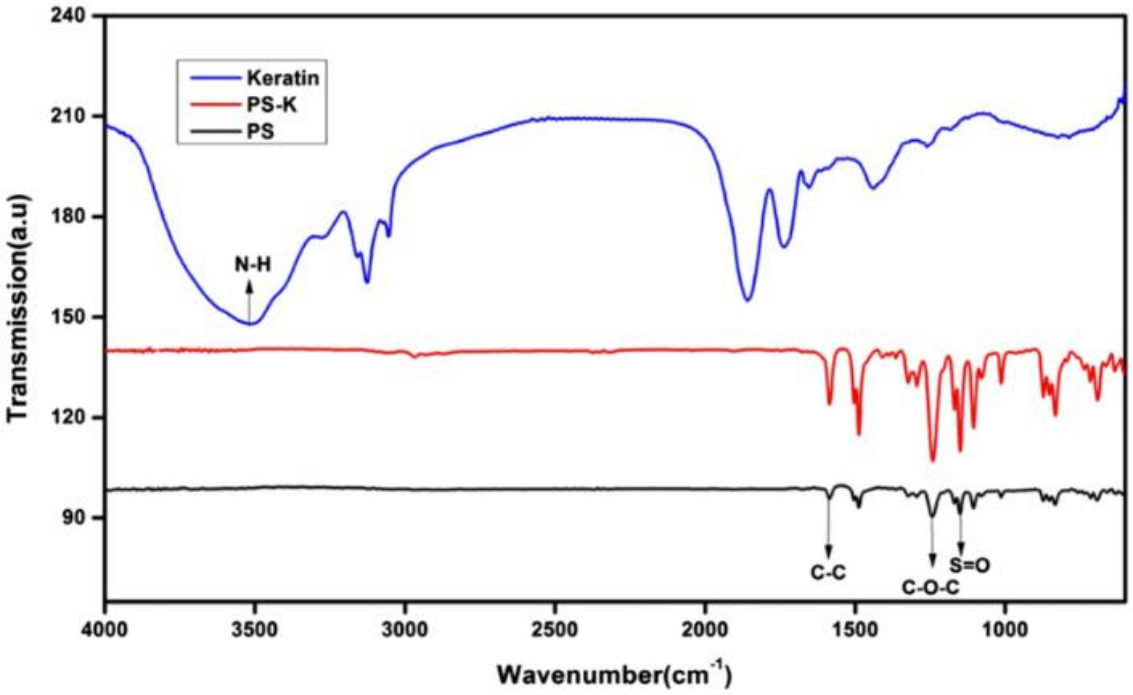

Fig.1 FTIR Spectra of Pure Keratin Extracted from Human Hair

After performing the FTIR from JC Bose Institute, Kolkata and the spectra shows that the keratin we extracted from human hair is reliable and pure.

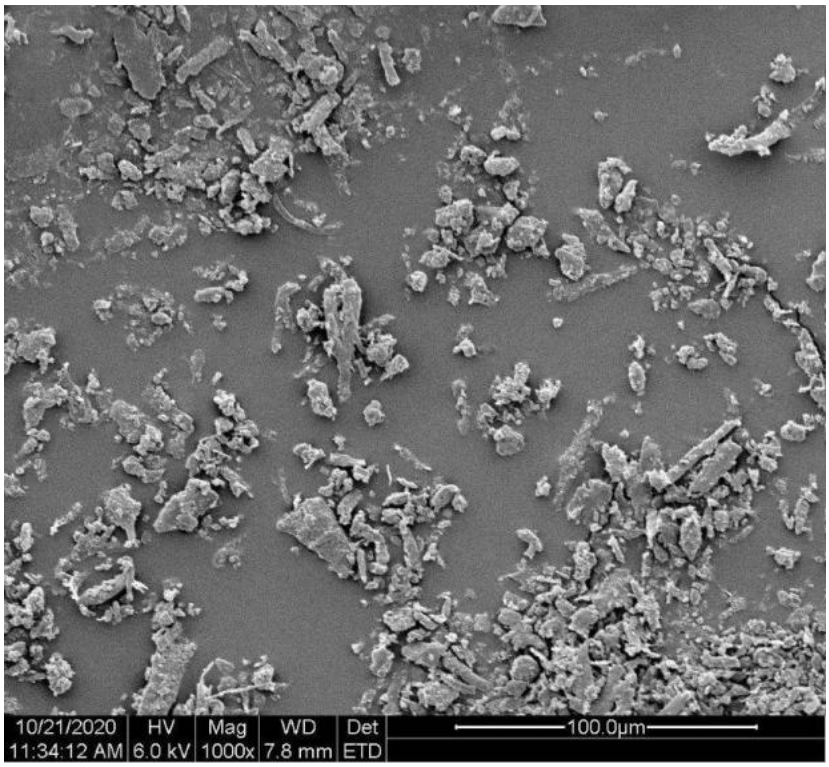

Fig.2 SEM Image of Keratin Fibers

Figure 2 shows the image of keratin fibers by SEM and the aspect ratio of fibers was calculated as 2.04.

Clindamycin phosphate was found to be white or almost white, crystalline powder. For solubility it is freely soluble in water and very slightly soluble in ethanol (~750 g/L) TS and acetone R, practically insoluble in dichloromethane R. The calculated melting point of Clindamycin phosphate was found to be 114-degree C. This result is the same as reported in reference and helps in the identification and purity of the drug powder used in the study.

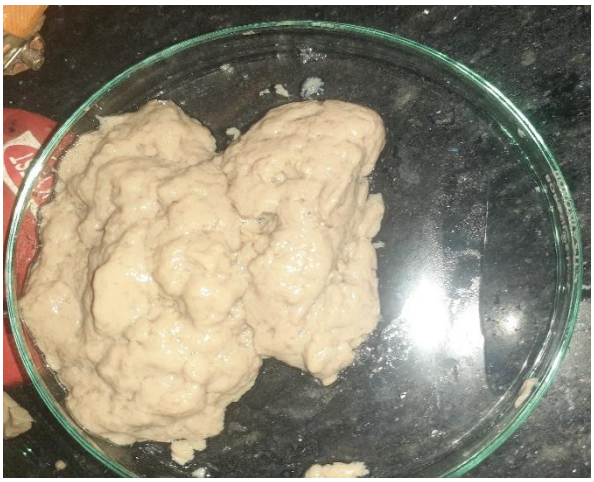

Fig.3 Fibrous Keratin

Fig.4 Drug Loaded Chitosan Bio Film

4. CONCLUSION

After successfully performing the research work, we can conclude that this type of natural biofilm is very much useful for bio medical application like wound healing and various types of external injuries. As keratin itself a protein and having fast cell re-growth ability, it is very much usefully natural polymer in pharmaceutical sector. Beside this keratin we also incorporate clindamycin drug having, broad-spectrum antibiotic, anti-inflammatory and anti-microbial properties, so it is also useful in tissue engineering.

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