FORMULATION OF WOUND HEALING PRODUCT FROM KERATIN PROTEIN

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LIST OF ABBREVIATION

F1	Formulation one
F2	Formulation two
F3	Formulation three
F4	Formulation four
W0	Weight of sample before immersion in water
W24	Weight of sample after 24 hours immersion in water
FTIR	Fourier transform infrared spectroscopy

LIST OF SYMBOLS

%	Percentage
ml	Milliliter
g	Gram
^{0}C	Degree Celsius
h	Hour

PERUMUSAN PRODUK PENYEMBUHAN LUKA DARIPADA PROTEIN KERATIN

ABSTRAK

Setiap tahun berjuta ayam diternak dan diproses di seluruh dunia. Ayam-ayam yang diproses itu akan meninggalkan berjuta bulu ayam yang tidak digunakan. Jadi, cabarannya adalah untuk menjadikan bulu-bulu ayam yang tidak digunakan itu kepada produk yang berguna dan bernilai. Di dalam penyelidikan ini, protin keratin akan diambil daripada bulu-bulu ayam yang terbuang dan seterusnya akan digunakan untuk formulasi produk menyembuhkan luka. Penyelidikan ini bertujuan untuk mencapai tiga tujuan yang utama. Tujuan yang pertama adalah untuk membuat formulasi produk penyembuhan luka yang mengandungi protin keratin, untuk menghasilkan produk yang dapat menyembuhkan luka, dan untuk mengenalpasti rumusan terbaik untuk menghasilkan produk penyembuhan luka. Keseluruhan proses untuk menghasilkan kepingan hydrogel adalah seperti berikut. Proses yang pertama adalah menyediakan setiap bahan utama. Bahan-bahan utama adalah chitosan, polyvinyl alcohol (PVA) dan polyvinyl pyrrolidone (PVP). Kemudian, semua bahan-bahan itu akan dicampur bersama sambil dikacau secara berterusan di atas plat pengacau panas selama 2-3 jam. Selepas itu, semua sampel akan dikeringkan di dalam ketuhar pada suhu bilik. Kepingan hydrogel yang telah kering sepenuhnya dicabut dari bekas petri menggunakan forcep.

FORMULATION OF WOUND HEALING PRODUCT FROM KERATIN PROTEIN

ABSTRACT

Every year billion of chickens will be commercially grown up and processed worldwide. These chickens when processed will leave billion pounds of unused feathers. The challenge is to turn the white plumes into usable items or valuable products. In this research, keratin protein will be extracted from the unused chicken feathers and then will be used in the formulation of effective wound healing product. This research intends to achieve three main goals. Firstly, to formulate wound healing product contains keratin protein, to produce an effective wound healing product to heal the wound and to determine the best formulation of wound healing product. The overall process to produce a hydrogel sheet are as the following. First of all, the main solutions need to be prepared one by one. The main solutions are chitosan, polyvinyl alcohol (PVA) and polyvinyl pyrrolidone (PVP) solutions. Next, the hydrogel sheet was produced by mixing all the solution together with the continuos stirring on a hot plate stirrer for about 2-3 hours. After that, the samples were dried in an oven at room temperature. The fully dried hydrogel sheets were removed from the petri dish by using a forcep.

CHAPTER 1

INTRODUCTION

1.1 Background of Proposed Study

Keratin protein is a natural protein which is present abundantly in hair, nail and the other skin parts. Keratin has a high cysteine concentration compare to the other protein. The sulphur atoms in the cysteine residues tend to cross-link with one another and this makes the protein tough, strong, and lightweight.

The keratin protein will be extracted from chicken feathers for a further used. Chicken feathers consist of about 91% keratin, 1.3% fat, and 7.9% water. In order to use this protein, the number of chemicals needed must be identified to reduce the chicken feathers into the smaller amount of protein. After that, the protein obtained from the process can be used to produce many usable items such as wound healing product, shampoo, cosmetic product and so on.

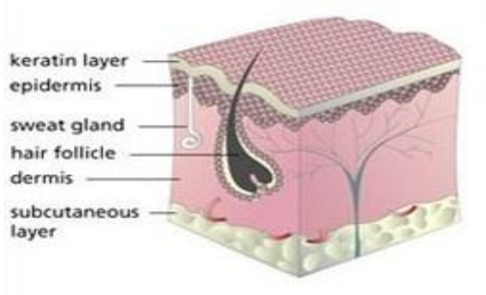

Figure 1.1: Human skin structure

Apart from that, skin is the largest organ in the body. It performs many vital roles as both a barrier and a regulating influence between the outside world and the controlled environment within our bodies. There are two main layers of skin that are epidermis and dermis. Epidermis is the outermost layer of the skin. The main skin cell that makes up the epidermis is called the keratinocyte. The keratinocyte will produce a tough protein called keratin. Keratinocytes arise in the deepest level of the epidermis and new cells are constantly being produced.

Hence, we can develop a product having a high percentage of keratin either in the form of cream or bandage to promote the healing process. By applying a wound healing product that contains a rich amount of keratin, a faster wound healing process can be achieved. This is because the wound can absorb keratin protein from the product and the production of the new cells to replace the old and the dead cells will take place.

1.2 Problem Statement

Every year billions of chickens will be commercially grown up and processed worldwide. These chickens when processed will leave billion pounds of unused feathers. The challenge is to turn the white plumes into usable items or valuable products. In addition, healing of wounds may be difficult and may result in problems such as ulcers and septicaemia. A proper treatment of these conditions is very important to maintain our health. Besides, attempts have been made to provide improved wound dressing by using biological materials such as growth factors. However, it has been proven very costly and shown a longer time for healing. As for now, a wound healing product is to be produced from keratin protein obtained from chicken feathers.

Furthermore, there is a lot of wound care products sold in the market. However, not all products can heal the wound perfectly. Certain products may leave some effects to the skin especially in case of the serious wound if the healing process is delayed. The effects such as larger scar area, skin discoloration will take place after the healing process end. Hence, this study intends to produce an effective wound healing product that accelerates the time taken for the wound to heal so that the side effects which may occur due to the delayed wound healing process can be avoided.

1.3 Research Objectives

This study is guided by the following research objectives:

1.3.1 To produce wound healing product contains keratin protein from chicken feathers.

1.3.2 To produce an effective wound healing product to heal the wound.

1.3.3 To determine the best formulation of wound healing product.

1.4 Scope of Proposed Study

The scope of the study is to produce an effective wound healing product from keratin protein. The keratin protein is obtained from the unused chicken feathers. The keratin protein will be extracted from the chicken feathers and then will be processed and purified in order to obtain the pure keratin. After that, the purified keratin protein will be further used as the main material in producing the wound healing product. As the outcome, the product is expected to be an effective wound healing product that promotes the healing process and reduce the time for the wound to heal.

1.5 Significance of Proposed Study

The production of wound healing products from keratin protein obtained from chicken feathers is an economically process. This is because the feathers can be obtained at lower cost and sometimes free of cost. So, when the cost of the production is low, the profit margin is very high. In addition, the difficulty to heal some chronic wounds can be overcome. Furthermore, the time taken for some wounds to heal also can be reduced.

1.6 Conclusion

In conclusion, this chapter briefly explained about the research. This chapter is divided by seven subtopics that are background of proposed study, problem statement, research objectives, scope of proposed study, expected outcome, significance of proposed study and conclusion of the chapter.

CHAPTER TWO

LITERATURE REVIEW

2.1 Wound

In easy words wound can be defined as a type of injury where the skin is torn, cut or punctured. According to Boateng et al (2008), a wound is defined as a defect or break in the skin, resulting from physical or thermal damages.

2.2 Wound Healing Process

Boateng et al (2008) stated that wound healing process is a series of independent and overlapping stages. In these stages both cellular and matrix compounds will work to re-establish the integrity of damaged tissue and replacement of the lost tissue. In addition, Guo and Dipietro (2010) stated that wound healing is a dynamic process consisting of four continuous, overlapping, and precisely programmed phases. The events of each phase must happen in a precise and regulated manner. Interruptions, aberrancies, or prolongation in the process can lead to delayed wound healing or a non-healing chronic wound. Deep wounds heal firstly through the formation of granulation tissue and then through epithelialisation. Shallow wounds where only the epidermis has been damaged heal through epithelialisation only.

These overlapping series can be classified in five stages (Boateng et al, 2008):

- Haemostasis and inflammation
- Migration
- Proliferation
- Maturation

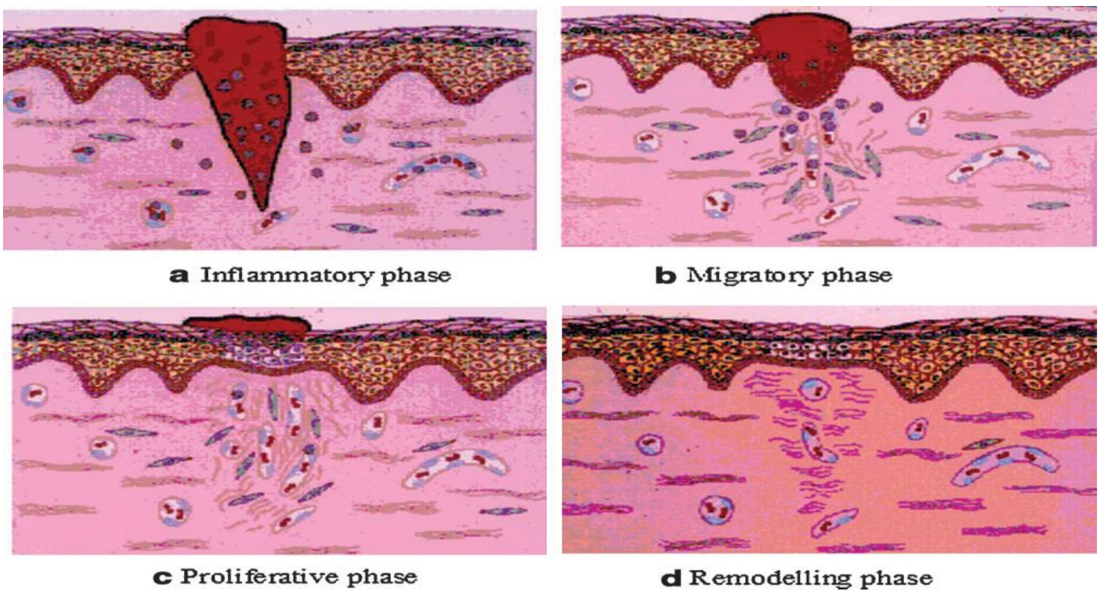

Figure 2.1: Wound healing process (Boateng et al, 2008)

2.2.1 Haemostasis

The first response to injury is bleeding. Bleeding is an effective way to wash out bacteria that are on the surface of skin. Afterwards, bleeding activates haemostasis stage that is initiated by clotting factors. The clot dries out and creates a hard surface over the wound that protects tissues underlying (Boateng et al, 2008).

2.2.2 Inflammation

This stage starts almost at same time as haemostasis. It occurs from between few minutes to up to 24 minutes after injury. In this stage histamine and serotonin are released into wound area and activate phagocytes to enter the wound area and engulf dead cells (Boateng et al, 2008)

2.2.3 Migration

In this stage the reestablishment of wound begins. The epithelial cells and fibroblasts move into the injured area and grow rapidly under the hard scab to replace the damaged tissue (Boateng et al, 2008).

2.2.4 Proliferation

This stage has three characteristics. First, the granulation tissue is formed by growth of capillaries. Second, lymphatic vessels enter into wound and the third one, synthesis of collagen starts providing form and strength to the injured tissue (Boateng et al, 2008).

2.2.5 Maturation

In this stage, the shape of the final scar is determined by formation of cellular connective tissue and strengthening of the new epithelium (Boateng et al,2008).

2.3 Wound Healing Dressings

According to Rahman et al (2006), one dressing agent is not suitable for the management of all types of wound and only a few of them are ideally suited for the treatment of a single wound during all stages of the healing process. Therefore, successful wound management depends to the selection and the use of products based on the understanding of the healing process combined with the knowledge of the properties of the various dressings available. The process of dressing selection is determined by a number of factors including the nature of the wound, location of the wound, and the range of materials available. However, in most situations the cost of treatment is also a major factor. In developed country, many sophisticated dressings are available which are made from a wide range of materials including polyurethane, salts of alginic acid and other gelable polysaccharides such as starch and carboxymethylcellulose. These materials are combined to form wound healing products such as film, foam, fibrous product, hydrogel and hydrocolloid dressing.

In addition, dressings are classified in a number of ways depending on their function in the wound, type of material employed to produce the dressing, and the physical form of the dressing. However, Boateng et al (2008) classified wound healing dressing as traditional and modern dressings. Traditional dressings include cotton wool, natural or synthetic bandages and gauzes. They may be used as a primary or secondary dressing or as a part in a several dressings by performing a specific function. While modern wound dressings includes hydrocolloid dressing, alginate dressing, hydrogel dressing, foam and film dressing. The main aim of modern wound dressing is to create a moist environment around the wound to enhance the healing process. Each modern dressing has their own characteristics that differ from one another. Some of the modern dressings with their own characteristic are as follow.

2.3.1 Hydrocolloid Dressing

This dressing is a combination of hydrocolloid materials that are gel forming agents and other materials such as elastomers and adhesives. It is widely clinically used because they can adhere to both dry and moist surface. These types of dressing are adhesive, occlusive and comfortable dressing.

2.3.2 Foam dressing

This dressing is porous polyurethane foam or polyurethane foam film. It is highly absorbent and preferred to gauze in term of pain reduction, patient acceptability and nursing time.

2.3.3 Hydrogel Dressing

Hydrogel is swellable hydrophilic material. It is made from synthetic polymers such as polymethacrylate or polyvinylpyrrolidone. Hydrogel can be produced in two shapes that are amorphous or solid sheet or films. If hydrogel is applied to the wound as gels, it needs a second cover such as gauze. On the other hand if it is applied as films to the wound, it can be used both as a primary and secondary dressing.

2.4 Formulation of Wound Healing Product

There are a lot of formulations used in order to produce wound healing products that have the ability to enhance wound healing process. One of the formulations is by using honey as the main material. According to Hollis (2007), the use of honey has renewed interest in human particularly for treatment of chronic and infected wounds. It has been shown that the antimicrobial effect of honey can fight against over 70 strains of bacteria commonly found in the wounds. Research has identified some of the topical effects of honey on wounds such as antibacterial and antimicrobial autolytic debridement, wound deodorising, stimulation of growth of wound tissues, anti-inflammatory activity including reduction in pain and oedema, and moist wound healing.

Collagen also can be used as one of the formulation to enhance wound healing process. Based on the research made by Kale et al (2011), collagen plays a major role in haemostasis in order to promote wound healing. Collagen binds to the specific receptor site on platelet membrane which helps in haemostasis stage. Apart from that, collagen also provides support for the growth of new capillaries that essential for the deposition of the new fibres. It also supports the growth, attachment, differentiation and migration of keratinocytes directly. Moreover, collagen provides a provisional matrix for keratinocytes migration by binding with fibronectin and it also helps in wound remodelling.

In addition, Kale et al (2011) also stated that besides collagen, gelatine also can be used to promote wound healing. By having similar structures and properties as of collagen, it also can be used as haemostatic and for wound remodelling. Gelatin has been used in wide variety of wound dressing. Recently gelatin has shown to exhibit activation of macrophages and high haemostatic effect. Furthermore, gelatin is practically more convenient than collagen because a concentrated collagen solution is extremely difficult to prepare and gelatin is far more economical than the collagen.

Tonin (2010) studied the biocompatibility of keratin protein. The research has identified that keratin-based materials can be used in biotechnological and biomedical fields for tissue engineering and the production of affinity membranes. This is due to their biocompatibility, their ability to support fibroblast growth and absorb heavy metal ions and volatile organic compounds. Moreover, Rouse and Van Dyke (2010) stated that over the past century the characterizations of keratin have led to the development of a keratin-based biomaterials platform. This is because keratin has intrinsic biological activity and biocompatibility like the other naturally-derived biomolecules. In addition, extracted keratins are capable of forming self-assembled structures that regulate cellular recognition and behaviour too. These qualities have led to the development of keratin biomaterials with applications in wound healing, drug delivery, tissue engineering, trauma and medical devices.

2.5 Keratin Protein in Chicken Feather

A large quantity of chicken feathers is available as a waste product in Malaysia. Chicken feathers have high percentage of keratin protein content and can be a suitable protein source. Based on the research by Tonin (2010), keratins represent a group of fibrous proteins with high sulphur content produced in some epithelial cells of vertebrate such as reptiles, birds and mammals. There are two kinds of keratins that are “hard-keratin” and “soft-keratin”. They are classified according to their physical and chemical properties particularly the sulphur content in them. Apart from that, soft keratins are found in the *stratum corneum* of the skin containing <3% wt of the sulphur content whereas the hard keratins having >3% wt sulphur content are found in hair, wool, feather, nails and horns.

According to Fan (2008), the main chemical structure of chicken feathers is keratin protein. Since the chicken feather fiber is mainly made up of the structural protein keratin, its chemical durability is primarily determined by keratin. Based on the research, chicken feathers could potentially be used for protein fiber production. The keratin protein contains in chicken feathers have some advantages compare to the other proteins. First of all, the fibrous feather keratin can stretch approximately 6% before breaking, unlike hair keratin that can stretch to only twice of its length. Feather keratin is also a special protein. It has a high content of cysteine (7%) in the amino acid sequence and cysteine has -SH groups that causes the sulfur–sulfur (disulfide) bonding. This high content of cysteine makes the keratin stable by forming network structure through joining adjacent polypeptides by disulfide cross-links. The feather keratin fiber is semi-crystalline and made up from a crystalline fiber phase and an amorphous protein matrix phase linked to each other. Moreover, the feather fiber shows good durability and resistance to degradation too. This is because keratin has extensive cross-linking and strong covalent bonding within its structure. Due to these advantages, feather keratin can be a good use to produce many better products.

Furthermore, according to Schmidt as cited in McGovern (2000), feather protein contained common properties with the other fibrous materials and feathers are keratin just like wool. However, the surface area is larger than the wool because of the diameter difference. So the fiber can absorb more than wool or cellulose fiber. Furthermore, the crystal structure of feather fiber makes them naturally stable and durable too. Besides, the properties that make feather fiber valuable are the keratin protein content in them. Hence, due to these properties, feathers can be put to good use in the manufacture of consumer goods, replacing wood pulp and other expensive fibers.

In addition, it is recognised that the chicken feathers consist about 90% of keratin. According to Wrześniewska-Tosik and Adamiec (2007), keratin from chicken feathers is a by-product which is available in great amounts but only used in a small degree. Furthermore, the keratin in chicken feathers is a very inconvenient and troublesome waste product of the poultry industry. Therefore, many researches have been made on how to turn this waste product into usable items. Wrześniewska-Tosik and Adamiec (2007) stated thatby considering the hydrophilic properties of the keratin, it can be used further for manufacturing fibres with increased sorption features that can be used to produce sanitary and medical applications.