# FORMULATION OF WOUND HEALING HYDROGEL BASED ON KERATIN DERIVED FROM CHICKEN FEATHER

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# ABSTRACT

A novel cross-linked keratin hydrogel dressing was developed by incorporating keratin from chicken feather into hydrogel dressing formulation. Keratin fibers from feathers are non-abrasive, eco-friendly, biodegradable, insoluble in organic solvents and also have good mechanical properties, low density, hydrophobic behaviour and finally low cost. In the matter of fact, keratinocytes (epidermal cells that manufacture and contain keratin) migrate from wound edges to cover the wound during healing process. These characteristics open a new approach to do research on keratin based hydrogel which derived from chicken feather for wound care product. In this study, the physical properties of keratin based hydrogel and its wound healing efficacy on small wounds in rabbits were assessed. Keratin based hydrogels have been done for five types of formulation. Optimum concentration of keratin has been identified from previous studies. Some of analysis of keratin based hydrogel includes spectroscopic analysis using Fourier transform infrared and Raman, microbial count analysis and heavy metal test using Inductively Coupled Plasma Mass Spectrometer (ICP-MS). XRD analysis was performed to analyse the crystallinity index of the hydrogel which lied between 30-50% of crystallinity. Morphological details are observed with optical, transmission and scanning electron microscopy. Swelling and solubility tests are carried out on the hydrogel to observe the solid content and water absorbance capacity of the hydrogel. Application of keratin hydrogel dressings significantly enhanced ($P < 0.05$) wound closure and accelerated the rate of re-epithelialization as compared to control hydrogel and other film dressing. Overall, this product is safe to use and acted as an effective wound healing product with appropriate hydrogel characteristics. Thus, the studies reviewed can be the scaffolding to increase the use of this protein for wound care product as the keratin from chicken feathers are shown as a novel eco-friendly material which is very beneficial and profitable.

# ABSTRAK

Kerja ini membentangkan satu penjagaan luka produk berkesan dan ekonomi yang dibuat daripada keratin yang diperolehi daripada bulu ayam . Keratinosit ( sel-sel epidermis yang mengeluarkan dan mengandungi keratin ) berhijrah dari tepi luka untuk menutup luka semasa proses penyembuhan . Bulu ayam digunakan sebagai sumber keratin mana ia mengandungi jumlah yang tinggi protein keratin dan ia adalah murah . Lima jenis perumusan dilakukan dalam bentuk hidrogel . Analisis kiraan mikrob dilakukan dan ia menunjukkan bakteria sifar dalam hidrogel selepas pensterilan . The Hidrogel diuji pada tikus untuk melihat masa yang diambil untuk menyembuhkan luka-luka . Ia menunjukkan bahawa keratin hidrogel berasaskan menyembuhkan luka-luka dengan lebih cepat berbanding dengan lain Hidrogel penjagaan luka . Bengkak dan kelarutan ujian dijalankan ke atas hidrogel untuk melihat kandungan pepejal dan keserapan air hidrogel itu . Hidrogel PVA - PVP berasaskan mempunyai 50-60 % daripada bengkak keupayaan yang boleh diterima untuk hidrogel untuk menyerap eksudat dari luka . Sementara itu, Hidrogel terkandung 40-70 % kandungan kelarutan untuk menyediakan komponen penting bagi kawasan yang cedera . Analisis SEM pada lima jenis Hidrogel menunjukkan jenis topografi yang poros , serpihan kristal , tidak rata dan licin . Analisis FTIR menunjukkan bahawa kewujudan ikatan hidrogen dalam hidrogel yang memegang hidrogel dengan rapi. Ujian logam berat ( ICMPS ) telah dilakukan dan menunjukkan tiada apa-apa logam berat yang berbahaya dalam hidrogelnya. Secara keseluruhan, produk ini selamat untuk digunakan dan bertindak sebagai produk penyembuhan luka berkesan dengan ciri-ciri hidrogel yang sesuai.

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# LIST OF ABBREVIATIONS

| | |
|---|---|
| $A_{crystal}$ | area under curve that have peak |
| $A_{total}$ | total area under curve |
| *API* | active pharmaceutical ingredients |
| FTIR | Fourier Transforrm Infrared & Raman |
| IR | Irradiation |
| mm | millimeter |
| μm | micro meter ($10^{-6}$) |
| PEG | polyethylene glycol |
| PPG | polypropylene glycol |
| PVA | polyvinyl alcohol |
| PVP | polyvinyl pyrollidone |
| SEM | Scanning Electron Microscopy |
| SIFP | Sulphonated Intermediate Filament Protein |
| w/w | ratio of weight of component with total weight of components |
| UV | ultra violet |
| XRD | X-Ray Diffraction |

# LIST OF SYMBOLS

| | |
|---|---|
| % | Percentage |
| °C | Degree Celcius |
| Θ | Angle of Diffraction |
| Ø | Porosity |

# 1 INTRODUCTION

## *1.1 Motivation and statement of problem*

Many products have been developed using biomaterial in wound care product to enhance recovery of wounds. Usage of biomaterials in the product is due to development of a matrix or scaffolding system that mimics the structure and function of native tissue. For this purpose, many researchers have discovered the benefit of natural macromolecules in medicinal field for their intrinsic ability to perform very specific biochemical, mechanical and structural roles. In particular, protein-based biomaterials have become the interest among researchers for many biomedical and biotechnological applications due their ability to function as a synthetic extracellular matrix that facilitates cell-cell and cell-matrix interactions. The protein components contain a defined, three-dimensional microstructure that aids in cellular proliferation and cell-guided tissue formation, which are essential characteristics for biomaterial scaffolds (Roland et al., 2008). In addition, the strong bioactivities and diverse physiochemical properties of proteinaceous macromolecules are attractive for other biomedical applications for which biocompatibility are crucial, such as medical devices, bioactive surfaces, hygiene products, etc. Several proteins have been investigated in the development of naturally-derived biomaterials, including collagen, albumin, gelatin, fibroin and keratin. Of these, keratin-based materials have shown promise for revolutionizing the biomaterial world due to their intrinsic biocompatibility, biodegradability, mechanical durability, and natural abundance (Rouse & Van Dyke, 2010).

There are various of wounds made on human's skin. It depends on cause and infection that occured on the patients' body. To assess the wound and identify the correct treatment, patients' body condition is essential to consider (Baranoski,2001). Wound healing is a complex biological process that consists of haemostasis, inflammation, proliferation, and remodeling. Multiple factors can cause impaired wound healing by affecting one or more phases of the process and are categorized into local and systemic factors. Many suffered from wound healing problem. Single or multiples factors may play a role in any one or more individual phases, contributing to the overall outcome of the healing process. Conducive environment around the wound is essential for healing process. To have an effective healing process, best dressing should be provided where it can protect the injured tissue, maintains a moist environment, water permeable, maintains microbial control and delivers healing agents to the wound. As the healing progress, the treatment for the wound will be differ. For example, as the drainage slows, the dressing choice will no longer be one that absorbs exudates, but possibly one that donates or retains moisture. Intact keratin protein fraction, particularly sulphonated intermediate filament protein is extracted from chicken feather. This is due to keratin protein characteristics which is biocompatible and possess some structural form of skin. Thus, it has the capability to serve the wound by being so-called skin over it and provide an appropriate environment for the healing process to occur. This study is intended to produce an effective wound healing product that accelerates the time taken for the wound healing process.

When assessing the wound and the patient, the best step to make first is determining the etiology of the wound. Recognising the factors that affect patient's ability of healing determines the right treatment and overcome the slow healing and incomplete healed wound (Kerstein, 1994). There are many types of wounds which need to different treatment and dressing according to the conditions. The wounds are acute wounds, chronic wounds, pressure ulcer, lower extremity wounds, lymphedema, suspected deep tissue injury, Kennedy terminal ulcers, surgical site incision and skin tears (Kane & , 2001).

According to (Barone, 2009), 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 , 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 (Blanchard et al., 1997).Other than that, chicken feathers is available abundantly as a poultry waste, thus it becomes another advantage to produced as environmental friendly product.

To promote the healing process, aloe vera, chitosan, and honey are included in the formulation due to their healing property (Tao Wang et al., 2012). Chitosan exihibits lack of toxicity and allergenicity, and its biocompatibility, biodegradability and bioactivity make it a very attractive substance for diverse applications as a biomaterial in pharmaceutical and medical fields (Sevda S et al., 2004). Honey is one of the most enduring materials to be used in wound care, attributed to its antibacterial, anti-inflammatory, and antioxidant properties (Molan, 1999). Aloe vera gel has been used throughout history for its therapeutic healing effect on burns, insect bites, and other human and animal injuries (Viswanathan et al., 1993).

As conclusion, the response of materials to wound exudates and the biochemical environment provided by keratin protein becomes very essential for their performance in the wound. An optimum wound dressing protects the injured tissue, maintains a moist environment, water permeable, maintains microbial control , delivers healing agents to the wound, easy to apply, does not require frequent dressings and non-toxic and non-antigenic (Boateng et al., 2007). Addition with healing agents, the wound healing gel showed an effective healing result on wound it's applied.

## *1.2 Objectives*

The following are the objectives of this research:

- To provide wound healing product containing keratin protein.
- To provide an effective wound healing product in aspects of time taken for the wound to be healed and normality of the wound area

## *1.3 Scope of this research*

The following are the scope of this research:

i) The formulation of wound healing gel is carried out.

ii) The product is characterized for their thermal physiochemical properties.

iii) The healing power of the product determined by handling clinical and toxicological test.

iv) The best formulation is chosen by observing the result on clinical test on animal.

## *1.4 Main contribution of this work*

The following are the contributions:-

- An effective wound healing product for society
- Production of a cheap and environmental friendly wound care product

## *1.5 Organisation of this thesis*

The structure of the reminder of the thesis is outlined as follow:

Chapter 2 provides information of the applications and benefits of biomaterial in medicinal field. A general description on skin structure, types of wound encountered by human being and factors affecting the wounds on skin, formation of hydro gel and healing process of the wound. This chapter also provides a brief discussion of keratin protein and its characteristics of healing ability when applied on wounds. Information about other healing agents are also covered which is included in the hydro gel formulation.

Chapter 3 discussed about the method used to make solution of chemicals used in hydro gel formulation. There are five formulation proposed in three different concentration of substance respectively. Then, the best formulation of hydro gel is identified by undergoing several testing on pH value, morphological details using scanning electron microscope, x-ray diffraction, heavy metal test, microbial count, Fourier transform infrared and Raman and testing. Toxicology testing is done by applying the hydro gel on artificial wound on rabbit in order to find the healing power of the hydro gel.

Chapter 4 is report on method and methodology and also includes results and discussion on five types of hydrogels that formed from keratin derived from chicken feather.

Chapter 5 draws together a summary of the thesis and outlines the future work which might be derived from the model developed in this work.

# 2 LITERATURE REVIEW

## *2.1 Overview*

In this study, biomaterial that used in the wound care product is keratin protein which presents in a wide range of biological tissue, performing a structural role in skin, hair and feathers. Non-healing wounds constitute a major problem for society, as they lead to social isolation of the patient, prolonged inability to work and have considerable effects on quality of life. Chronic wounds affect around millions of patients globally. It is claimed that an excess of billions is spent annually on treatment of chronic wounds and the burden is growing rapidly due to increasing health care costs, an aging population and a sharp rise in the incidence of diabetes and obesity worldwide. Thus, research on keratin proteins is conducted on wound healing product to give an effective result on all types of wounds. It is identified that keratin have regulatory functions and participated in wound healing. Moreover, chicken feather is used as keratin source as it consists of high amount of keratin protein, hence decrease the cost of this product. To conduct this experiment, the chemicals are prepared in desired concentration and mixed according to the formulations. Freeze and thaw method is handled to keratinize the wound healing gel. The hydro gel is tested on rats to observe the time taken to heal the wounds. It showed that keratin based hydro gel healed the wounds more quickly compared to other wound care product. Overall, this product ensured providing a moist environment around the wound to enhance the healing process. This can be achieved by having keratin protein component which acted as secondary skin for the wound. Thus, healing agents are easily delivered to wounded area and maintains microbial control.

## *2.2 Introduction*

Skin is the largest organ that protects our body from outside environment. It helps to protect our inner organ and regulates our body temperature. It plays a vital part in every living thing. One small cut or sore on our body can disturb our skin's function. Other than that, it can spoil one's appearance. The major problem for the skin is wounds. Wounds can be due to surgery, burns, abrasions, septicemia, skin grafts, ulcers and traumatic injury. The importance for treatments for these conditions is increasing as the population ages. The biochemical processes involved in wound healing are homeostasis and inflammation, granulation tissue formation and reepithelization and remodeling.

These processes are disrupted if the wound is prolonged and the release of the destructive enzyme by inflammatory cells.

Conducive environment is essential for healing process. Many products have been developed to enable faster recovery of wounds. The materials used for the dressings are biocompatible to some extent and include polylactic acid, chitin, alginate derivatives and collagen. The response of these materials to wound exudates and the biochemical environment provided by these materials are very essential for their performance in the wound. An optimum wound dressing protects the injured tissue, maintains a moist environment, water permeable, maintains microbial control , delivers healing agents to the wound, easy to apply, does not require frequent dressings and non-toxic and non-antigenic.

In this study, a wound healing gel is developed based on keratin protein. Keratin protein is presents in a wide range of biological tissue, performing structural role in skin, hair and other materials. The core components of keratin fibre are specifically the high sulphur concentrated intermediate filament proteins and the matrix proteins present there plays a crucial part within the fibre which is reflected in their tertiary structure and amino acid composition. To invent the keratin based hydro gel, firstly, the keratin should be isolated from the keratin source by using a wild method for not damaging the protein and create the cystine modification that is reversible. Chicken feather is used as keratin source as it is cheap unwanted biomaterial yet has high keratin protein. Besides that, it is environmental-friendly and economical.

## *2.3 Wound*

Generally, wound is well known as injury. It is usually involving separation of tissue or rupture of the integument or mucuos membrane, due to external violence or some mechanical agency rather than disease. More scientifically, wound is a physical injury to the body consisting of a laceration or breaking of the skin or mucous membrane, or an opening made in the skin or a membrane of the body incidental to a surgical operation or procedure. Wounds may be acute or chronic trauma resulting from an injury where, the injury does not heal because of a number of factors. Acute wounds can be a occurred in planned or unplanned event, and healing typically proceeds in an orderly and timely fashion. Examples of acute wounds include a cut, graze or burn. Examples of chronic wounds include leg ulcers, pressure wounds and diabetic wounds (Lee et al., 2006).

## 2.4 *Types of wounds*

There are various of wounds made on human's skin. It depends on cause and infection that occured on the patients' body. To assess the wound and identify the correct treatment, patients' body condition is essential to consider (Baranoski S & Lippincott). As the healing progress, the treatment for the wound will be differ. For example, as the drainage slows, the dressing choice will no longer be one that absorbs exudates, but possibly one that donates or retains moisture.

When assessing the wound and the patient, the best step to make first is determining the etiology of the wound. Recognising the factors that affect patient's ability of healing determines the right treatment and overcome the slow healing and incomplete healed wound (Black, 2003). Types of wounds as follows:-

I. Acute Wounds
II. Chronic Wounds
III. Pressure Ulcer
IV. Lower Extremity Wounds
V. Lymphedema
VI. Suspected Deep Tissue Injury
VII. Kennedy Terminal Ulcers
VIII. Surgical Site Incision
IX. Skin Tears

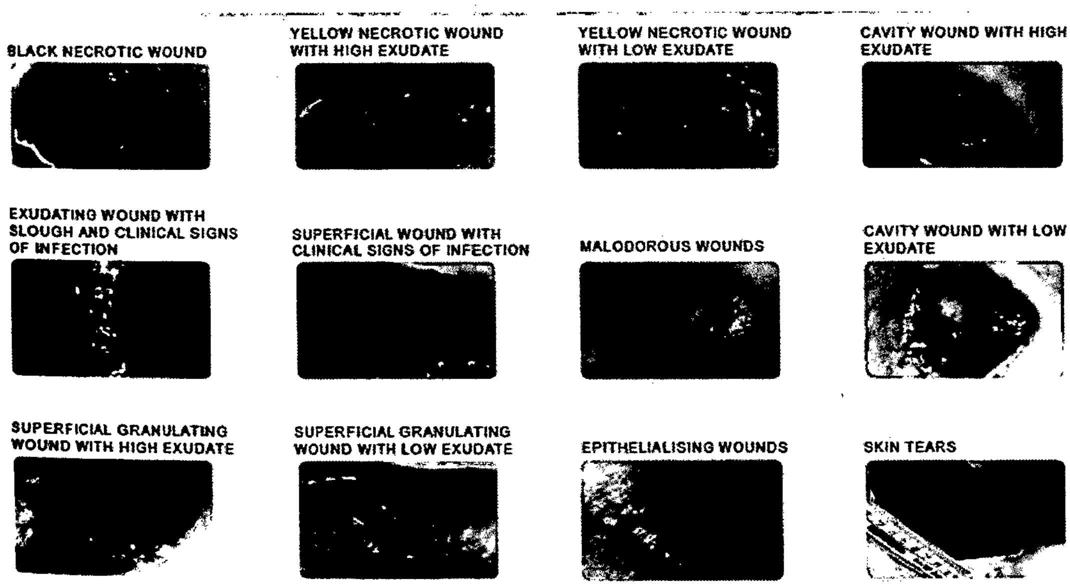


Figure 2-1: Illustration of the types of wounds by Smith (1991)

## 2.5 *Factors affecting the wound*

Multiple factors can lead to impaired wound healing. In general terms, the factors that influence repair can be categorized into local and systemic. Local factors are those that directly influence the characteristics of the wound itself, while systemic factors are the overall health or disease state of the individual that affect his or her ability to heal. Many of these factors are related, and the systemic factors act through the local effects affecting wound healing involved. The factors discussed include oxygenation, infection, age and sex hormones, stress, diabetes, obesity, medications, alcoholism, smoking and nutrition. A better understanding of the influence of these factors on repair may lead to therapeutics that improve wound healing and resolve impaired wounds.

Table 2-1: Illustration of factors affecting the wound healing. (Cutting and White, 2002)

| Local Factors | Systemic Factors |
|---|---|
| **Oxygenation** | Age and Gender |
| **Infection** | Sex Hormones |
| **Foreign Body** | Stress |
| **Venous Sufficiency** | Ischemia |
| | Obesity |
| | Nutrition |
| | Diseases: diabetes, keloids, fibrosis, heredity healing disorders, jaundice,, uremia |
| | Medications: glucocorticoid steroids, non-steroidal anti-inflammatory drugs, chemotherapy |
| | Alcoholism and Smoking |
| | Immunocompromised conditions: cancer, radiation therapy, AIDS |

## 2.6 *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 reestablish the integrity of damaged tissue and replacement of the lost tissue. In addition, Armstrong and Ruckley (1997) 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.

There are three phases of wound healing:

- inflammatory (destructive)
- proliferative (regenerative)
- maturation (reparative)

During all of these phases there are a number of cells that are essential to the process of the healing including platelets, neutrophils, macrophages and fibroblasts. Some of the cells which you may think are only present for one particular phase of the healing process are there from the very beginning of the wound, through to the ultimate healing of the wound. The critical thing is that the phases of healing are a continuum. Each phase continues in a steady process merging with the next phase. In fact, one wound may be in more than one phase at one time (Krasner, 1995).

## *2.7 Phases of Healing*

### *2.7.1 The inflammatory phase*

The inflammatory phase is normally the shortest, and is manifest at the beginning of the wound itself where a wound is either surgically created, caused by trauma or some other reason. There will be bleeding, and a clot will develop to induce haemostasis (clotting). The tissue around the wound may often be red, hot, sore and swollen. This does not indicate infection, it is merely the inflammatory process itself and, consequently, it is not always necessary to apply topical antiseptics or topical antibiotics. Wound exudates is often copious during the inflammatory phase. It is not a passive component of the healing process, but serves to nourish the tissues and to flush out necrotic tissue and foreign debris from the wound. The inflammatory phase uses the exudates as a support medium for enzymes, antibodies and the various cells necessary for wound cleansing. Exudates are also contains a number of growth factors produced by the body which are critical in the promotion of healing. If the inflammatory phase is impaired, wound healing may be slowed or halted. The continued presence of foreign material, necrotic tissue, excessive antimicrobial use or other continued disruption of the wound can result in prolonged inflammation thereby preventing the onset of proliferation and maturation. In turn, this may lead to fibroses tissue. Some of the factors that will affect the inflammatory phase include continued tissue destruction due to pressure not being relieved, dryness and desiccation, poor blood supply, clinical infection, or thermal shock during the inflammatory phase. The most important cells are the platelets whose task is not only haemostasis, but also the production of a growth factor which is important in stimulating the healing process.

During this phase the destruction of non-viable tissue and bacteria occurs. Neutrophils are involved in phagocytosis (killing) of bacteria as well as aiding in the extra-cellular release of protease – an enzyme used by the body for the destruction of necrotic tissue. The macrophages that are involved in phagocytosis of micro-organisms and necrotic tissue also release a growth factor important in the healing cascade.

### *2.7.2 The Proliferative Phase*

During this phase the new vascular bed is formed by angio-genesis. Capillary buds are formed which link up with the existing capillary network and allow oxygenated blood to provide a lush bed of capillary vessels. During the proliferative phase collagen is deposited by the fibroblasts. Collagen is the essential framework for the connective tissue which will eventually fill the wound. Fibroblasts also synthesise proteoglycans, or ground substance. It is collagen and ground substance which form the scaffolding for wound repair. Collagen is laid down in a wound over a period of weeks, after which time no new collagen is produced. The fibroblasts produce enzymes which help break down the collagen. The collagen then realigns itself by cross-linking, resulting in tensile strength in the wound. There are a number of elements essential for collagen production and deposition including vitamin C, oxygen, iron and zinc. A deficiency in these nutrients could lead to the development of a weakly bonded matrix, and ultimately to dehiscence of the wound.

During the proliferative phase, wound contraction occurs. This is an action of the myofibroblasts, contractile cells which pull the wound margins together. Contraction and granulation are the processes by which the wound becomes smaller. During the latter stage of the proliferative phase, epithelialisation occurs. This involves the growth of the epidermal cells over the surface of the granulation tissue, a process which is completed most efficiently in a moist, clean environment.

### *2.7.3 The Maturative Phase*

The maturation phase is the final stage of healing. During this stage the fibroblasts decrease in number, vascularisation decreases, and the tensile strength of the wound increases. Maturation is the most misunderstood phase of healing. It is assumed that a wound is healing once the epithelium has closed the surface of the wound. Tensile strength of the wound may in fact take quite a considerable time to develop, and in some patients it can take up to twelve months. It is often incorrect to view a re-ephithelialised wound and think that the wound is totally healed beneath the surface. The lack of tensile strength in a wound will increase the risk of breakdown that may be related to tension of the tissue below the surface.

Under the influence of ADP (adenosine diphosphate) leaking from damaged tissues the platelets aggregate and adhere to the exposed collagen3. They also secrete factors which interact with and stimulate the intrinsic clotting cascade through the production of *thrombin*, which in turn initiates the formation of *fibrin* from *fibrinogen*. The fibrin mesh strengthens the platelet aggregate into a stable hemostatic plug. Finally platelets also secrete cytokines such as *platelet-derived growth factor* (PDGF), which is recognized as one of the first factors secreted in initiating subsequent steps. Hemostasis occurs within minutes of the initial injury unless there are underlying clotting disorders.

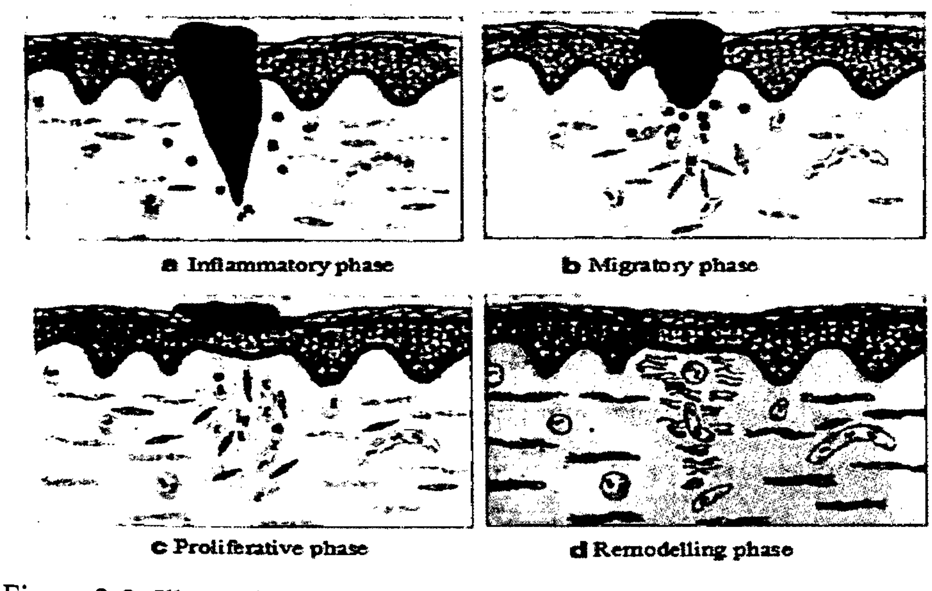


Figure 2-2: Illustration of the types of wounds by Krasner (1995)

## 2.8 *Wound Healing Dressing*

Several factors apart from the choice of wound dressings need to be considered to ensure successful wound healing. In the case of chronic wounds, underlying factors such as disease, drug therapy and patient circumstance must all be reviewed and addressed before a particular wound dressing is applied. Several methods are employed for wound debridement including: surgical removal using scalpel and scissors, hydrotherapy or wound irrigation and autolytic removal by rehydration of necrotic tissue, for example using hydrogel dressings, enzymatic removal using bacterial derived collagenases or preparations such as streptokinase. The various types of debridement in terms of their advantages and disadvantages as well as the basis for their clinical efficacy and safety are studied (Fallabela, 2006). There has been a resurrection of the ancient use of maggots for the debridement of wound surfaces and these insect larvae are now bred under aseptic conditions in the laboratory for such use. Maggots debride necrotic and sloughy wounds by dissolving only dead and infected tissue (Stoddard, 1995). This is achieved by the secretion of proteolytic enzymes that liquefy necrotic tissue. and allows them to absorb the dead tissue in a semi-liquid form over the course of several days. In addition to removing necrotic tissue, maggots disinfect wounds by killing bacteria and also stimulate faster wound healing especially for chronic wounds. It has been suggested that maggots also stimulate the production of granulation tissue (Wollina et al. 2000). A key objective in the choice of a dressing is to 'provide an environment at the surface of the wound in which healing could take place at the maximum rate consistent with a completely healed wound, having an acceptable cosmetic appearance' (Thomas, 1990). In most cases, a combination of dressings is needed in order to achieve complete wound healing in a reasonable time. Some dressings such as gauze and saline are useful for the initials stages of wound healing for absorbing blood and exudates, cleansing and debridement. Other dressings provide a moist environment during the latter stages of wound healing, whilst some medicated dressings and biomaterials can take active part in all the stages of wound healing and a detailed discussion is found in the ensuing sections.

It is important to remove necrotic tissue or foreign material from areas around the wound to increase the chances of wound healing and this process is known as wound debridement. Debridement of the wound area is important because the open wound bed cannot be observed and assessed effectively with necrotic tissue. The presence of necrotic tissue or foreign material in a wound also increases the risk of infection and sepsis and also prolongs the inflammatory phase.

According to Chang et al. (1998), one dressing agent is not suitable for the management of all types of wounds 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 characteristic that differ from one another. Some of the modern dressings with their own characteristic are as follow.

I. Hydrocolloid dressing
II. Foam dressing
III. Hydrogel Dressing

## *2.9 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 formulation is by using honey as the main material. According to Sherlock et al. (2010), 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 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, Stoddard et al (1995) 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.

Douglas (2003) 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, Tanabe et al. (2002), 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 behavior too. These qualities have led to the development of keratin biomaterials with applications in wound healing, drug delivery, tissue engineering, trauma and medical devices.

## *2.10 Keratin Protein in Chicken Feathers*

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 Huda and Yang (2008), 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 Berg et al. (2002), 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