FORMULATION OF WOUND HEALING HYDROGEL FROM KERATIN PROTEIN

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ABSTRACT

Keratin is a natural protein extracted from the chicken feather. In the developed country, the usage of keratin in the personal care products is widely used. The personal care product produced from the keratin protein is conditioning shampoo, anti aging cream, facial cleanser and others. There are some differences between the personal care products already produced by the other developed country because the raw materials to extract the protein is sheep wool while in this research, the extraction of keratin protein is from chicken feathers. Keratin solution, polyvinyl alcohol. Polyvinyl pyrrolidone, glutaraldehyde and ammonium thioglycollate was mixed intimately. The combination of the solution was then cast and hardened through a freezing-thawing process for 1 hour and this process was repeated up to 7 times to obtain a hydrogel. The related test was conducted to test the effectiveness of the wound healing product.

ABSTRAK

Keratin ialah protein semulajadi yang di ekstrak daripada bulu ayam. Di negara membangun, penggunaan keratin dalam produk pengajaan diri sangat meluas. Produk pengajaan diri yang di buat menggunakan keratin ialah shampoo, krim anti menuaan, pencucui muka, ubat menyembuh luka dan sebagainya. Terdapat beberapa perbezaandi antara produt penjagaan diri yang di buat di negara menbangun kerana mereka menggunakan ekstrak keratin daripada bulu kambing biri-biri manakal di dalam kajian ini mneggunakan ekstrak keratin daripada bulu ayam. Keratin, polyvinyl alcohol, polyvinyl pyrrolidone glutaraldehyde dan ammonium thioglycollate di campurkan. Campuran tersebut melalui proses beku dan rendamselama satu jam. Proses ini di lakukan sebanyak tujuh kali untuk mendapatkn gel. Ujian terhadapgel tersebut di lakukanbagi menguji keberkesanan gel tersebut.

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LIST OF ABBREVIATIONS & SYMBOLS

FTIR	Fourier-Transform Infra-Red
PVA	Polyvinyl Alcohol
PVP	Polyvinyl Pyrrolidone
SIFP	S-Sulfonated Keratin Intermediate Filament Protein
SHSP	S-Sulfonated Keratin High Sulfur Protein

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Chicken feather wastage is made up approximately eleven million pound from the commercial poultry processing plant annually. The disposal process for chicken feather is expensive. It is also can be difficult because the chicken feather is burning up with the incinerator plant, buried in the soils and also recycled as a low quality of poultry foods. These processes mostly give the bad effects to the environment, especially the burning of chicken feather which will release the green house gasses in the air. There are several alternatives invented based on the chicken feather application, but the wastage of chicken feather is still does not change as much as possible because of its low requirement.

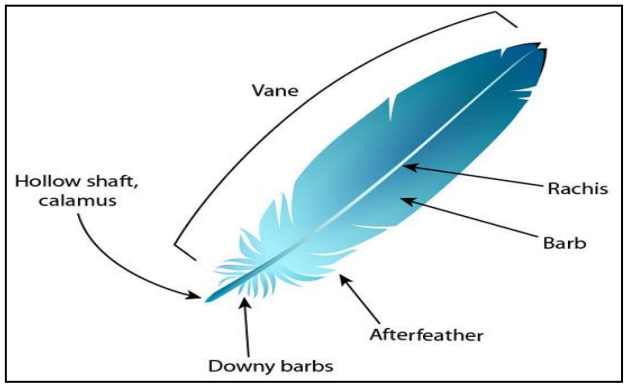

Figure 1.1: The anatomy of a chicken feather

Keratin is a natural protein extracted from the chicken feather as shown in Figure 1.1. In the developed country, the usage of keratin in the personal care products

is widely used. The personal care product produced from the keratin protein is conditioning shampoo, anti aging cream, facial cleanser and others. There are some differences between the personal care products already produced by the other developed country because the raw materials to extract the protein is sheep wool while in this research, the extraction of keratin protein is from chicken feathers. These two materials will produce two different sequences of amino acids.

1.2 Problem Statement

Nowadays, the demand of the chicken is increasing throughout the year from the consumer. Poultry slaughterhouses produce large amount of feather. Further, burning feather in special installations is very economically ineffective. The uncontrolled disposal of feather leads to pollution in our environment. Five percent of the body weight of poultry is feather.

Demand from consumer for keratin based product such as wound healing is increasing. There are variety of events that wounds and lesions can be caused such as surgery, traumatic injury, burn, abrasion and also skin grafts. The different result and problem will occur in healing process (Kelly et al. 2010).

1.3 Research Objectives

1.3.1 To extract the keratin protein from the chicken feather.
1.3.2 To prepare the formulation of biomedical product from keratin protein.
1.3.3 To produce the wound care product containing keratin from chicken feather.
1.3.4 To analyse the wound care product.

1.4 Scope of Research

To achieve the objective of this research, the scopes have been identified in this research. The scopes of this research are listed as below:-

1.4.1 Study the extraction of keratin protein from chicken feather

1.4.2 Freezing-thawing method will be used to produce hydrogel wound care product.

1.4.3 Testing on the animal will be conducted to analyse the wound care product effectiveness.

1.5 Significance of Study

The research is to be done to develop new product which is wound care product. The research try to develop new formulation of wound care product that containing natural keratin protein extracted from chicken feather. Natural keratin protein extracted from chicken feather has a wide range of use in biomedical product such as wound care product that contains beta keratin and acid amino composition.

CHAPTER 2

LITERATURE REVIEW

2.1 Feather

Chicken feather is a material which produced keratin protein. “The structures of the chicken feather consist of beta keratin as its major structural instead of alpha keratin (R.H. Sawyer et al., 2000)”. The keratin protein is used in bio-medical and cosmetic products and this research is about the development of these products with the usage of chicken feather as a keratin source.

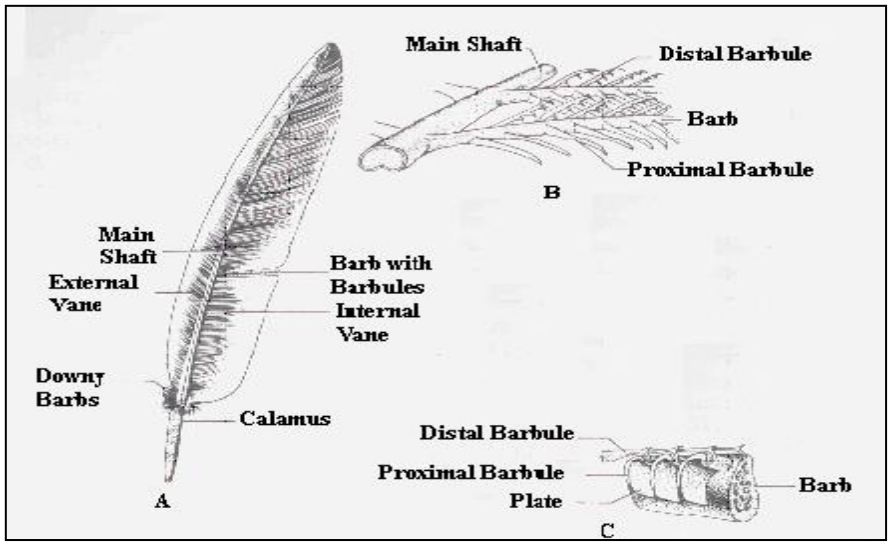

Figure 2.1: Structure of chicken feather

2.2 Protein

Proteins play a big role in the human system daily life which provides the structure to the human body system and also transporting oxygen in the human blood regulating system. Human bodies depending on proteins to perform the life better and protein also have its composition and structure. The differences of each protein classification play its different role in the human body.

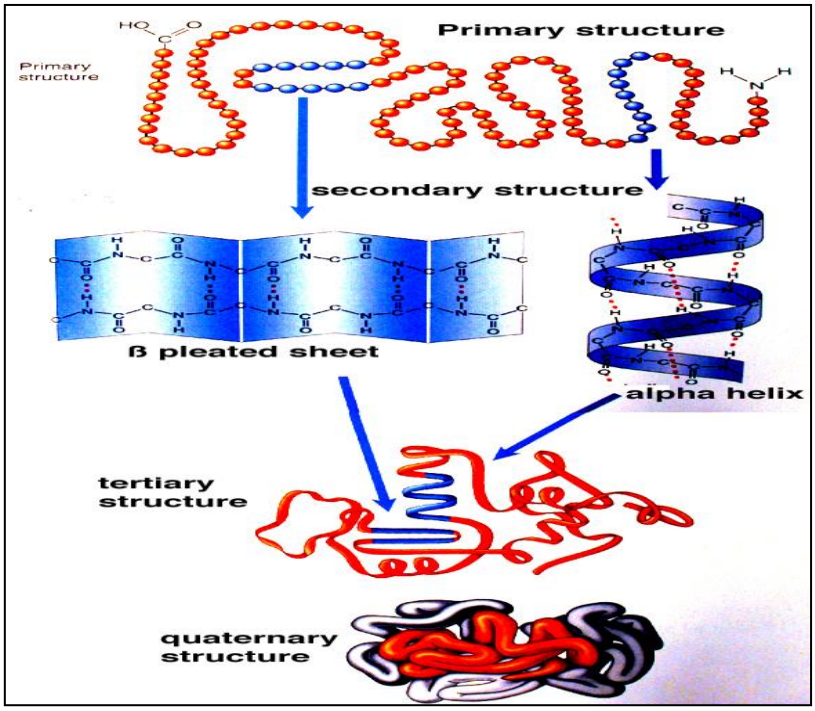

Figure 2.2: The protein structure

2.3 Keratin

The term ‘keratin’ originally referred to the broad category of in soluble proteins that associate as intermediate filaments (Ifs) and form the bulk of cytoplasmic epithelia and epidermal appendage structures (hair, wool, horns, hooves and nails) (Jillian G. Rouse, Mark E. Van Dyke, 2009).

As per Claudio et.al (2010) keratin represent a group of fibrous protein with high sulphur content produces in some epithelial cell of vertebrate such as reptiles, birds and mammals. In particular, the cysteine amino acid residues form inter and intra molecular disulphide bonds (cysteine residues) that give rise to a compact three-dimensional structural that confers to keratin proteins a high resistance to chemical and enzymatic attacks (Dawling et.al 1986).

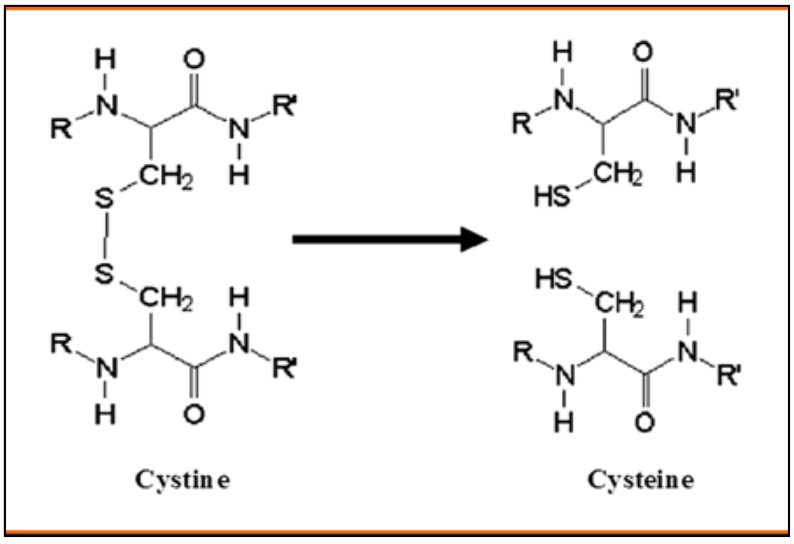

Figure 2.3: The reduction of disulfide bond

There are two kinks of keratins according to the physical and chemical properties (particularly the sulphur content) which is ‘hard-keratin’ and ‘soft-keratin’.

The content of sulphur in 'hard-keratin' found in hair, wool, feathers, nails and horns is > 3% whereas in 'soft-keratin' is < 3% (Fraser et.al 1972).

Table 2.1: The Amino Acid Composition of Chicken Feathers

Amino Acid	uM/mg Protein*[1]	% Amino Acid in Protein
Aspartic Acid	0.358	4.76
Threonine	0.345	4.11
Serine	1.292	13.57
Proline	0.875	1.01
Glutamic Acid	0.624	9.18
Glycine	1.008	7.57
Alanine	0.411	3.66
Valine	0.618	7.24
Cystine	0.088	2.11
Methionine	0.017	0.025
Isoleucine	0.376	4.93
Leucine	0.570	7.48
Tyrosine	0.102	1.85
Phenylalnine	0.267	4.11
Lysine	0.039	0.57
Histidine	0.001	0.016
Arginine	0.377	6.57

*Based on sample as 100% protein
[1]Micro mole per milligram of protein

The keratin are the proteins of epidermal and skeletal tissues which are insoluble in the normal protein solvents, neither do digested by trypsin or pepsin and have high cystine content. It is shown in "a study on keratin" journal by David R. Goddard and Leonor Michaelis that chicken feather keratins can be converted to natural protein soluble in alkali or acid and digestible by trypsin and pepsin. This was accomplished by breaking the disulfide bonds of the keratin. The β-keratins of reptiles and birds have β-pleated sheets twisted together, then stabilized and hardened by disulfide bonds. So by breaking these disulfide bonds, strength of the keratin in the chicken feathers can be reduced thus become soluble and converted to natural protein.

Keratin is insoluble in water, weak acids and bases as well as in organic solvents. The amino acid content of keratin is characterised by a high cyctine content(and at the same time sulphur), which may change within 2% wt and 18%wt, a significant amount of hydroxyaminoacids, especially serine (about 15 % wt), and a lack of hydroxyproline and hydroxylisine, among other substances. The chemical activity of keratin is connected in a significant degree to the crytine content. The disulphide bonds which is formed between two cysteine molecules is responsible for the high strength of keratin and its resistance against the action of proteolitic enzymes. On the other hand, keratin is very reactive, as cystine can easily be reduced, oxidised, and hydrolysed. In order to precisely determine the possible future applications of keratin, it is necessary to learn in detail the structure the potential possibilities of this valuable protein. (Krystyna Wrzesnieska-Tosik, 2007).

2.4 Biomedical Product

Robert James Kelly et. all., (2010) studied the composite materials containing keratin. In their findings, the application represents materials derived from keratin proteins in combination with polymers, either as intimate mixtures with water soluble polymer, or as chemically bound copolymers. An amino acid composition of keratin fractions: S-sulfonated keratin intermediate filament protein (SIFP), pepetides derived from S-sulfonated keratin intermediate filament protein (SIFP-pep), S-sulfonated keratin high sulfur protein (SHSP), peptides derived from S-sulfonated keratin high sulphur protein (SHSP-pep), S-sulfonated keratin peptide (SPEP) as used in the invention. The conversion is confirmed using Fourier-Transform Infra-Red (FTIR) spectroscopic studied as the S-sulfonated group gives rise to a strong and sharp absorbance at 1022 cm-1 which is observed to disappear on exposure of the S-sulfonated to the reagents described. Using a freezing-thawing process during the constructing composite films or membranes can improve of the physical and mechanical properties of the keratin-co-polymer composites.

Alisa Dawn Roddick-Lanzilotta et. all. (2010) studied the wound care products containing keratin. This invention relates to would care product that provides a biochemical environment around a wound to promote wound healing. In their inventions to provide a keratin protein fraction that is intact and S-sulfonated for use in wound care. This invention also provides a material for treating a wound including a keratin protein fraction in which the protein fraction is fraction is from the among filament protein family. A material for treating a wound includes a keratin protein fraction in which the protein fraction is s-sulfonated. The protein fraction may be hydrolysed, preferably s-sulfonated, from high sulphur protein family and an among filament protein.

Sierpinski et al and Apel et al demonstrated that keratin-based hydrogels were neuroinductive and capable of facilitating regeneration in a peripheral nerve injury model. Keratin-filled hydrogels were shown to accelerate nerve regeneration as evidenced by improved electrophysiological recovery and increased axon density at early time points. The keratin gel used in these experiments acted on the injury site by instigating thrombus formation and by forming a physical seal of the wound site that acted as a porous scaffold to allow for cellular infiltration and granulose tissues formation. The ability for keratin-based biometerials to be translated into the human clinical setting is dependent on further research to elucidate the mechanism by which these materials regulate hemostasis and nerve regeneration.

2.5 Wound

A wound is a type of injury in which skin is torn, cut or punctured or where blunt force trauma causes a contusion and also can be classified into chronic wounds and acute wounds. In pathology, it specially refers to a sharp injury which damages the dermis of the skin. Wounds may be grouped according to the cause, the environment in which they occur, their extent and whether they are clean or contaminated. The

microorganisms that typically infect wounds and the skin depend on what is present in the environment, the state of the person's immune system and the depth of the wound.

2.6 Wound Healing Process

Wounds required good blood flow and good access to rich blood with oxygen in order to heal. Dietary nourishment is important for rapid recovery. There are four stages or step in wound healing process which is inflammation, migration, proliferation and maturation. The wound healing was considered finished when the skin surface has gained its natural form and strength. (Boeteng *et al*,. 2008)

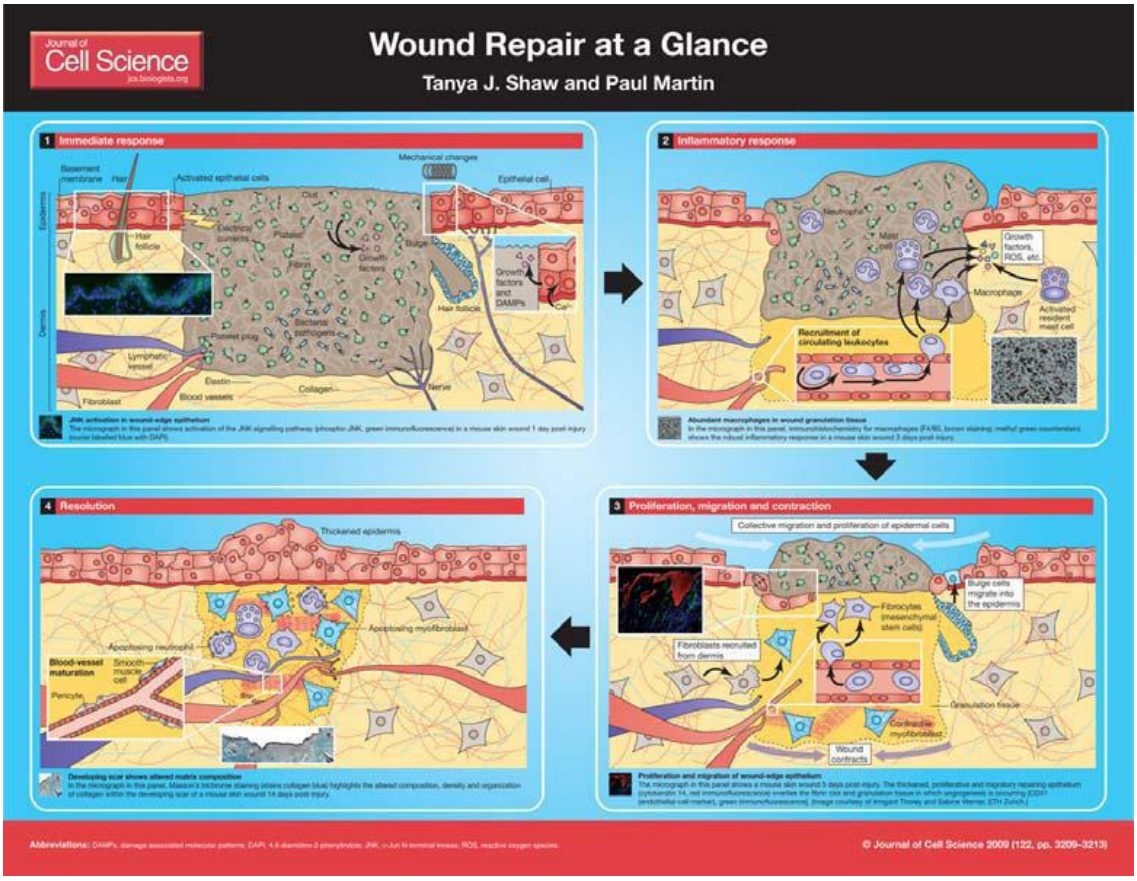

Figure 2.4: Stages of wound repair

Source: (Shaw and Martin, 2009)

2.7 Hydrogel by Freezing-Thawing Process

Freezing-thawing method was developing to avoid the cross linking processes that potentially lead to the release of some polymers. (Christie M. Hassan, 2002). Result from this process shows the freezing thawing process with chemical of PVA are high tensile strength, high water content and high light transmittance.

CHAPTER 3

RESEARCH METHODOLOGY

3.1 Introduction

The method to extract the chicken feather and production of biomedical product will be discussed in this chapter. The methods of this research will be start on the production of the keratin protein from chicken feathers. Keratin protein extracted from the chicken feather then will be use for developing biomedical product which is wound care product. These products are to be analysed.

3.2 Material and Apparatus

3.2.1 Material and Reagents

3.2.1.1 Material and Reagent for Protein Production

i. Chicken feather
ii. Ether
iii. Sodium Sulfide
iv. Sodium Hydroxide
v. Ammonium Sulfate
vi. Potassium Hydroxide
vii. Copper Sulfate

3.2.1.2 Material and Reagent for Wound Care Product

i. Polyvinyalcohol
ii. Polyvinylpyrrolidone

iii. Glutaraldehyhe
iv. Ammonium Thioglycollate
v. Keratin Protein
vi. Water

3.2.2 Apparatus

i. Beaker
ii. Conical falask
iii. Volumetric flask
iv. Centrifuge tube
v. pH meter
vi. Thermometer
vii. Magnetic stirrer
viii. Schott bottle
ix. Forcep
x. Sample bottle
xi. Spatula
xii. Bottle reagent
xiii. Micropipette
xiv. Wash bottle
xv. Peri dish

3.2.3 Equipment

i. Centrifuge
ii. Hot and stirrer plate
iii. Water bath
iv. Ultrasonic water bath
v. Freezer
vi. Rheometer
vii. Mechanical stirrer

3.3 Research Methodology

3.3.1 Research methodology of Protein Production

3.3.1.1 Feathers Treatment

a) Soak the chicken feather in ether for 24 hours.
b) Wash the feathers with the soap water and dry the wet feathers under the sunlight.
c) Collect all the dried feathers and blend the feather then keep the blend feathers in the sealed plastic bag carefully.

3.3.1.2 Dissolving of Chicken Feather

a) Prepare the sodium sulphide solution in the conical flask.
b) Weight the blend feathers and add in into the sodium sulphide solution
c) Stir the solution for 6hours and maintain the condition of solution at 30°C and pH range of 10-13.
d) Filter the solution and centrifuge the solution at 10000 rpm for 15 minutes.
e) Filter the solution to get the supernatant liquid.
f) Place the supernatant liquid in a beaker and stir the solution.

3.3.1.3 Protein Precipitation

a) Add an ammonium sulphate solution into the solution and centrifuge the solution at 10000 rpm for 5 minutes.
b) Filter the solution to get supernatant liquid and solid particles.
c) Repeat step a) and b).

3.3.1.4 Protein Purification

a) Pour the deionized water into the solid particles and stir the solution
b) Centrifuge the solution at 10000 rpm for 5minutes and filter the solution to get supernatant liquid and solid particles.
c) Use sodium hydroxide solution to dissolve the solid particles.
d) Centrifuge again the solution at 10000 rpm at 5 minutes. Collect the liquid and discard the solids.
e) Repeat steps 1 to 4 for three times.

3.3.1.5 Biuret Test

a) Prepare the copper sulphate solution and potassium hydroxide solution.
b) Mix the protein solution with potassium hydroxide solution by 1:1 ratio.
c) Add three drops cooper sulphate solution in the mixture solution.
d) Observe and record the change in the solution.
e) Analyze the solution under UV-Visual to obtain its absorbance.

3.3.1.6 Analysis of the Sample

a) Analyze the protein solution in FTIR to obtain the wavelength graph.
b) Mix the ammonium sulfate with protein solution, collect and weight the solid particles.

3.4 Research Methodology of Wound Healing Hydrogel Production

A 10% keratin solution was prepared using keratin powder dissolved in distilled water with gradual addition of 1M NaOH over 2 hours under mechanical stirring. The pH was maintained in the range 8.0-9.5 and finally adjusted to 8.5. The keratin protein solution was centrifuged at 10000 rpm for 10 minutes in order to remove any air bubbles and undissolved material. The resulting keratin protein solution was cast into a peri dish and the solvents evaporated wider ambient conditions to leaves keratin membrane. The solvent can also include some percentage of organic based aqueous can also include some percentage of organic based aqueous miscible solvent, such as an alcohol.

A 10% keratin solution that prepared as describe above intimately mixed with water soluble polymer which is polyvinyl alcohol (PVA) comprising 20% solid content and polyvinyl pyrrolidone (PVP) comprising 10% solid content to achieved a optimum rheology and optimal composition (keratin: PVA: PVP = 100: 60: 40 (w/w, %)) for creating hydrogel. The cross-linking agent which is 0.05% to 0.1% of glutaraldehyde was added into the blended solution. The solution then mixed with the final component of chemical which is 1% of 0.25M ammonium thioglycollate solution. The combination

of the solution was then cast and hardened through a freezing-thawing for 1 hour. This freeze-thaw cycle was repeated up to 7 times to obtain a hydrogel. This resulting hydrogel was washed with distilled water multiple times to remove any unreacted keratin and polymers.

The hydrogel get from freezing-thawing process then proceed with the rheology test, toxicity test and testing on animal and the rate of healing of wound was observed.

Table 3.1: Formulation of Hydogel

	Formulation,% w/w	
Compound	F1	F2
Polyvinylalcohol	20	15
Polyvinylpyrrolidone	15	15
Ammonium Thioglycollate	8	8
SIFP	10	10
Water	30	30
Glularaldehyde	-	0.05