DEVELOPMENT OF CERAMIDE AND HONEY BASED BIODEGRADABLE DRESSING MATERIALS FOR THE APPLICATION TO BURN SKIN

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M.Sc. ENGINEERING THESIS

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DECLARATION

I hereby declare that the study reported in this thesis entitled as above is my own original thesis work and has not been submitted before anywhere for any degree or other purposes. Further, I certify that the intellectual content of this thesis is the product of my own work and that all the assistance received in preparing this thesis and sources have been acknowledged and cited in the reference section.

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ABSTRACT

Development of Ceramide and Honey Based Biodegradable Dressing Materials for the Application to Burn Skin

Ceramide is widely recognized for its skin barrier function whereas hydrogel is an ideal one for wound healing where honey is used for antibacterial activity and burn wound treatment from ancient ages. This research work proposes to develop a ceramide-loaded honey-based hydrogel biomaterial for burn wound healing. The biodegradable hydrogels have been prepared by the solution casting method. The developed hydrogel membranes were characterized by using Fourier Transform Infrared Spectroscopy (FTIR), Field Emission Scanning Electron Microscopy (FESEM), and UV Spectrophotometer. Besides that, swelling behavior in Physiological Solution, Moisture Retention capability, Gel Fraction, Water Vapor Transmission Rate (WVTR), Porosity, and Anti-microbial activity were studied and evaluated thoroughly. Then, ceramide hydrogel (C2) and ceramide & honey Hydrogel (CH) were assessed for burn wound healing activity in the mice model. Both C2 and CH hydrogel showed significant wound healing rates while only CH hydrogel showed significant antibacterial activity against *Staphylococcus aureus*. Other properties like physicochemical, swelling, WVT, porosity, gel fraction and data obtained from FTIR, FESEM, and UV spectrophotometer were identical to confirm the presence of ceramide & honey in the hydrogel. This newly prepared ceramide loaded honey based hydrogel plays an important role during application on burn wounds and scars and further healing process. Current outcomes of this research can be used for further investigation on the future biological assays to declare this as a potent candidate for the treatment of burn patients.

সারসংক্ষেপ

Development of Ceramide and Honey Based Biodegradable Dressing Materials for the Application to Burn Skin

সেরামাইড (ceramide) ত্বকের ন্যায় দেহে অণুজীব প্রবেশের প্রধান প্রতিবন্ধক হিসেবে কাজ করে যা শরীরের প্রাথমিক প্রতিরক্ষা স্তর (first line defense) নামে স্বীকৃত। হাইড্রোজেল (hydrogel) ক্ষত সারানোর জন্য একটি আদর্শ উপাদান। মধু প্রাচীন কাল হতে ক্ষত নিরাময় ও জীবাণুরোধী (anti- microbial) হিসেবে ব্যবহৃত হয়ে আসছে। তাই আমাদের এই গবেষণার উদ্দেশ্যে সেরামাইড এবং মধু ভিত্তিক হাইড্রোজেল তৈরি করা হয়েছে যা আগুনে পুড়ে যাওয়া ক্ষত নিরাময়ের জন্য কার্যকরী হবে। হাইড্রোজেল দ্রবণ ঢালাই (solution casting) পদ্ধতিতে তৈরি করা হয়েছে। আমাদের তৈরিকৃত হাইড্রোজেল এর বৈশিষ্ট্য বিভিন্ন বর্ণালিবীক্ষণ (spectrophotometer) ও আণুবীক্ষনিক (microscopic) যন্ত্রের এর মাধ্যমে পরীক্ষা করা হয়েছিল। পাশাপাশি শরীরবৃত্তীয় (physiological) দ্রবন্রে হাইড্রোজেলের শোষণ (swelling) ক্ষমতা, আদ্রতা ধরে রাখার (moisture retention) ক্ষমতা, ভঙ্গুরতা (gel fraction), জলীয় বাষ্পের স্থানান্থরের (water vapor transmission) হার নির্ণয়, ছিদ্রের (porosity) উপস্থিতি এবং জীবানুরোধী বৈশিষ্ট্য সমূহ পর্যবেক্ষণ করা হয়েছিল। পরবর্তীতে সেরামাইড হাইড্রোজেল (C- hydrogel) ও সেরামাইড- মধু হাইড্রোজেল (CH- hydrogel) এর ক্ষত নিরাময়ের ক্ষমতা পর্যবেক্ষণের জন্য ইঁদুরের উপর পরীক্ষা চালানো হয়েছিল। উভয় হাইড্রোজেলের ক্ষত নিরাময়ের হার তাৎপর্যপূর্ণময় ছিল। শুধুমাত্র CH- হাইড্রোজেলের *Staphallycoccus aureus* অনুজীবের বিপক্ষে জীবানুরোধী বৈশিষ্ট্য পরিলক্ষিত হয়েছিল। অন্যান্য ভৌত রাসায়নিক (physicochemical) পর্যবেক্ষণের পরীক্ষালব্দ ফলাফল গ্রহণযোগ্য ছিল এবং আমাদের প্রস্তুতকৃত হাইড্রোজেলে সেরামইড ও মধুর কার্যকারিতা পরিলক্ষিত হয়েছিল। আমাদের এই নতুন তৈরিকৃত সেরামাইড এবং মধু ভিত্তিক হাইড্রোজেল ক্ষত নিরাময় প্রক্রিয়ায় (wound healing process) গুরুত্বপূর্ণ ভূমিকা পালন করবে।

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ATP	Adenosine Triphosphate
DNA	Deoxyribonucleic Acid
ECM	Extracellular Matrix
FTIR	Fourier Transform Infrared Spectroscopy
H & E	Hematoxylin- Eosin
IM	Intramuscular
IPA	Iso Propyl Alcohol
NMR	Nuclear Magnetic Resonance
NSAID	Non-Steroidal Anti-Inflammatory Drugs
PEG	Polyethylene Glycol
PLGA	Poly Lactide-co-Glycolide
PU	Polyurethane
PVA	Poly Vinyl Alcohol
REDOX	Reduction and Oxidation
RGD	Arginine-Glycine-Aspartate
SEM	Scanning Electron Microscope
TSST-1	Toxic Shock Syndrome Toxin-1
UV-VIS	Ultra Violet Visible
WVTR	Water Vapor Transmission Rate

CHAPTER 1

INTRODUCTION

1.1 Background of the Study

Burns are severe health problems causing skin loss and a large amount of fluid loss that may lead to life-threatening complications. So the use of traditional dressing such as bandages and gauze which are made of cotton wool may cause bacterial infection, painful administration to patients, and destroy newly generated tissue. So an ideal wound dressing needs to act as a barrier to microorganisms, take away extra fluid loss, be non-toxic, non-allergic, and be non-adherent (be eliminated effortlessly without pain). Generally, no single material can meet all the requirements required for the ideal wound dressing and wound recovery process. Therefore, we make a hydrogel biomaterial combination of ceramide, honey, and gelatin. Where ceramide is well informed for its skin barrier function and from many years ago honey is popular for antibacterial activity and burn wound healing. On the other hand, for burn wound treatment hydrogel itself is an ideal one because it helps to absorb exudates and keep moist to the wound area **Enas M. A. (2015)**.

1.2 Problem Statements

Millions of people go through fire-induced burns, chemical burns, radiation burns, electric burns, and other incarnations who face problems in bacterial infection in burn wound treatment as a consequent healing the wound has been in a challenging process. The bacterial infection of the burn wound is one of the major challenges in wound care treatment. Besides they face other problems like delaying wound healing, organ loss, expensive and not available etc.

1.3 Objectives of the Thesis

The objectives of this study are:

- to develop ceramide and honey-based hydrogel dressing materials
- provide a moisturized wound-healing microenvironment
- to protect the skin from burn infection
- to study in- vivo experiment
- characterized Hydrogel by FTIR, SEM, and Physicochemical parameter

1.4 Motivation of the Thesis

Burn is one of the serious health problems which cause disability & mortality in Bangladesh. According to data provided by the Fire Service and Civil Defense of Bangladesh, at least 2,308 people were killed in burns across the country between 2004 and 2020, where the year 2019 saw the highest number of 24,074 burn incidents while 2020 the second highest of 21,073 **The daily independent (2021)**. In Dhaka burn victims were 25%, in rural areas were 55% and in the rest other cities were 20% as per report on 2020. According to the Sheikh Hasina National Institute of Burn and Plastic Surgery, Dhaka, in 2020, 2,854 victims were admitted to the institute. Only at the Sheikh Hasina National Institute of Burn and Plastic Surgery, Dhaka, Bangladesh 2,854 victims were admitted in 2020. Total 7,344 people were burnt in 2019 as per a report from daily newspaper, 45% from villages, 30% were from the mega city Dhaka, and 25% from rest of other cities **Dhaka tribune (2020)**. After burn injuries, in most cases, the skin loses its barrier functions to microorganisms leading to the potential risk of infection. Wound healing delayed

due to burn infection and leading pain, scarring and even death. Denaturation of protein and cell death either by necrosis or apoptosis is very common in burn injuries.

Wound dressings like gels, creams, ointments, alginates, foams, films, composites, hydrocolloids, and liquid wound washes are abundantly found to treat different types of burn injuries and wounds. These dressings are very laborious to remove, difficult to handle, difficult to store, require saline solution or sterilized water or oil for removing the dressing, are more expensive, unpleasant odor, adherence to wound bed result disturb new epidermal tissue, and causes pain, non-absorbent cause permitting excess wound exudates accumulation and impermeable for proteins and drugs again semipermeable for gasses, do not absorb blood or exudates, healing may take a longer time, opaque layer formation may complicate wound tracking, not suitable for dried wounds, and bad balance and the opportunity of bacterial invasion. On the other hand, these dressings contain silver, bismuth, and chlorhexidine which received criticism by the Food and Drug Administration (FDA) including certain risks of use and potential risk mitigation measures. To minimize these risks and disadvantages our prime motivation behind this current research is to develop biodegradable dressing materials like hydrogels containing honey together with plant-derived ceramide for the resuscitation of burns.

1.5 Organization of the Thesis

The structure of the thesis is composed of a total of five chapters, namely chapter 1: introduction, chapter 2: literature review, chapter 3: experimental procedure, chapter 4: result and discussion, and chapter 5: conclusion and future recommendation. The details of the organization of the thesis are as follows:

Chapter 1 comprises introduction to the thesis topic and the background behind the research. Also, the motivation and objectives of the thesis have been included in this chapter. Finally, the chapter is concluded with a mention of the structure or organization of the thesis in this book.

Chapter 2 consists of the theoretical studies that are required for the research work. It describes details of hydrogel, ceramide, honey, biomaterials, burn, wound healing process, etc.

Chapter 3 describes the methods and materials of the research work. This chapter included the name of materials used in the thesis, preparation method of hydrogel dressing materials, and physicochemical characterization methods of hydrogel dressing (e.g. water vapor transmission rate, moisture retention capability, porosity evaluation, gel fraction, swelling behavior, SEM, UV, and FTIR) and biological evaluation (e.g. antimicrobial activity, in vivo study in mice model and histological analysis.

Chapter 4 provides outcomes and observations of the research work and discussions of the significance of the experiments and relative contributions of the work to the field of study. The outcomes and observations have been displayed in graphs and tables where it is required. Other results such as wound healing process in mice, histological analysis, disc diffusion test for antimicrobial activity, SEM, and UV analysis have been demonstrated with a figure with proper illustration and marking.

Chapter 5 concludes the research work where the output and findings of the research work have been summarized. Also, some of the recommendations have been provided in this chapter to carry forward the research in the future.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Hydrogel promotes wound healing better than traditional dressing because traditional dressings like bandages and gazes have many types of limitations **Zheng P., Huijun Y., Decheng W. (2021)**. Traditional dressings are not suitable for irregularly shaped wounds and sensitive to bacterial infection. They have no healing capability in burn or wound injury; they may also cause secondary tissue damage and are nearly effective much less for burn wound restoration. They are only effective in controlling hemorrhage and in some cases in protecting from further external friction**.** On the other hand, as an ideal wound dressing biomaterial hydrogel is one of the best choices for burn wound healing due to the fact that they possess almost all the properties which are required for the healing purpose. Hydrogel wound dressings are antibacterial, biocompatible, biodegradable, nontoxic, responsive, and injectable. Ceramide is an important structural chemical element of the stratum corneum which developed 50% of the epidermis layer of human skin **Coderch, L., López, O., de la Maza. A., Parra, J. L. (2003)**. It can stimulate the activity of the skin for burns and people having dry skin. It performs a vital position in skin care by making a barrier that protects from bacterial infection, environmental stress, and dehydration **Feingold KR, Elias PM. (2014)**. On the other hand, honey possesses antimicrobial activity which keeps a safe burn wound area from microbial contamination and performs an important position in wound recovery. It also helps to reduce inflammation and soothe burn wound areas. Gelatin is used as a gelling agent in medication like soft gelatin capsules, hydrogel sheets for wound healing, etc. It could absorb more than 5 to 10 instances of water as its weight itself

Budavari, S. (1996). Gelatin allows to soak up extra watery exudates composed of serum, wound fluids, and cellular particles which promote wound healing and faster new tissue growth **Tanaka, A.., Nagate, T. and Matsuda, H. (2005)**. PVA is used to prepare various hydrogels which includes wound dressing fabric due to their excellent binding and filling properties which are found to be beneficial and possessed water retention properties, biocompatibility, biodegradable, non-toxic, swelling properties, curative, non-carcinogenic and excellent film forming ability **Kawai, F. and Hu, X. (2009)**.

In this study, therefore, ceramide, honey, PVA, and gelatin had been selected to prepare hydrogel sheets (C1, C2, CH) as wound dressings. The properties of the hydrogel sheet, such as WVTR, moisture retention capability, gel fraction, porosity evaluation, swelling behavior, SEM, UV and FTIR, antibacterial activity, and in vivo wound healing potential were investigated**.**

2.2 Biomaterials for Burn Wound Healing

There are several biomaterials like composite biomaterials; scaffold biomaterials; hydrogel biomaterials for wound healing are available.

2.2.1 Hydrogel Biomaterials

Hydrogels are biomaterials with a cross-linked polymeric network that consists of a swollen water network of cross-linked polymer chains that are being widely investigated for biomedical applications such as drug delivery. For wound dressings hydrogels are powerful biomaterials as well for tissue engineering they are also significant biomaterials, reason they exchange fluid, hydrating necrotic tissues. When used as a burn wound dressing, hydrogel not only forms a physical barrier and removes excess exudates but also provides a moist environment that

promotes the wound healing process. The biomaterial can absorb the secretions from burn wound bed reasons this type of dressing having capability to swell, having expanding capability by cross-linking within the chains of polymer**.**

2.2.2 Criteria of Ideal Hydrogel Biomaterials

Additionally, a hydrogel can flawlessly fill irregularly formed wounds and cope with deep bleeding correctly. Moreover, following all criteria make hydrogel biomaterials one the ideal candidate for wound healing **Rosiak, J. M. and Yoshii, F. (1999), Enas, M. A. (2015)**.

a) The best biodegradability without the formation of poisonous species following the degradation.

b) The very best durability and stability inside the swelling surroundings and during storage.

c) The highest absorption potential (most equilibrium swelling) in saline. The preferred charge of absorption (preferred particle length and porosity) relies upon the application requirement.

d) Having large compatibility, similarity and non-poisonous with living tissue additives of the skin.

e) Have shown extremely good ability as one of the finest encouraging groups of biomaterials.

f) The lowest price, biocompatible, pain-free for patients, and non-toxic.

g) Minimal frictional irritation in the surrounding tissues on implantation.

h) Photostability, superior biocompatibility & good oxygen permeability, pH- neutrality after swelling in water, colorless, odorless, and surely non-poisonous, moderate protein adsorption and little cellular adhesion.

i) Watery surface environment to defend cells and therapeutic agents (peptides, proteins, oligonucleotides, DNA), ease of surface change with particular biomolecules.

j) Soft and tissue-like physical properties & micro-porous structure for additional delivery channels.

2.2.3 Hydrogels Biomaterials- Classifications

Hydrogels are three-dimensional (3D) network of hydrophilic polymers that can swell in water and hold a large amount of water while maintaining the structure due to chemical or physical cross-linking of individual polymer chains. They can be categorized in several ways depending on the synthesize strategies, conditions of ion, origins or sources, nature of swelling with modifications within the environment, rate of biodegradation, or the character of cross linking. Depending on ionic charges hydrogel are four types: nonionic, cationic, anionic & ampholytic, depending on physical properties they are two types: smart & conventional, depending on biodegradability they are two types: biodegradable & non-biodegradable, depending on cross linking they are two types: physical & chemical, depending on source they are three types: natural, hybrid & synthetic, depending on preparation methods they are three types: homopolymers, copolymers & interpenetrating, again copolymers are two types: triblock and multi block.

Different types of hydrogel biomaterials have been drawn in **Figure 2.1**:

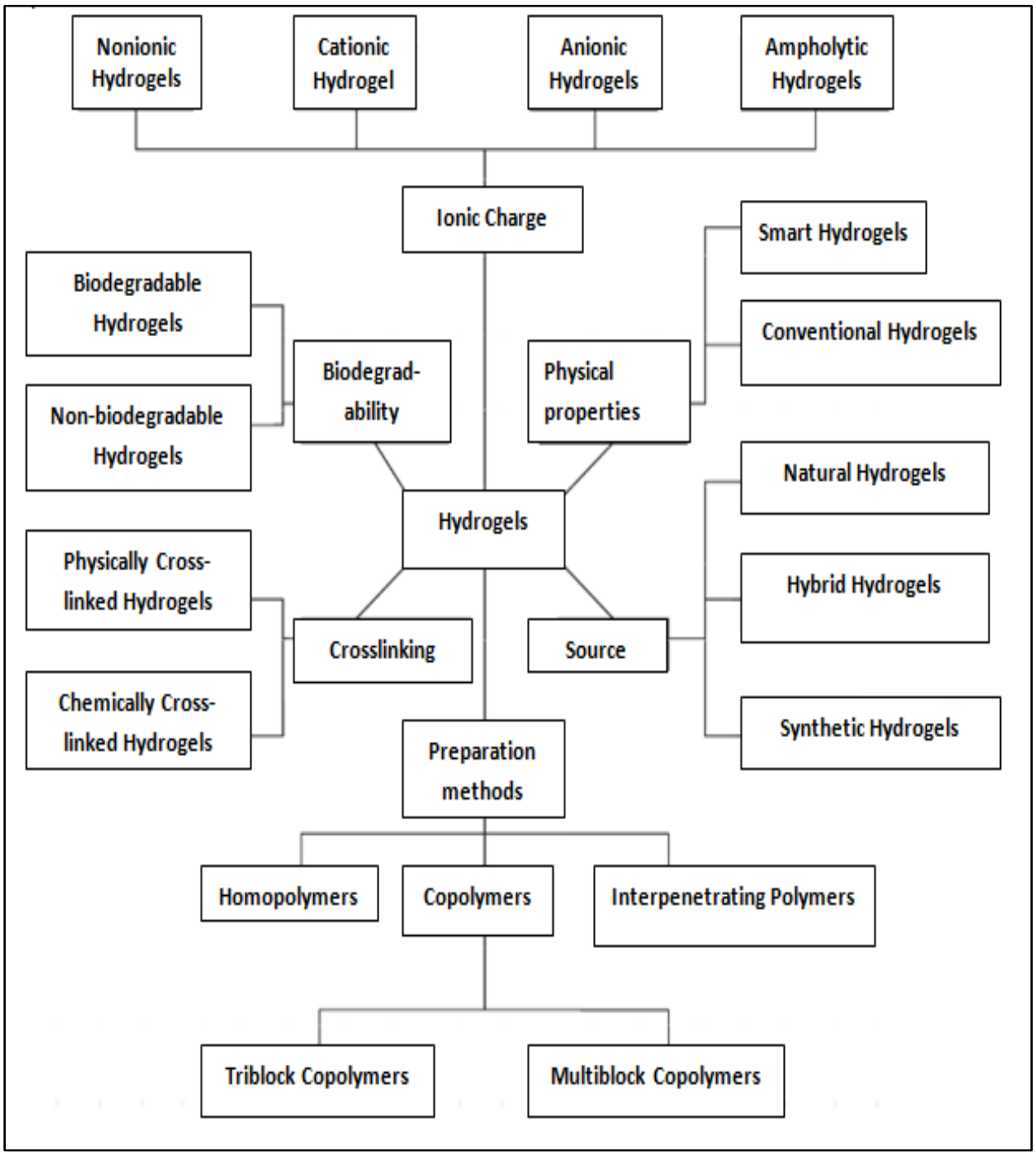

Figure 2.1: Classifications of Hydrogel Biomaterials.

(Source: https://www.intechopen.com/chapters/17653)

2.3 Wound Dressing

A wound dressing is a pad or a form of material carried out to a wound to promote recovery and shield the wound from similar damage. A wound dressing is designed to be in direct touch with the wound, as prominent from a bandage, which is most often used to hold a dressing in place. Many modern dressings are self-adhesive.

2.3.1 Characteristics of Ideal Wound Dressing

An ideal wound dressing fabric or biomaterial should have the following criteria to preserve a wet environment around the wound **Lin, S. Y., Chen, K. S., Run-Chu, L. (2001)., Boateng, J. S., Matthews, K. H., Stevens, H. N. E., Eccleston, G. M. (2008)**.

a) Eliminate excess exudates, shield the wound from microorganisms, infections, or contaminations and reduce the wound surface necrosis, stop the wound desiccation and stimulate the growth factor.

b) Smooth and relax to do away from skin, non-allergic, non-poisonous and biocompatible, biodegradable and elastic.

c) Resource to lessen the ache from the wound and commercially perfect.

d) Can shield the wounded area from repeat trauma and owning mechanical protection.

e) Low adhesion, easy removal, and minimal frequency of dressing change.

f) Effortlessly sterilized, smooth to use, long shelf-lifestyles, comfy and conformable, and cost-effectiveness.

2.3.2 Classification of Wound Dressing

Depending on their nature of action the dressing can be commonly divided into three extensive groups: i) Inert/passive ii) Interactive and iii) Bioactive.

i) Passive Dressing

These type of dressings which can be usual dressings, consisting of light cloth, simply were used to cowl and disguise the wound while repairing underneath and have a minimal position in the restoration manner, preventing contamination by means of building a shield against bacterial colonization **Vanesa, A., Gracia, M. and Manuel, A. (2015)**.

ii) Interactive or Biodegradable Dressing

Interactive dressings enhance debridement, improve granulation and re-epithelialization, and reduce exudates layer and bacterial colonization levels to adhere to the wound surface and optimize healing. They can modify the body structure of the wound environment **Vanesa, A., Gracia, M. and Manuel, A. (2015)**. These dressings are apparently permeable to water vapor and oxygen, but impermeable to bacteria. **Mayet, N., Choonara, Y. E., Kumar, P., Tomar, L. K., Tyagi, C., Du Toit, L. C., Pillay, V. A. (2014), Sharma, S., Dua, A. and Malik, A. (2014)**. Some of the goods together within the class of interactive dressings are hydrocolloids, alginates, collagen, hyaluronic acid (HA) products, foams, hydrogels, and semipermeable films.

iii) Bioactive Dressing

These products deliver activities along with antimicrobials and antibiotics that have the immediate function of altering the chemical and cellular environment of the adjacent wound and stimulating the recovery cascade **Sharma, S., Dua, A. and Malik, A. (2014)**.

2.3.3 Classification of Interactive Biodegradable Dressing

Biodegradable dressings are mainly collagen-based biological dressing, other polymeric-based non-biological or artificial dressing, and traditional or conventional dressing. It should be safe, mechanically stable and provide a comfortable environment for tissue repair. Example: Hydrogel, Film, Gauze. Depending on the use for burn and wound biodegradable dressings are categorized into traditional, biological and artificial **Elbadawy, A. K., El-Refaie, S. K. and Xin, C. (2017)**.

i) Biological Dressings

Collagen-based dressings are organic dressings which might be impermeable to microorganisms and make an excellent physiological interface between the burn wound surface and the surroundings. Factors in this association derive from collagen-like systems such as elastin and lipids **Kearney, J. N. (2001)**.

Biological bandages have other advantages in that they are easier to apply than traditional bandages and are natural, non-immunogenic, non-pyrogenic, hypoallergenic and painless.

This bandage relies on intact, fresh skin provided by a foreign body, such as a human or animal. A fundamental drawback of these materials is that they may be insufficient to provide pores and skin elements to deep or large wounds, ultimately leading to a search for new tissue donors. **Edwards, J. V., Yager, D. R., Cohen, I. K., Diegelmann, R.F., Montante, S., Bertoniere, N. et al. (2001)**. A biological bandage called an "autograft" is considered the most acceptable material for the complete healing of deep chronic wounds and burns.

ii) Non-Biological or Artificial Dressings

Non-biological or artificial bandages are made from synthetic materials such as non-biological materials or polymers that are not part of the skin's constituents. Synthetic dressing compositions should be biodegradable and provide a suitable environment for wound healing. Recently, there has been a great demand for polymeric membrane materials used in wound dressings. Polymeric wound dressings have recently been used in various forms such as films, foams, hydrogels, alginates and hydrocolloids. The following describes the classification of artificial polymeric wound dressings by shape, including criteria, indications, benefits, and limitations.

2.3.4 Conventional Dressings

The best known example of this category are gauze or gauze fabrics, bandages, cloths, or cotton composite bandages that have been supplied since the middle of Nineteen Seventies **Edwards, J. V., Yager, D. R., Cohen, I. K., Diegelmann, R. F., Montante, S., Bertoniere, N. et al. (2001)**. Gauze dressings are the most normally used and comfortably available wound dressings out there today. They may be made from woven or nonwoven silk, linen, polyester, rayon, or cotton causing them to be pretty permeable.

Gauze dressing can come in many diverse sizes and styles that may be without problems tailored to fit the wound. There are sterile and non-sterile sorts. There are also people with or without an adhesive border. Gauze sponge, this sort of gauze is normally crafted from hundred percent cotton and is usually used to soak up blood or other fluids. They may be normally cheap, and are terrific for all-cause use inside the cleaning, dressing, packing, and prepping of all kinds of wounds. Gauze dressing roll is also applied for all types of wounds; the gauze roll may be used as a primary layer or additional layer on a burn wound. The hundred percent cotton roll may be

wrapped round limbs on the pinnacle and is specially useful for the wounds which are in an area that isn't always easy to get dressed. They may be suitable for minor accidents consisting of grazes, cuts, or regions of sensitive skin.

2.3.4.1 Advantages of Traditional or Conventional Dressing

This form of fabric is non-occlusive meaning it does not seal on the pores and skin, permitting blood, water, and air to effortlessly bypass through. This dressing without difficulty allows the wound to dry out and minimizes exuding fluid leaking from the wound. Those dressings are relevant to something from a small finger harm to a wound that extends throughout the body. It is able to be used on any sort of wound. Inexpensive, smooth to use and fabrication, effectively reachable in maximum clinics and surgical centers.

2.3.4.2 Limitation of Conventional Dressing

Conventional dressing materials have some limitations like dry and uncontrollable moisture levels, low efficiency when becoming saturated with exudates, and mechanical trauma when removed. These dressing materials are a mile’s absorbent capability of wound exudates which causes speedy dehydration and promotes bacterial growth and contamination. It can adhere to a burn wound bed and losses of newly generated tissue during removal, after remedy the quilt removal is really difficult that reasons bleeding or harm to the renewed epithelial flora. Adhesiveness to tissues and exudates fluid solidifies give rise to pain.

2.3.5 Hydrogels

Hydrogels are hydrophilic dressings with very excessive water content, capable of donating water to the burn and rehydrate dry eschar or necrotic slough. It is able to be synthesized from

each herbal and synthetic polymer. It has an excessive absorption ability and is successful to soak up exudates from the wound surface. It could be used as a calming, cooling and soothing agent for skin wounds, suitable for dry burns, which want a few debridement. Hydrogels are proper for all burn depths however in particular mid-dermal to deeper burns. Hydrogels are very effective for burn or wound healing, it non-harmful to granulation tissue or epithelialization, have high exudates capacity, are non-adherent, easily eliminated from the wound, boost up the recovery, ache, and anti-inflammatory reduction, are much less costly, easily developed and dealt with, cover burn wounds without much discomfort does not cause any irritation, suitable for use in sensitive skin, and helps to slow down bleeding, The main limitations of hydrogels are semi-transparent, semi-permeable to gasses and water vapor, poor bacterial barrier, and sometimes have poor mechanical stability.

2.3.5.1 Hydrogel as a Dressing for Burn Wound Healing

Hydrogel dressings has the capability to save you bacterial infection, keep water (moisture retention capability), remove easily from wound area without losing newly generated tissues, meet the basic condition of biocompatibility, counter to adjustments within the microenvironment on the burn wound surface, raise right microenvironment for angiogenesis, recruitment of fibroblasts, and cell proliferation. Hydrogel dressings can keep as much as 600 instances their original volume of water, which include fluid-based totally wound exudates **Tavakoli S., Klar A. S. (2020)**.

The unique properties of hydrogel membranes as wound dressings to advise skin recovery and to shield the skin disease region from bacterial contamination have been regularly examined and carried out inside the medical sections for the reason that early Eighties. The hydrogel bandage

mimics its 3D interconnected network of extracellular matrix fibers in human skin. The tight mesh size of the hydrogel structure protects the wound from contamination and stops microorganisms and bacteria to reach the wound vicinity. But, hydrogel structure lets in transporting of bioactive molecules e.g. antibiotics, prescribed drugs, pharmaceuticals to the wound center. Such molecules may be entrapped into hydrogel networks in the course of the gelling process, while these molecules can be exchanged by means of absorbing the wound exudates throughout the sustainable launch method after contacting hydrogels with the wound surface. The big tissue-like water content of hydrogels provides the wished flexibility and elasticity to evolve to wounds located in different body regions. In this study, we have incorporated ceramide and honey into the hydrogel for chemical, physical, biological, and in vivo assessment of hydrogel dressing for burn wound restoration.

2.3.5.2 Physicochemical Characteristics of Hydrogel Dressings

Hydrogel bandages or dressings show off physical or chemical cross-linking. Both physical and chemical characteristics of hydrogels bandages have been described below:

a. Physical Characteristics

Hydrogel dressings are to be had in a distinct kind of shape like film/ sheet, amorphous, impregnated, or sprayable foams. Film/ sheet-shape hydrogel dressings are non-adhesive in opposition to the wound and are powerful in healing second degree burn wounds. Amorphous hydrogels are greater powerful inside the remedy of third degree burn wounds than sheet-form dressings due to the fact they can comply with the form of the wound bed and facilitate self-degrading debridement. Impregnated hydrogel dressings are dry dressings (e.g. gauzes) saturated with an amorphous hydrogel. Nebulizable or sprayable hydrogel dressings consist of amorphous

hydrogels which rapidly increase in viscosity after software. Nebulizable or sprayable hydrogels have additionally been shown to boom the penetration and efficacy of therapeutic agents **Tavakoli S., Klar A. S. (2020)**.

Physically cross-linked hydrogel assemblies are assembled through hydrogen bonding, ionic interactions, crystallization or hydrophobic interactions, physically cross-linked hydrogels degrade with local changes in ionic strength, temperature and pH **Ulijn, R. V., Bibi, N., Jayawarna, V., Thornton, P. D., Todd, S. J., Mart, R. J., Smith, A. M. and Gough J. E. (2007)**.

b. Chemical Characteristics

This cross-linking involves the formation of covalent bonds between polymer chains. Chemically cross-linked hydrogel assemblies are prepared using chain-climbing polymerization, step-climbing polymerization, radiation polymerization or enzymatic polymerization. Synthetic bandages containing nanoparticles such as PVA and PEG are assembled using a chemical cross-linking mechanism. Natural bandages containing polysaccharides and proteoglycans/proteins form 3D networks through physical cross-linking **Hennink, W.E. and van Nostrum, C. F. (2002)**.

Cross-linking of soluble hydrophilic monomers bureaucracy a 3D insoluble netted structure which could include a large amount of water. The 3D polymeric network of hydrogels is noticeably hydrated with 90-99% water w/w; it is capable of binding many times more water molecules when assembled than in the uncross-linked state **Tavakoli, S. and Klar, A. S. (2020)**.

Hydrogels can be formed through a self-assembly process in which monomers diffuse in solution and then form non-covalent interactions. Hydrogels used in wound dressings can be self-assembled upon the addition of divalent metal cations or electrically-charged polysaccharides due to electrostatic interactions. Self-assembly via hydrophobic interactions can be induced in amphiphilic polysaccharide-based gels by the addition of water; it can also be induced in non-amphiphilic polysaccharide-based hydrogels by the addition of hydrophobic grafts.

2.3.5.3 Types of Hydrogel Dressing

Hydrogel dressings are categorized into three groups: i) Naturally-derived hydrogel dressings ii) Synthetic hydrogel dressings and iii) Bio-hybrid hydrogel dressings. These subgroups are elaborated as follows:

i) Naturally-Derived Hydrogel Dressings

Polysaccharide-based hydrogel dressings have been synthesized from polymers consisting of hyaluronic acid (HA), chitin, chitosan, alginate, and agarose. Naturally-derived protein/proteoglycan hydrogel dressings have been synthesized from polymers which include collagen, gelatin, kappa-carrageenan, and fibrin.

ii) Synthetic Hydrogel Dressings

Synthetic hydrogel dressings can be derived from synthetic polymers together with PVA, PEG, PU, and PLGA. Synthetic hydrogel dressings will also be fashioned from designer peptides.

iii. Bio-Hybrid Hydrogel Dressings

Hydrogels may be changed to contain metal cations (e.g. copper- II), degradable linkers (e.g. dextran), and adhesive functional groups (e.g. RGD). Integrating organic derivatives into

synthetic hydrogels allows manufacturers to tailor binding affinities and specificity, mechanical properties, and stimuli-responsive properties.

2.3.5.4 Applications of the Hydrogel Dressing

The hydrogel dressings are applicable and effective on various wound types. They are powerful dressings for chronic wounds like pressure ulcers, diabetic ulcers, and venous ulcers. Previous studies have shown that hydrogel can accelerate recuperation in partial and full-thickness burn wounds of various sizes **Mohd, Z. R., Abu, B. Z., Zuki, Y., Norimah, M. M., Noordin, A. and Muhammad, N. H. (2012)**. Other studies have shown that hydrogel dressings accelerate healing in radioactive skin accidents **Jiang, X., Sun, R. and Li, J. (2018)**. For traumatic skin injuries, hydrogel dressings decrease the recovery time to average 5.28 days and reduce the pain suggested by sufferers **Chen, L. (2015)**. A few hydrogel dressings possess intrinsic antibacterial properties e.g. antibacterial peptides and chitosan-based hydrogel dressings have antibacterial activity **Salomé, V., Ana, S. and Joel P. (2013)**. Incorporation of metallic nanoparticles, antibiotics, or other antibacterial agents into hydrogel also helps to increase the antibacterial activity of hydrogel dressing **Salomé, V., Ana, S. and Joel P. (2013)**. Silver and gold nanoparticles also can be incorporated into hydrogel dressings to boost up antimicrobial activity **Salomé, V., Ana, S. and Joel, P. (2013)**. Some hydrogel dressings have antibiotics inclusive ciprofloxacin and amoxicillin integrated into their structure that are unloaded into the wound as fluid is exchanged **Salomé, V., Ana, S. and Joel, P. (2013)**. A few hydrogel dressings have included stimuli-responsive nitric oxide-freeing agents and different antimicrobial agents **Salomé, V., Ana, S. and Joel, P. (2013)**.

2.4 Skin

The outer protecting of the body is the biggest organ of the integumentary system made from a couple of layers of ectodermal tissue and guards the underlying muscles, bones, ligaments, and internal organs with three major features: protection, regulation, and sensation. The skin is the biggest organ of the body, with a total region of about twenty square feet. The skin covers us from microbes and the elements, enables modification of temperature, and allows the sensations of touch, heat, and cold. The skin is made of water, protein, fats and minerals. Some diseases are associated with skin like rash, dermatitis, eczema, psoriasis, dandruff, acne, cellulitis, skin abscess, rosacea, warts, melanoma, basal cell carcinoma, seborrheic keratosis, actinic keratosis, squamous cell carcinoma, herpes, hives, tinea versicolor, viral exanthem, shingles, scabies, ringworm etc. All mammals have some hair on their skin, even marine mammals like whales, dolphins. Skin plays an important immunity role in protecting the body against pathogens and excessive water loss.

2.4.1 Anatomy of the Skin

Skin is composed of three primary layers; they are:

a) Epidermis,

b) Dermis and

c) Hypodermis.

The details of the skin layers have described below with **Figure 2.2** & **Figure 2.3**:

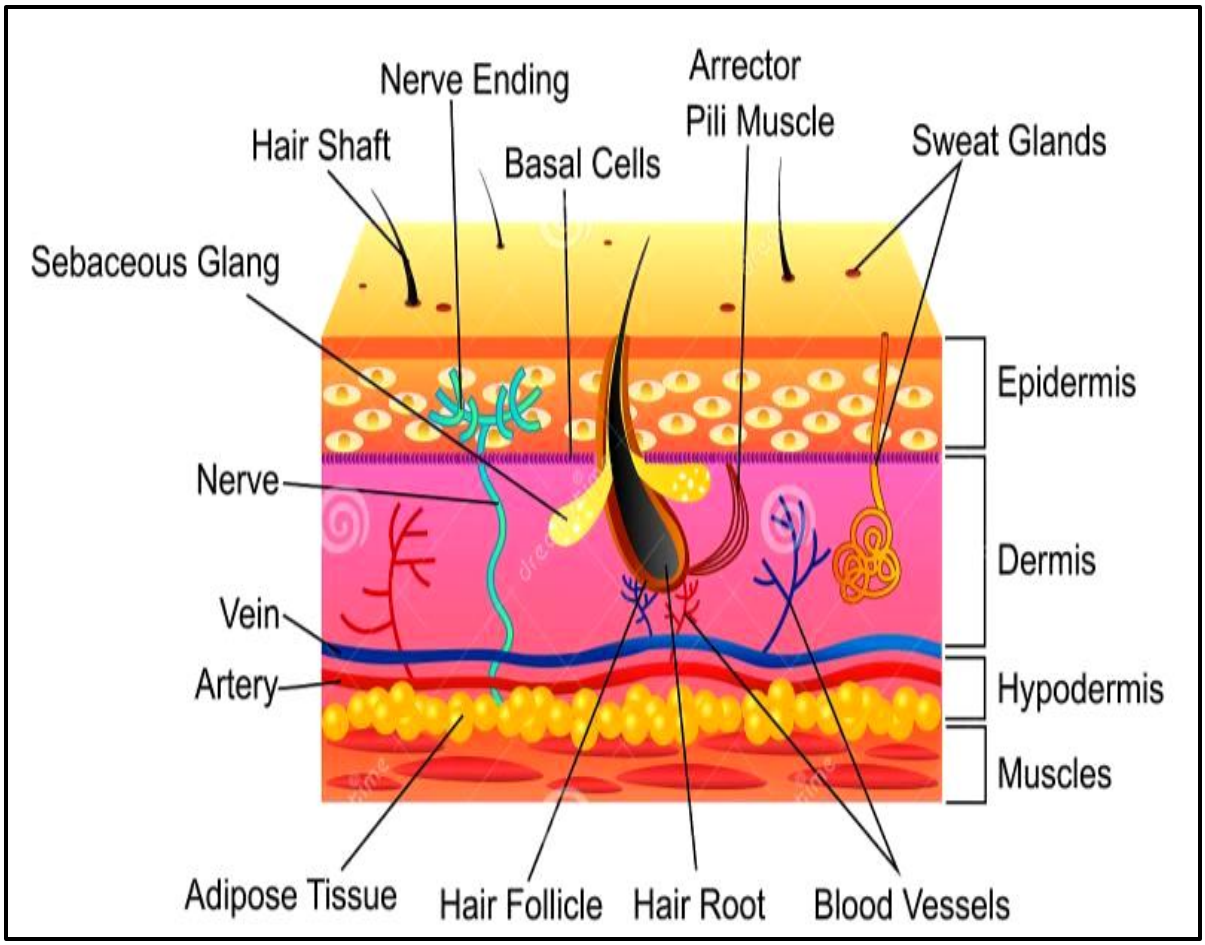

Figure 2.2: Structure of Skin. (Source: https://www.dreamstime.com/)

i) The Epidermis:

The outermost layer of the skin provides a water-resistant barrier and creates our skin tone. The epidermis allows the skin to modify body temperature. The primary kinds of cells that make up the epidermis are Keratinocytes, Melanocytes, Langerhans cells, and Merkel cells.

The epidermis is split into the following 5 sub-layers: a) Stratum Corneum b) Stratum Lucidum c) Stratum Granulosum d) Stratum Spinosum e) Stratum Basale (also called "Stratum Germinativum")

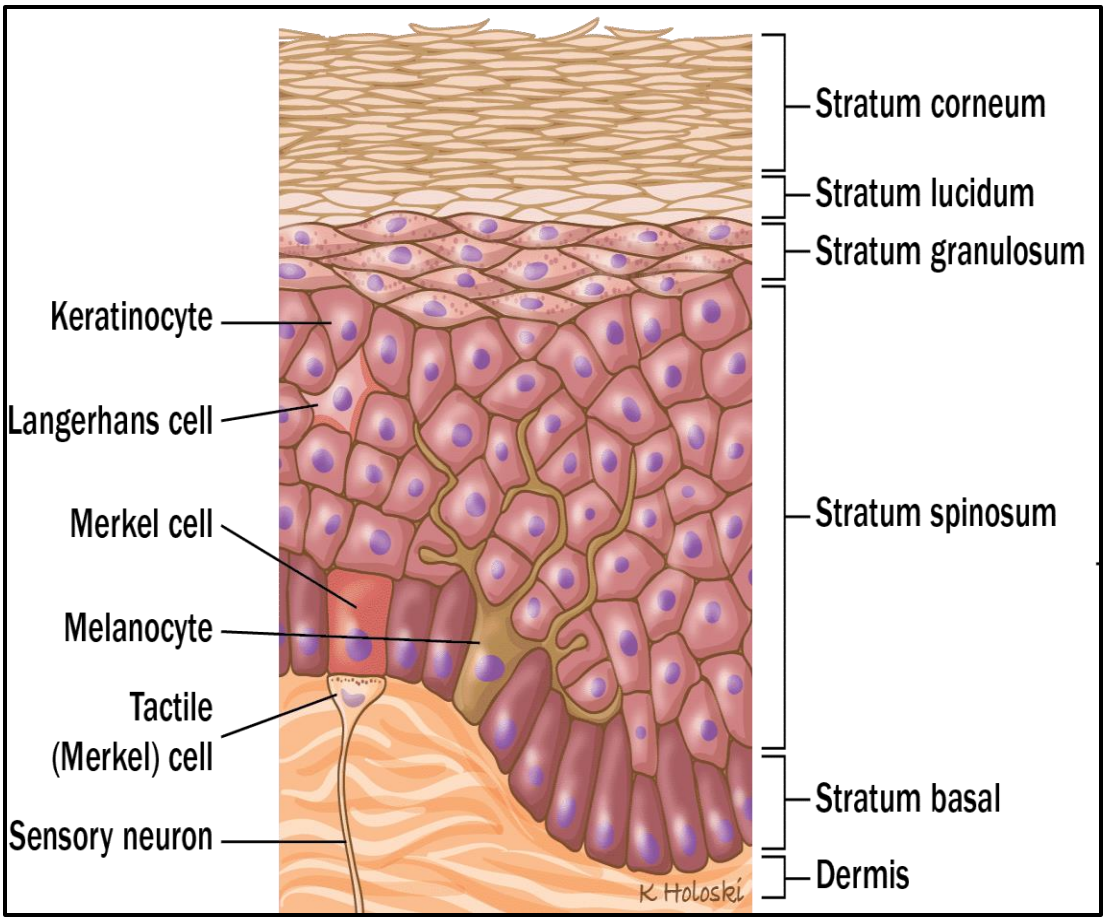

Figure 2.3: Sub layer of the Epidermis and Main Types of Cells of the Skin. (Source: https://thancguide.org/cancer-types/skin/anatomy/)

a) **Stratum Corneum**: The outermost layer of the epidermis, capabilities of the layer are to form a barrier to shield underlying tissue from contamination, dehydration, chemical compounds, and mechanical stress.

b) **Stratum Lucidum**: A thin, clear layer of lifeless skin cells in the epidermis, it's composed of 3 to 5 layers of dead, flattened keratinocytes.

c) **Stratum Granulosum**: A skinny layer of cell in the epidermis mendacity above the stratum spinosum and under the stratum corneum.

d) **Stratum Spinosum**: The layer of epidermis observed between the stratum granulosum and stratum basale, the layer is composed of polyhedral keratinocytes, and it contains Langerhans cells.

e) **Stratum Basale**: The deepest layer of the five layers of the epidermis, also known as stratum germinativum, divide to shape keratinocytes of the stratum spinosum, which incorporates melanocytes and Merkel cells.

ii) The Dermis:

The dermis is the center layer of the skin, this layer below the epidermis consists of connective tissue; it is far linked to the epidermis by using a basement membrane. It carries hair follicles, sweat glands, apocrine glands, blood vessels, lymphatic vessels, collagen bundles, fibroblasts, nerves, and sebaceous glands. The blood vessel of the dermis gives nourishment and waste elimination from its personal cells and from the stratum basale of the epidermis. The middle layer of the skin is split into two areas: the papillary region and the reticular region.

iii) Hypodermis:

This layer is also called subcutaneous tissue; the subcutaneous fat layer is the deepest layer of skin. It is made from fat and connective tissue. It consists of a network of collagen and fat cells, free connective tissue, adipose tissue, and elastin. The principal cell sorts are fibroblasts, macrophages, and adipocytes. It helps preserve the body's heat and protects the body from harm via acting as a shock absorber.

2.4.2 Function of Human Skin

Human skin is the out covering and biggest organ of the body, which protects against heat, injury, and contamination. The skin additionally acts as an anatomical cover from bacteria and any damage between the internal and external environment, and performs a key role in shielding the body against immoderate water loss **Madison, K. C., (2003), Proksch, E., Brandner, J. M. and Jensen, J. M. (2008)**. It helps to regulate body temperature. The skin can act as a storehouse of water & fat (lipid). Skin can synthesize vitamin D when exposed to the sun (UV rays). It excretes urine with the aid of sweating is at most a secondary characteristic to temperature regulation. Many varieties of medication can be topically administered via the skin e.g. cream or ointments. The skin is a vital site of transport in lots of other organisms. It contains a variety of nerve endings that react to warmness and cold, touch, pressure, vibration, and tissue injury. The skin acts as a water-resistant barrier so crucial nutrients are not washed out of the body.

2.5 Burn

Burn is one of the serious health problems which causes disability & mortality worldwide **Kumar, S., Ali, W., Verma, A. K., Pandey, A. et al. (2013)**. There are numerous types of burns because of thermal, radiation, chemical, or electrical touch.

i. Thermal Burns: These burns are because of warmness sources that enhance the temperature of the skin and tissues. They motive tissue cell death or charring. When hot metals, scalding liquids, steam, and flames come into touch with the skin, they can cause thermal burns.

ii. Cold Exposure Burns (frostbite): Damage to the skin is caused by exposure to cold.

iii. Radiation Burns: These burns are because of extended exposure to UV- rays of the solar, or to other sources of radiation, which includes X-rays.

iv. Chemical Burns: These burns are due to strong acids, alkalis, detergents, or solvents that come into contact with the skin, eyes, mouth, or GI- tract.

v. Electrical Burns: These burns are from electrical current.

In burn injuries, protein denaturation and cell death either by necrosis or apoptosis are very common. Some current therapy for acutely burned patients is based on sufficient regeneration, quick wound debridement, closure, the aid of post-burn hyper metabolic response, and control of infection.

2.5.1 Burn Wound and Burn Infection

Whilst skin or organs are damaged by means of an electrical shock, heat, chemical compound, or flammable agent effect the burn wound occurs. Burn reasons changes in vascular permeability, extravasation of plasma proteins, aggregation of platelets, and extended fibrinolysis.

These wounds or injuries are devastating accidents and cause disability & mortality worldwide **Kumar, S., Ali, W., Verma, A. K. and Pandey, A. et al. (2013)**.

After burn injuries, in most cases, the skin loses its barrier functions as a result there is a potential chance of bacterial infection by microorganism. Microbial contamination may also delay recovery and growth pain, and chance of scarring to skin and even lead to death. The wet nature, high temperature, and nutrient-wealthy surroundings of a burn wound create a perfect territory for the growth of bacteria. But, the predominant subject whilst faced with an infected

burn is that the contamination is hard to diagnose. The signs and symptoms associated with burn accidents, hyperthermia, tachycardia, and hyperventilation, are also common in sufferers with infected wound sites. The similarity in signs displayed makes the prognosis of infection much much greater difficult. *Staphylococcus aureus* and *Pseudomonas aeruginosa*, others may additionally consist of *Streptococcus pyogenes* are the most not unusual pathogens isolated from burn wounds location, they produce several virulence elements together with proteinases and collagenases, a variety of exotoxins, such as TSST-1 as well as a range of endotoxins.

2.5.2 Types of Burn

Depending on the severity and how deep they penetrate the skin, burns are classified into four groups. All types are given & described below with **Figure 2.4**

A. First Degree Burn or Superficial Burn.
B. Second Degree Burn or Partial Thickness Burn.
C. Third Degree Burn or Full-Thickness Burn.
D. Fourth Degree Burn

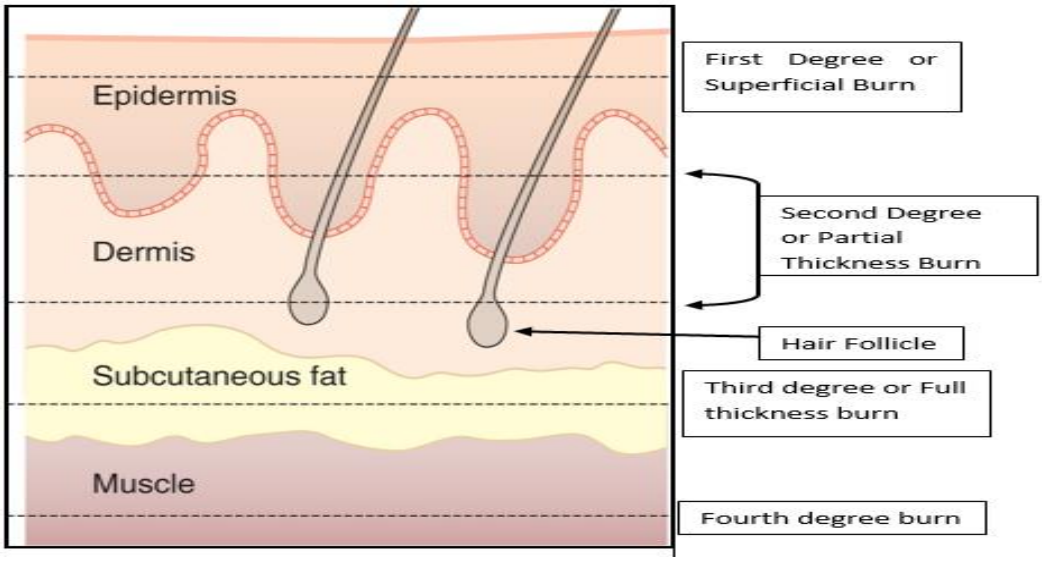

Figure 2.4: Different Types of Burn. (https://www.elsecier.com)

A. First Degree Burn

A first-degree burn (**Figure 2.5**) is also known as a superficial burn or wound.

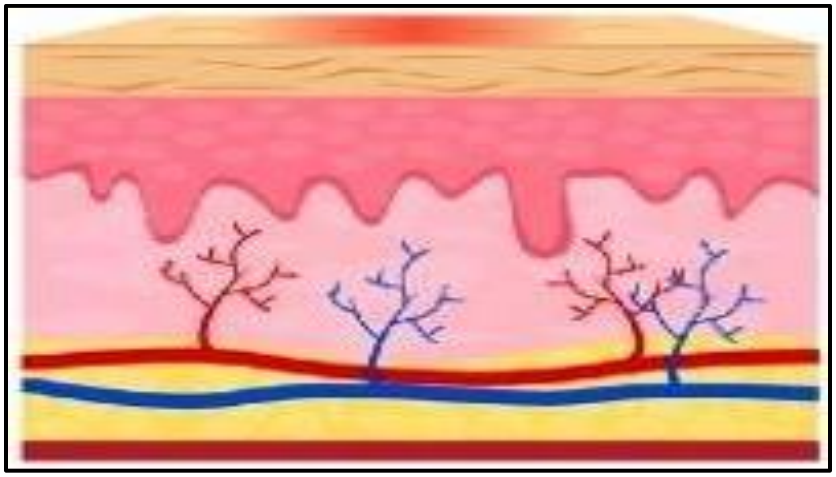

Figure 2.5: First-Degree Burn with Redness.
(https://www.parkwayeast.com.sg/healthplus/article/treating-minor-burns)

Sunburn is a kind of first-degree burn. It's one of the mildest forms of skin injury and it usually doesn't require medical treatment. It causes minimal skin damage. The outer layer of skin or the first layer of skin or epidermis is the only skin layer that is affected. The skin is red, dry, and painful, and the area may swell slightly but blisters will not be visible.

B. Second Degree Burn

Sometimes called a partial thickness burn, swelling with clear or yellow color fluid. It affects both the epidermis and the second layer of skin called the dermis. This type of burn involves the dermis, the second layer of skin. The skin is red, peeling, and painful, as with a first-degree burn. The skin will often start to blister and pain can be severe. This can take the time or it can begin immediately after receiving the burn. Second degree burn has been shown in **Figure 2.6**

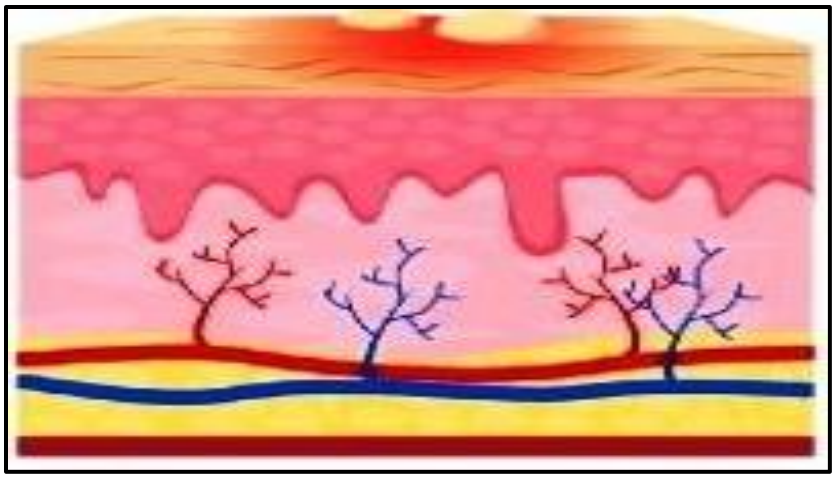

Figure 2.6: Second-Degree Burn with a Blister.

(https://www.parkwayeast.com.sg/healthplus/article/treating-minor-burns)

C. Third Degree Burn

A 3rd-degree burn (**Figure 2.7**) is also called a full or complete-thickness burn. Third degree burns expose the dermis and epidermis to the underlying adipose tissue or fat. These types of burns can ruin nerves, so the location can become numb. The skin may look or feel different, appear white or brown, or even become leathery. It may become hard.

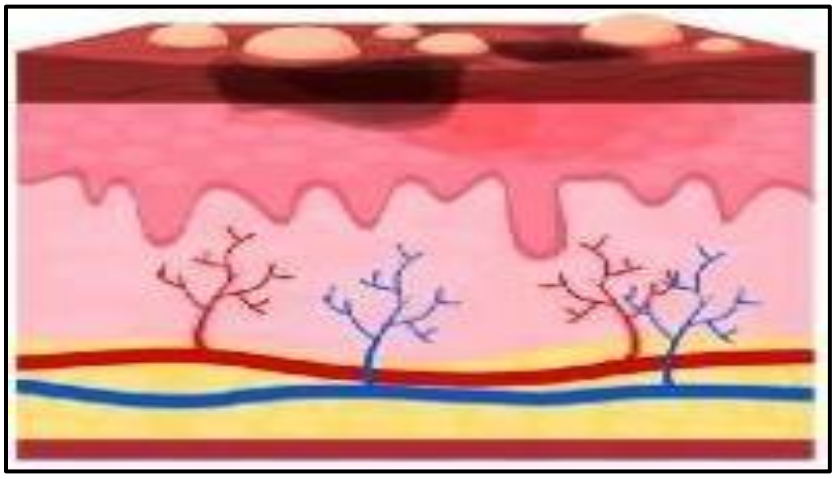

Figure 2.7: Third-Degree Burn with Dryness and Discolored.

(https://www.parkwayeast.com.sg/healthplus/article/treating-minor-burns)

D. Fourth Degree Burn

A fourth-degree burn (**Figure 2.8**) is the most intense type of burn.

Figure 2.8: Fourth-degree Burn with Capillary Absent and Black Color.

(https://www.parkwayeast.com.sg/healthplus/article/treating-minor-burns)

This kind of burn involves both layers of the skin and underlying tissue, and possibly deeper tissue associated with muscle and bone. The skin may be blackened or absolutely burned away, the affected area has a charred-looking appearance. There is often nerve damage (the nerve endings are destroyed) with a fourth-degree burn, so the affected person might not experience within the affected area or experience no ache.

2.6 Burn Wound Healing

Burn wound restoration is a complicated method that goes through a sequence of cellular and biochemical events. The foremost feature of this technique is to prevent the body from being infected through wounds, promote wounds to heal with a minimum scar, and go back skin feature quickly **Martin, P. (1997)**.

2.6.1 Burn Wound Healing Process

The process includes four tiers: a) Hemostasis b) Inflammation c) proliferation and d) remodeling **Boateng, J. S., Matthews, K. H., Stevens, H. N. E. and Eccleston, G. M. (2008)**.

a) Hemostasis:

The hemostasis process starts immediately after injury. Platelets play a role in forming a clot to seal the damaged site and stop bleeding and the following help to wound healing response **Davie, E. W., Fujikawa, K. and Kisiel, W. (1991)**. The process continues for 24 to 48 hours. Platelet growth factors are released.

b) Inflammation:

Inflammations begin after 24 hours of injury. Neutrophils (platelet growth factor) are released in this stage to clean and destroy bacteria, dead tissue, pathogens, and cell debris by the phagocytosis mechanism. It also plays a role in activating fibroblasts, keratinocytes, and endothelial cells **Hübner, G., Hu, Q., Smola, H. and Werner, S., (1996)**.

c) Proliferation:

Proliferation, also known as the regenerative phase, started on the third day of post-wounding. The level may be characterized via the presence of fibroblast, red tissue, replacement of dermal tissue, and subdermal tissue in the wound **Nissen, N. N., Polverini, P. J., Koch, A. E., Volin, M. V., Gamelli, R. L. and DiPietro, L. A. (1998)**.

d) Remodeling:

The very last level of the wound recovery process is the remodeling phase. It can last more than two years after beginning at about 2 to 3 weeks **Liu, D., Li, X., Li, J., Yang, J., Yokota, H. and**

Zhang, P. (2015). The new epithelium is formed along with the transition of granulation tissue to a mature scar that has high tensile strength.

2.6.2 Factors Affecting Burn Wound Healing

Many elements controlling the efficacy, velocity, and way of wound restoration fall underneath two sorts:

i. Local Factors and
ii. Systemic Factors

i) Local Factors: A local factor that affects burn wound healing is oxygenation. Hypoxic wounds that do not restore oxygenation have impaired recovery. Persistent or chronic hypoxia slows wound healing. Local factors are including: infection, foreign objectives and hydration.

- **Infection**: *Pseudomonas aeruginosa* and *Staphylococcus aureus* appear to play an important role in microbial infection of wounds.
- **Foreign Objects**: Small, sharp objects can pierce the skin and leave small scratches on the surface, causing internal injuries and bleeding.
- **Hydration**: Keeping the wound moist rather than dry can help it heal faster and significantly reduce pain and scarring. Other: mechanical factors, edema, ionizing radiation, defective wound closure technique, venous insufficiency, ischemia and necrosis, perfusion

ii) Systemic Factors that Influence Healing: Systemic factors that influence burn are: age, sex hormones in elderly individuals, diabetes, nutrients and medications.

- **Age**: aging is a major risk factor for impaired wound recovery. Numerous medical and animal researches at the cellular and molecular levels have tested age-related modifications

and delays in wound recuperation. Although it is widely accepted that the effects of aging delay wound healing in healthy older adults, there is no real impediment to the quality of healing.

- **Sex Hormones in Elderly Individuals**: research suggests that estrogen can improve the age-associated impairment in recuperation in both male and female, whilst androgens modify cutaneous wound restoration negatively.
- **Diabetes**: People with diabetes reveal decreased functionality inside the healing of acute wounds and are at risk of growing chronic diabetic foot ulcers, a severe complication of diabetes that impacts fifteen percent of human beings with diabetes and debts for eighty-four percent of all diabetes-related lower leg amputations. This impaired recuperation involves hypoxia, fibroblast, and epidermal cell dysfunction, impaired angiogenesis and neovascularization, high stages of metalloproteases, harm from reactive oxygen species and AGEs (advanced glycation end-products), reduced host immune resistance, and neuropathy.
- **Nutrients**: Nutrients are very critical factors that have an effect on wound recuperation. Deficiency of nutrition or malnutrition has a marked effect on wound restoration. Nutrients which include vitamin A, C and E, minerals like magnesium, copper, zinc, and iron besides proteins, carbohydrates, arginine, glutamine, polyunsaturated fatty acids all play giant roles in wound restoration. Fats and carbohydrates offer most people of the strength required for wound restoration. Glucose is the primary fuel source and is used to create cellular ATP that provides energy for angiogenesis and new tissue deposition. Due to the complex nutritional needs of each patient and associated wound, it is recommended that customized nutritional support can support acute and chronic wound restoration.

- **Medications**: many medical drugs, along with those which interfere with clot formation or platelet feature, or inflammatory responses and cell proliferation have the capability to have an effect on wound recuperation. Examples of few normally used medicines which have a huge effect on restoration:

  ✓ **Steroid Drugs**: Glucocorticoid steroids Drug-beclomethasone, betamethasone, budesonide, cortisone, dexamethasone, hydrocortisone, prednisolone: For the anti-inflammatory purpose

  ✓ **Non Steroids**: NSAID drugs- Acetaminophen, diclofenac: For the anti-inflammatory purpose

  ✓ **Chemotherapeutic Drugs**: Mechlorethamine, Cyclophosphamide, Chlorambucil, Melphalan, and Ifosfamide.

Other factors like Metabolic diseases, Immunosuppression, Connective tissue disorders Inflammation, Obesity, etc.

2.7 Ceramide

Ceramide is one of the important components of the stratum corneum, the epidermal layer of human skin. The major lipid components of the stratum corneum play important roles in regulating the homeostasis of the skin's water barrier and water protective ability. **Elias, P. M. and Menon, G. (1991)**.

Many products containing ceramide have already been investigated and found effective for skin care which made it potential in pharmaceutical industries for uses in skin care products.

Hydrophilic ointment enriched with 0.1 % ceramide was found to have good healing effects on patients with atopic dermatitis (and hydrocolloid dressing containing 0.3% ceramide accelerated skin erosion **Tsuchiya, S., Ichioka, S., Sekiya, N., Tajima, S., Iwasaki, T. and Numata, S. (2012)**. Ceramides are also very powerful in recovering the water content of dry skin and in alleviating atopic eczema **Hara, S., Takahashi, H. and Tomiya, Y**. **(1999)**.

The ceramides are positioned in the stratum corneum of the skin and play important roles in preserving barrier characteristics and defending the skin in opposition to numerous overseas damages. An examination by way of Imokawa et al. showed that the signs and symptoms of atopic dry skin were stepped forward by using topical application of the ceramides **Imokawa, G. and Ishida, K. (2014)**. Meanwhile, Lati et al. mentioned that plant ceramides are useful as anti-allergic and antioxidant by inhibiting free radical effect and inhibition of elastase, collagenase, and tyrosinase **Lati, E. (1995)**.

As a result, the experimented ceramide is suitable to be used as a complement for prevention against aging and rejuvenating stressed skin. The impact of rice-derived glycosphingolipid on mouse itch model brought about by way of compound 48/80 and degranulation from sensitized mast cells was tested. Those exams suggest that rice-derived glycosphingolipid reduces histamine launch and itch of atopic dermatitis resulting from histamine.

These studies indicate that ceramide can be used as an active component in dermatological treatments, especially for the improvement of skin barrier function, atopic dry skin, and burn. Depending on the quantity of ceramide present in skin may regulate the condition of skin like adequate quantity of ceramide refer the healthy condition of ceramide on the other side inadequate of ceramide quantity of ceramide to be reason for unhealthy skin, dry skin and

damaged skin etc. The condition of skin depending on the quantity of ceramide presence in skin has been presented in **Figure 2.9**.

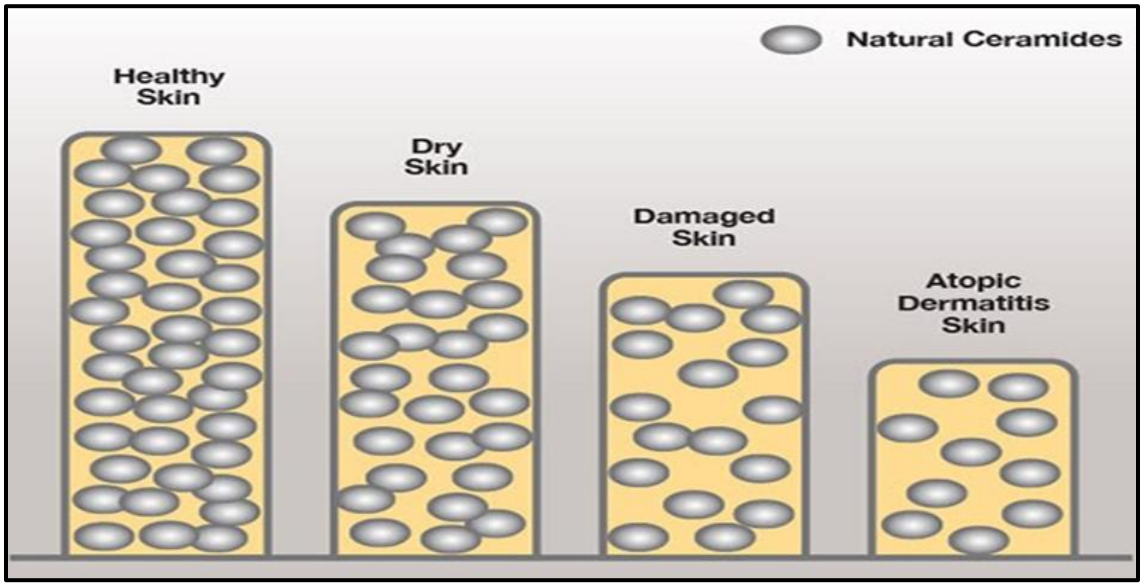

Figure 2.9: Quantity of Ceramide in Different Types of Skin. (Source: https://www.hollister.com/)

The animal glycosphingolipid is similar to the glycosphingolipid extract from rice bran; in which ceramide is the backbone of the glycosphingolipid including fatty acid in an amide linkage along sphingoid bases, and the hydroxyl group is substituted by glucose. As a result of the different fatty acid components and chemical structure of sphingoid bases, there are many types of glycosphingolipids present in nature. Till now, over twenty molecular sphingolipids species have been identified in rice bran. In this study, we have incorporated ceramides into the PVA & gelatin to form emulsions by constructing hydrogels. In the previous ceramide has been formulated or incorporated into hydrocolloid dressing or ointment but this is the first study in that ceramide has been incorporated into hydrogel.

2.7.1 Source & Uses of Ceramide

The word "ceramide" comes from the Latin sera and amide. Cera means wax and is the ingredient in cheese smear (*vernix caseosa*), the waxy or cheesy white substance found on the skin of newborn babies. This product is extracted with hexane and ethanol from rice bran and rice germ of *Oryza sativa* Linnaeus (Poaceae).

Naturally, ceramide is to be had within the skin, but synthetically ceramide has been collected from sweet potatoes, soy, wheat, rice & corn. The experimented ceramide changed into accrued from Oryza Oil & Fat Chemical Co., Ltd, Japan. The commercial name of the experimented ceramide is 'Oryza Ceramide- WSPC8'. From rice bran and rice germ of *Oryza sativa* Linne (Gramineae), 'Oryza Ceramide- WSPC8' is extracted with hexane and ethanol solvent. It consists of at least 8 % glycosphingolipid and 32% *Oryza sativa* (Rice) bran oil. This product is water soluble powder and cosmetic grade. The representative structure of ceramides in rice bran was set up by experiment of chromatography and NMR spectra, as demonstrated in **Figure 2.10** which is composed of a sphingoid long-chain base containing a fatty acid related to 2-amino group amide, it is an essential components of sphingolipids.

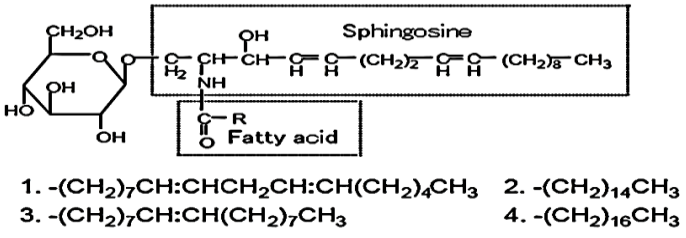

Figure 2.10: Structure of Ceramide (R= alkyl portion of Fatty Acid).

Topical skin drugs are used to complement the treatment of skin disorders such as eczema, atopic dermatitis, and skin sores. It is also used in beauty products such as soaps, shampoos, skin

creams and sunscreens. In addition, ceramide are being investigated as potential therapeutic agents for cancer. **Huang, W. C., Chen, C. L., Lin, Y. S., Lin and C. F. (2011)**. It helps to restore moisture and reduce skin irritation. It is also used for the treatment of psoriasis. Ceramide protect the skin from UV rays, bacteria, viruses, and chemicals and hold water molecules to keep skin moisture which is demonstrated in **Figure 2.11**.

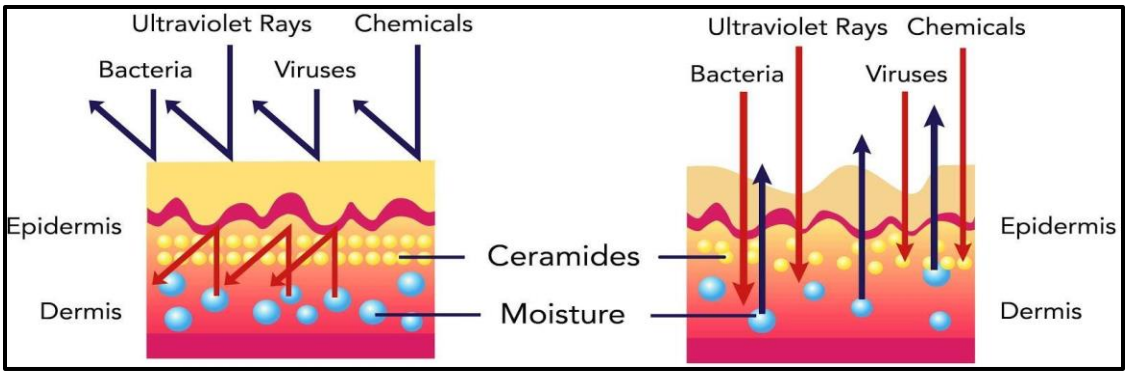

Figure 2.11: Role of Ceramide in Skin. (Source: https://www.gograph.com/vector-clip-art/ceramide.html)

2.8 Honey

Honey naturally contains eighty percent carbohydrates which include fructose, glucose, maltose, sucrose, etc; seventeen percent water, and the remaining three percent are protein, vitamin B, etc **Lee, D. S. and Sammy, S. (2011)**. Honey additionally consists of glycine, methionine, arginine, and proline, which might be all necessary for collagen formation and fibroblast deposition, the crucial elements wished for burn wound recovery **Tan, M. K., Hasan, A. D. S., Tumiran, M. A., Abdulla, M. A. and Yusoff, K. M. (2012)**.

Honey has natural antibacterial and anti-inflammatory properties, which promote granulation tissue formation and high ECM (collagen and hydroxyproline) deposition **Gupta, S. (2011)**.

The usage of honey in treating burns has the gain of making a moist environment, it saves the integrity of the burning surface as it is non-adherent, and it gives a bacterial barrier that forestalls cross-contamination and prevents infecting microorganism. Traditionally, honey was used as a natural medicinal wound dressing with multiple bioactivities, but a clinical explanation for its wound-restoration mechanism was not discovered until the 18th century. **Molan, P. C. (2011)**.

Now honey is approved by FDA for burn wound dressing applications and treatment of burn wounds and other wounds like ulcers & surgical wounds **Visavadia, B. G., Jan, H. and Martin, H. D. (2008), Subrahmanyam, M. (2015)**.

As a dressing application honey slowly releases H_2O_2 interacts with wound exudates, has antimicrobial properties, and at dilute concentrations promotes cell proliferation and angiogenesis, ultimately contributing to wound healing. **Tshukudu, G. M., Marilize van der, W., Quenton, W. (2010) and Al-Waili, N. S. (2005)**.

Honey has been shown to increase the wound healing rate when used as dressing material. Therefore, many researchers and scientists have incorporated honey into polymeric (e.g., PVA, PEO, Chitosan, and Silk) scaffold/matrix, skin graft, or hydrogel and used them as wound healing biomaterials. **Subrahmanyam, M. (2015), Arslan, A., Simşek, M., Aldemir, S. D., Kazaroğlu, N. M. and Gümüşderelioğlu, M. (2014), Wang, T., Zhu, X., Xue, X. and Wua, D. (2012), Reham, F. E., Reham, I. A., Dalia, A. and Elmazar, M. M. (2015)**. In our study, we have incorporated honey into PVA/ Gelatin with ceramide to develop a hydrogel dressing for burn treatment.

Honey hurries up wound recuperation whether or applied topically or administered systemically **Suguna, L., Chandrakasan, G. and Thomas, J. K. (1992)**. The healing results of honey have

been proven in the treatment of burns by helping wounds heal quickly with very little scarring **Subrahmanyam, M. (1991)**. Following materials like gelatin, PVA was used to develop hydrogel biomaterials where honey & ceramide were incorporated into the hydrogel biomaterials for burn wound healing purposes.

2.9 Gelatin

The matrix of polypeptides which is hydrolyzed from collagen, found widely in the bone and skin, pores, and other connective tissue of superior animals, and can be dissolved in warm water **Eong, B. and Gutowska, A. (2002)**.

It enables you to save fluid loss due to exudation, resulting inside the enhancement of its wound-recovery properties **Tanaka, T. N. and Matsuda, H. (2005)**. Gelatin is considered to be the most promising material for regenerative medicine applications due to its excellent biocompatibility and biodegradability.

Gelatin is without difficulty soluble in water at 37 °C, non-immunogenic, and reveals amphoteric behavior **Pierce, B. F., Pittermann, E., Ma, N., Gebauer, T., Neffe, A. T., Holscher, M., Jung, F. and Lendlein, A. (2012)**. Because of these properties, gelatin-based hydrogels are utilized in tissue engineering, and drug delivery systems. Additionally, the mechanical and chemical properties of gelatin can be changed by the usage of various varieties of crosslinking agents e.g., glutaraldehyde.

In addition, gelatin has been localized as a biomaterial that facilitates cell and tissue attachment and growth. For this reason, blends of biodegradable polymers have been used to provide applicable collagen-based hydrogel bandages.

2.10 Poly Vinyl Alcohol (PVA)

PVA is one of the renowned synthetic polymers because it possesses good biocompatibility therefore in several advanced biomedical applications like burn wound dressing it has been applied **Kenawyetal, E. K., Kamoun, E. A., Mohy, E. M. S., El-Meligy and M. A. (2014)**. PVA is also one of the maximum regularly synthetic polymers have no longer best been employed in wound dressing and management but also had been hired as drug delivery systems **Li, J. K., Wang, N. and Wu, X. S. (1998)** synthetic organs **Chen, D. H., Leu, J. C. and Huang, T. C. (1994)** and contact eye lenses **Hyon, S. H., Cha, W. I., Ikada, Y., Kita, M., Ogura, Y. and Honda, Y. (1994)**.

For hydrogel dressing membrane preparations PVA is one of the most crucial and carried out polymers. It can be attributed to its water solubility, biocompatibility, non-toxicity, and biodegradability. It possesses a simple structure and chemical amendment is also likewise clean. It well-known shows remarkable film-forming capacity and water retention properties **Peppas, N. A. and Merril, E. W. (1977)**.

The cross linking agent Glutaraldehyde is carried out to gelatin polymer with chemically cross linked PVA for biomedical research in the field of biomedical applications **Pal, K., Banthia, A. K. and Majumder, D. K. (2007)**.

Our attempt was made to develop a hydrogel membrane for burn wound healing by esterifying the -OH group of PVA with the -COOH group of Gelatin where ceramide and honey had been loaded. In this study, PVA/glycerol is applied as supporting other materials because of their curative dermal benefits, biocompatibility and swelling properties **Fluhr, J. W., Darlenski, R. and Surber, C. (2008)**.

CHAPTER 3

EXPERIMENTAL PROCEDURE

3.1 Materials

Ceramide was purchased from Oryza Fat oil and chemical company ltd, Japan. Polyvinyl alcohol (PVA), glutaraldehyde (GA), and glycerin were bought from Sigma Aldrich, Germany. Honey (Aussie Bee, Australia) was bought from the nearby marketplace in Bangladesh. Gelatin was collected from Global Capsules Ltd., Bangladesh, and Gelita Ltd., Germany. Mice (Swiss Albino) were bought from the Pharmacology Lab, Jahangirnagar University of Bangladesh. Distilled water and molecular biology-grade water have been used throughout the experiments.

3.2 Equipment

We have conducted different experiments in different labs and institutes using different materials and equipment. The preparation and physicochemical characterization of hydrogel was conducted in the Biomedical Engineering Lab of MIST. Scanning Electron Microscope (SEM) of hydrogel has been experimented with in the Chemistry lab of BUET. Fourier Transform Infrared Spectroscopy (FTIR) experimented at Wazed Miah Science and Research Institute, Jahangirnagar University. The antibacterial test was performed in the Tissue Engineering lab, MIST. In vivo experiments in mice, the model was evaluated in the Pharmacology lab, at Jahangirnagar University and the histological studies were experimented with in the Armed Forces Institute of Pathology. During this period, we used the following equipment throughout the research experiment.

- Hot plate magnetic stirrer
- Digital Electronic Balance Machine
- Freeze Drying Machine
- Oven
- Incubator
- Micro-Pipette
- Spectrophotometer
- SEM (Scanning Electron Microscope)
- FTIR (Fourier Transform Infrared Spectroscopy)
- Chemical Hood

3.3 Preparation of Hydrogel Membrane

The hydrogel dressing's membrane was prepared by following the solution casting method described in Hasan et al with slight modification **Hassan, A., Niazi, M. B. K., Hussain, A. and Farrukh, S. (2017)**. Briefly, a 10% gelatin solution was prepared by adding gelatin, glycerin & water in a ratio of 2.26:1:2 by using a hotplate magnetic stirrer with constant stirring. 3% honey solution was prepared by using a magnetic hotplate stirrer with constant stirring. Then the honey solution was dissolved into the gelatin solution. Different contents of ceramide ranging from 0, 0.25g, and 0.5 g were added in 10% PVA solution. PVA- Ceramide solution was poured into the mixture of gelatin and honey solution with constant stirring. The chemical crosslinking agent solution was prepared by adding 0.5 ml of GA and 0.05 ml of HCl in 10 ml of ethanol and added to the mixture with constant stirring. At last 2 ml of glycerin was added and the solution was poured into separated Petri dishes then the resulting solution mixture was left for 48 hrs at room

temperature to form hydrogel biomaterials. Heat and stirring continued for the whole process. The temperature was kept between 50°C to 70°C for the total process.

Five hydrogels biomaterials had been prepared for our experiment, which are:

a) C1 Hydrogel
b) C2 Hydrogel
c) CH Hydrogel
d) H Hydrogel
e) Control

C1 Hydrogel

To prepare 2.5% ceramide hydrogel, 0.25g ceramide was added into 10 ml distilled water and followed the above process. The prepared hydrogel was name C1 Hydrogel has been presented in **Figure 3.1**:

Figure 3.1: C1 Hydrogel (Containing 2.5% Ceramide).

C2 Hydrogel: To prepare 5% ceramide hydrogel, 0.5g ceramide was added into 10 ml distilled water and followed the above process. The prepared hydrogel was named C2 Hydrogel has been presented in **Figure 3.2**:

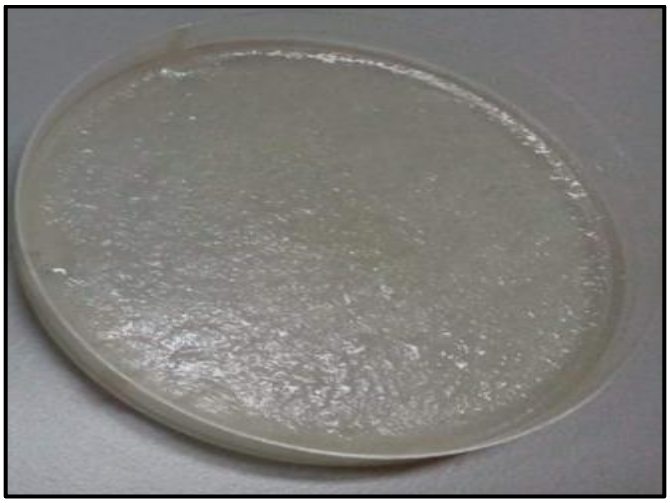

Figure 3.2: C2 Hydrogel (Containing 5% Ceramide).

CH Hydrogel: To prepare 5% ceramide and 3% honey hydrogel, 0.5g ceramide was added into 10 ml distilled water and 3ml honey was added into 100 ml distilled water then followed the above process. The prepared hydrogel was named CH Hydrogel has been presented in **Figure 3.3**:

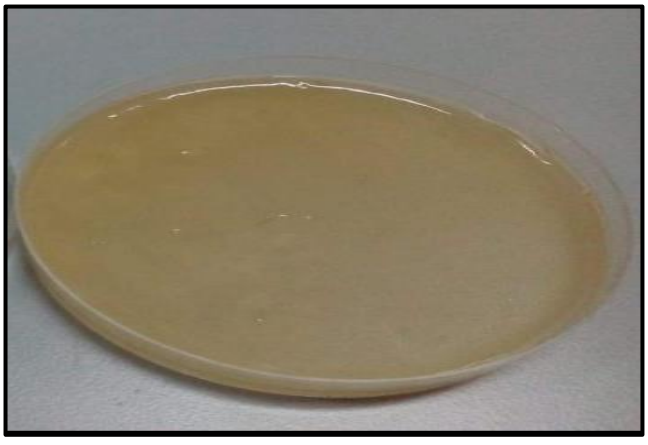

Figure 3.3: CH Hydrogel (Containing 5% Ceramide and 3% Honey).

H Hydrogel: 3% honey hydrogel, 3ml honey was added into 100 ml distilled water and followed the above process. The prepared hydrogel was named H Hydrogel has been presented in **Figure 3.4**:

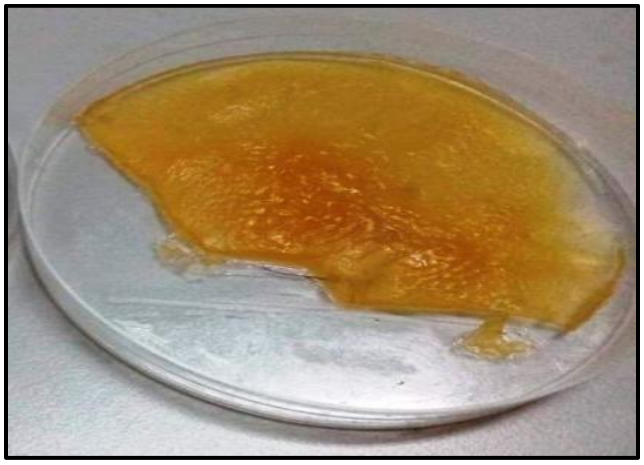

Figure 3.4: H Hydrogel (Containing 3% honey).

Control (No ceramide & honey, only PVA & Gelatin): To prepare control hydrogel, neither ceramide nor honey was added, only following the above process without adding ceramide and honey. The prepared hydrogel was named Control Hydrogel has been presented in **Figure 3.5**:

Figure 3.5: Control (Containing only PVA & Gelatin).

3.4 Water Vapor Transmission Rate (WVTR)

For WVT measurements, a test tube was taken with a 1.95 cm mouth diameter, and 10 ml of molecular biology-grade water into it. The open test tube was enveloped with hydrogel dressing's membrane and staunch by applying Teflon tape (**Figure 3.6)**. The test tube was weighed and at 40°C temperature placed in an oven for one day (24 hours). After 24 h taken out of the test tube was from the oven and took weight again. By using the following formula, the water vapor transmission rate (WVTR) was measured **Boonkaew, T. A. B., Suwanpreuksa, P., Cuttle L. et al. (2014)**:

$$\mathrm{WVT} = \frac{\mathrm{W_i} - \mathrm{W_f}}{\mathrm{A\ x\ 24\ x\ 10^6}}\ \mathrm{gm/m^2h}$$

Where, **A**= area of the round mouth of the test tube; $\mathbf{W_i}$ = initial weight of test tube; $\mathbf{W_f}$ = final weight of test tube

Figure 3.6: WVTR Experiment (Open Test Tube was Enveloped with Hydrogel).

3.5 Moisture Retention Capability

For moisture retention ability measurement, the hydrogel dressing membranes were cut into the same pieces and depth and weighed then placed in an oven for 5 h at 45°C. By using the following formula, the moisture retention capability was measured **Roy, N., Saha, N., Kitano, T. et al. (2011)**:

$$\text{Moisture Retention Capability}(\%) = \frac{W_f}{W_i} \text{x } 100$$

Where, $\mathbf{W_f}$ = weight before placing in an oven and $\mathbf{W_t}$ = weight after 5 h

3.6 Gel Fraction

Prepared hydrogel dressing membranes were cut into the same pieces then placed in a vacuum oven and waited to gain the constant weight. Then hydrogel membranes were weighed. Afterward, the hydrogel membranes were immersed in distilled water for 4 days. These hydrogel dressing's membranes were taken out of the water after 4 days and placed again in a vacuum oven to gain a constant weight. By using the following formula, the gel fraction was measured **Hago, E. E. and Li, X. (2013)**:

$$\text{Gel Fraction}(\%) = \frac{W_f}{W_i} \text{ X } 100$$

Where, $\mathbf{W_i}$= weight after placing in a vacuum oven and $\mathbf{W_f}$= weight when the wet samples were dried in a vacuum oven.

3.7 Porosity Evaluation

The porosity (P) of the hydrogel was determined by the liquid displacement method by following the equations as described below **Dhasmana, A., Singh, L., Roy P. and Mishra, N. C. (2018)**.:

$$P = \frac{S_1 - S_3}{S_2 - S_3} \text{ x } 100$$

Where, $\mathbf{S_1}$ = solvent volume (initial); $\mathbf{S_2}$ = solvent volume after the sample immersion; $\mathbf{S_3}$ = solvent volume after withdrawal of sample; **P** = porosity

3.8 Measurement of Swelling Behavior

The swelling behavior of the hydrogel dressing's membrane was measured by dissolving the hydrogel in different physiological solutions (e.g. Blood, Water, 0.9% NaCl, 5% Dextrose) has been demonstrated in **Figure 3.7**. The hydrogel dressing's membranes were cut and weighed to measure the swelling behavior. Then the hydrogel membranes were immersed in these physiological solutions for 1.5 hr & 3 hr. For the swelling ratio test in blood solution, hydrogels were immersed in blood solution with an anticoagulant (heparin). After a time interval, hydrogel membranes were withdrawn from the solution, and the surface of the hydrogel was dried with filter paper to dry and erase any droplets and take weight again. By using the following formula swelling behavior was calculated **Pal, K., Banthia, A. K. and Majumdar, D. K. (2007)**.

$$\text{Swelling Ratio } (\%) = \frac{W_s - W_d}{W_d} \text{x } 100$$

Where, $\mathbf{W_s}$ = weight of swelled membrane and $\mathbf{W_d}$ = weight of the dry membrane

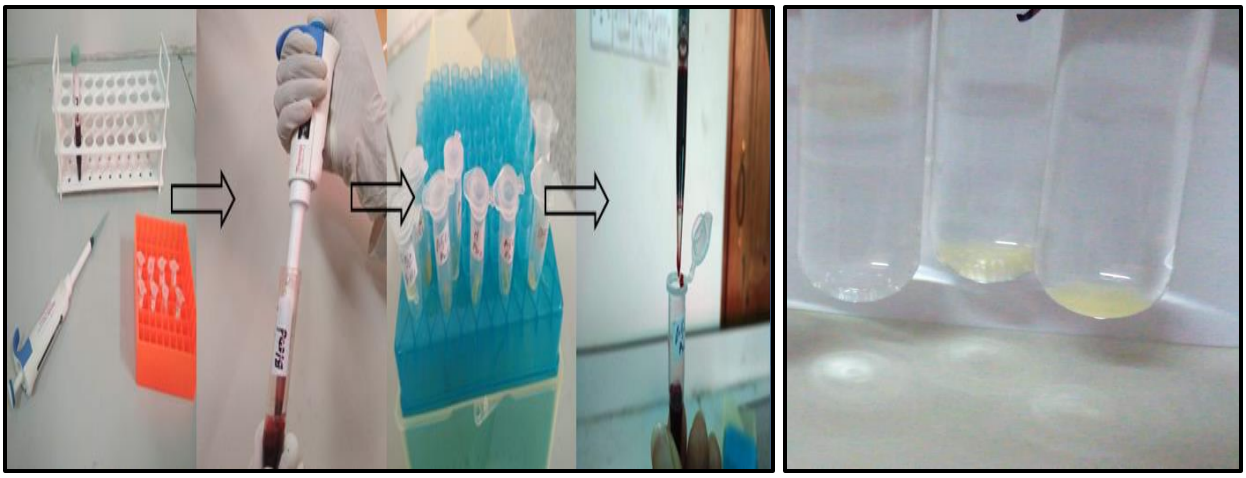

Figure 3.7: Hydrogels were immersed in the Blood (left) and in Water, 0.9% NaCl and 5% DA (right) for the Measurement of Swelling Behavior.

3.9 Evaluation of Antimicrobial Activity

The antimicrobial activity of hydrogels was determined by using the diffusion method against *E. coli* (ATCC 8739) and *Staphylococcus aureus* (ATCC 9749) as model gram (-) ve and gram (+) ve bacteria respectively and *Candida albicans* (ATCC 10231) as fungi **Bauer, A. W., Kirby, W. M., Sherris, J. C. and Turck, M. (1966)**. PVA- Gelatin hydrogels were used as a control.

The test specimens were C1 hydrogel, C2 hydro-gel, CH hydrogel & H Hydrogel. Test specimens were placed in an MHA medium inoculated with *Staphylococcus aureus*, *Escherichia coli* & *Candida albicans*. At 37°C temperature incubated for 24 h after which the 'Zone of Inhibition' was. After the incubation, the antibacterial and antifungal activity was evaluated on the basis of the zone of inhibition formed.

3.10 Scanning Electron Microscope (SEM)

The hydrogel was freeze-dried before being investigated by SEM. The structure of the surface of hydrogel membranes was experimented with by using FESEM (field emission scanning electron

microscope) (Model: JOEL JSM- 7600F). The sample was covered by a thin gold conductive layer before analysis. To create the layer a sputter coater was used. For observing the hydrogel surface, a secondary electron detector was used and the accelerating voltage was 5 kV. The structure of the surface of the hydrogel dressing's membrane was investigated by SEM

3.11 UV Spectroscopy

The hydrogel membranes solution prepared was characterized using ultra violet visible spectroscopy (Specord 205, Germany). For UV analysis spectra range was 200-600 nm, CH hydrogel was immersed in deionized water then ceramide particles and honey were eliminated into the deionized water sample from the swollen hydrogel samples, and for background correction, deionized water was used for UV spectra recording. Then from this medium sample was taken to measure the absorption spectra.

3.12 Fourier Transform Infrared Spectroscopy (FTIR) Analysis

FTIR (Shimadzu, IR Prestige- 21 PC) was performed to evaluate the major functional group, chemical structure, contents, and structural changes that happened in the hydrogel. The FTIR range was 4000 cm^{-1} to 400 cm^{-1} for the hydrogel membranes. In the case of hydrogel membranes (Control and CH Hydrogel), the samples were dried properly to make powder form. Then pellets were prepared including the KBr (infrared grade) by pressing and then taking the pellet in the FTIR machine for the experiment. At room temperature, all analysis was accomplished.

3.13 In- Vivo Burn Healing Experiment

3.13.1 Animal: Nine albino mice aged 8 weeks old and weight 30–35 g was collected for the experiment. All mice were separately kept in polyethylene cages at room temperature 23± 2 °C.

Throughout the study, all tested animals were fed on commercial pellets and supplied water ad libitum. The Animal Care, Handling, and Use Committee of Jahangirnagar University in Bangladesh approved the experimental protocol and the study was done following animal welfare guidelines and regulations.

3.13.2 Experimental Design: All mice were divided into 3 groups:

- **Group- I**: Control group (Negative). No treatment was applied.
- **Group- II**: Animal treated with CH- Hydrogel dressing.
- **Group- III**: Animal treated with C2- Hydrogel dressing.

Ketamine (80 mg/kg) and xylazine (5mg/kg) IM injection (Intramuscular) administered to anesthetized tested animals and used an electric clipper with shaving cream for removing hair on the back of the mice. Using 70% IPA disinfectant the shaved area. The burn wounds were created as reported in literature **Reham, F. E., Reham, I. A., Dalia, A. and Elmazar, M. M. (2015)**. Briefly, a 10 mm diameter cylindrical metal rod was heated over an open flame for 60 seconds then held and pressed to the shaved and disinfected dorsal mouse skin surface for 30 seconds under light ether anesthesia. Then experimented mice to be ready for taking measurement of the burn wound area by digital slide calipers.

3.13.3 Measurement of Wound Area

By digital slide caliper on 0, 7, 10, and 15 days before treatment application the reduction in burn wound edge diameter was measured. The diameter is measured in millimeter scale. The reduction of the initial burn diameter represented burn edge contraction. All data were recorded

for further analysis. Burn wound healing rate was calculated from this measured data recorded on mentioned days.

3.12.4 Determination of the Burn Wound Healing Rate

By using the following formula, the burn area reduction rate was calculated:

$$\text{Burn wound reduction rate (\%)} = \frac{A_1 - A_2}{A_1} \text{x } 100$$

Where, $\mathbf{A_1}$ = the initial area and $\mathbf{A_2}$ = the wound area at time **t**

3.14 Histological Analysis

Histological analysis was performed by using a light microscope. Tissue samples were collected from the skin of mice and fixed with 10% formalin. After fixation, samples were stained with H & E (hematoxylin-eosin), and all images were recorded by light microscope.

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1 Water Vapor Transmission Rate

After analysis of the WVT rate, the maximum rate was noted for C1 Hydrogel 97.23 $gm^{-2}h^{-1}$, after increasing ceramide the WVT rate was dropped for C2 Hydrogel 46.56 $gm^{-2}h^{-1}$. On the other hand, the minimum rate was noted for H Hydrogel 23.58 $gm^{-2}h^{-1}$. So the presence of honey helps to reduce the WVT rate drastically. When both ceramide and honey were added the rate was noted as 49.97$gm^{-2}h^{-1}$. From the WVT rate experiment, it has been cleared that higher ceramide lowers the WVT rate. For the control (neat hydrogel no ceramide and honey) the WVTR was noted at 99.12 $gm^{-2}h^{-1}$.

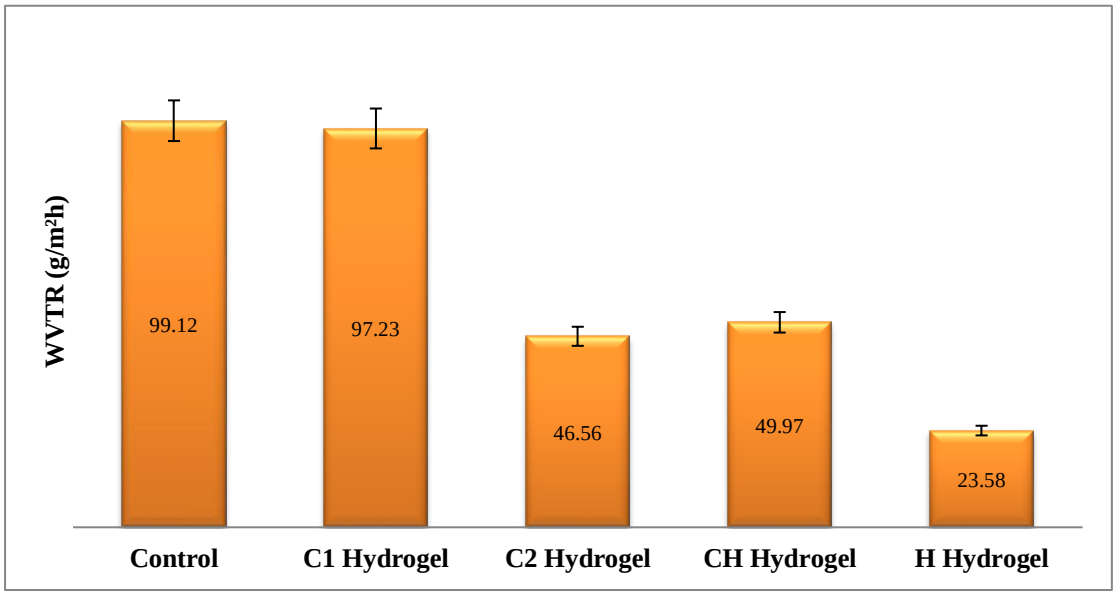

Figure 4.1: WVTR of Prepared Hydrogels.

Discussion on the Result: Too high value of WVTR may lead to rapidly drying the wound, resulting a scar formation, on the other hand too lower value of WVTR may lead to

accumulation of exudates, resulting risk of bacterial infection and a delayed healing process **Killion, J. A., Geever, L. M., Devine, D. M. and James, E. K. CLH. (2011)**. So an ideal hydrogel dressing's WVTR value should not have too much or too little and should control water evaporation from a wound at an optimal rate. An ideal dressing should also have less WVTR value than WVTR compared with 2nd and 3rd-degree burn in the skin, respectively which is 178.55 ± 4.5 $gm^{-2}h^{-1}$ and 143.2 ± 4.5 $gm^{-2}h^{-1}$ **Nilsson, G. et al. (1977)**.

In this study, the WVTR of four samples C1, C2, CH, and H were 97.23, 45.56, 49.97, and 23.58 g/m^2h respectively presented in **Figure 4.1**. The WVTR value of C2 and CH is comparable to Duoderm (UK) hydrocolloid dressings which have been reported to have 37.04±2 $gm^{-2}h^{-1}$ WVTR value **Fisher, P. W. A. C., FOO P. P., Queen, D. and Gaylor, J. D. S. (1995)**. Both results are also comparable with the WVTR value of PVA- Clay nanocomposite hydrogel for wound dressings is 35- 56 $gm^{-2}h^{-1}$ **Kokabi, M., Sirousazar, M. and Hassan, Z. M. (2007)**. So we can say that these two dressings could not only prevent dehydration but also protect the wound from excess hydration.

4.2 Moisture Retention Capability

The highest moisture rate was recorded for H Hydrogel at 47.30% then CH Hydrogel at 43.66% then C1 Hydrogel at 33.88% and the lowest recorded for C2 Hydrogel at 32.69%. For the control, the moisture retention rate was noted as 28.36%. The moisture retention rate of hydrogels and image after lost moisture from hydrogel are shown in **Figure 4.2** and **Figure 4.3** respectively:

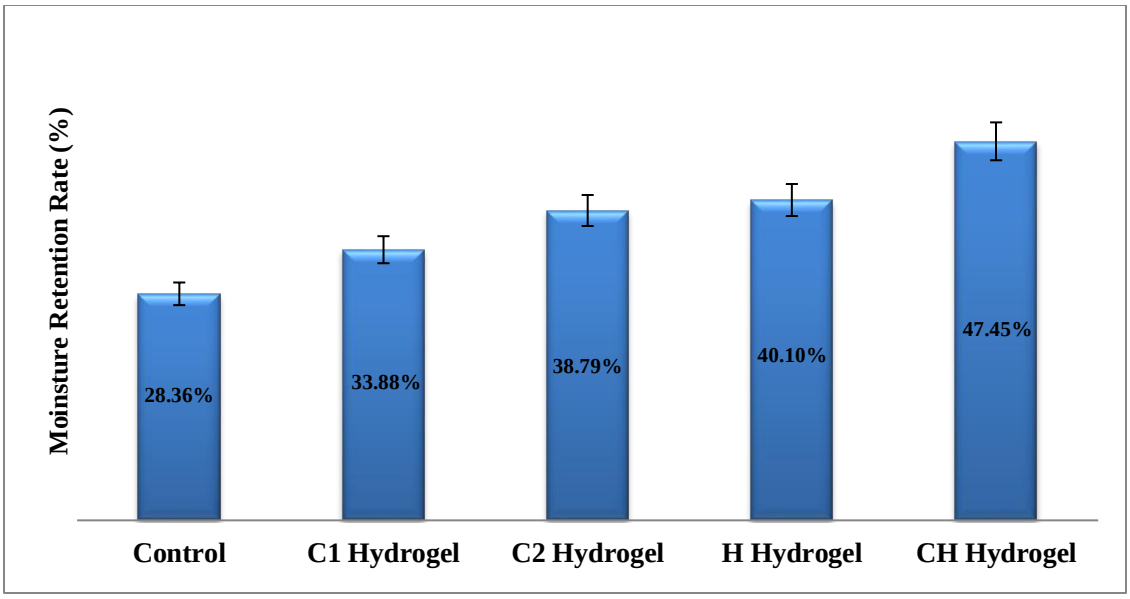

Figure 4.2: Moisture Retention Capability of Prepared Hydrogels.

Discussion on the result: Too much moisture loss from the wound may lead to a body temperature fall that may result in rises in metabolic rate and form a dry cell that may result in dead cells (the main component of the body is water so cell environment also moist) **Hoffman, A. S. (2002), Bryan, J. et al. (2004)**. On the other hand, too much accumulation of moisture may build up the risk of bacterial infection and bad odor. So ideal dressing should need to maintain optimal moisture content at the wound surface and it's very crucial in burn injury. In this study, we found too much less moisture retention capability for control (only PVA and Gelatin hydrogel) which was only 28%. After the addition and increasing quantity of only ceramide, the moisture retention capacity increased to 33.88% and 38.79% for C1 and C2 hydrogel dressing respectively presented in **Figure 4.2**. The moisture retention capability in both hydrogels was increased due to the addition of ceramide into the hydrogel. Oral ingestion of ceramide has already been described by Lati et al **Lati, E. (1995)**. Specifically, 20 mg of a wheat extract containing 3% by mass of ceramide derived from wheat is taken daily for 1 month to improve

the water retention function of the skin. The current investigation may suggest that the topical application of ceramide also contributes to improving moisture retention capability.

Again the addition of only honey also helps to increase the moisture retention of H hydrogel dressing was 40.10%. But in addition to both ceramide and honey together we have found an optimum moisture retention capability for CH hydrogel dressing which was 47.66%. The moisture retention capability of CH hydrogel dressing (47.45% at 40^0C in 5h) was comparable with the moisture retention capability of commercially available Geliperm sheet (Geistlich, Switzerland) was about 50% in 6h **Soler, D. M., Rodriguez, Y., Correa, H., Moreno, A. and Carrizales, L. (2012)**. So CH hydrogel dressing was recommended for burn injury which has been moderately losing moisture from the wound.

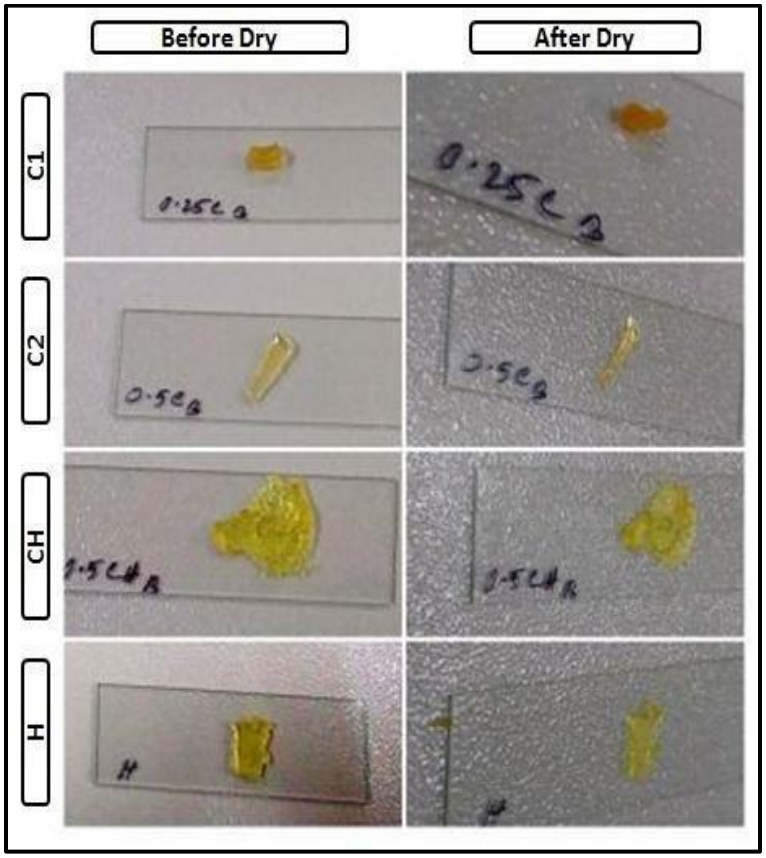

Figure 4.3: Moisture Retention Capability of HydrogelS (left: before dry, right: after dry).

4.3 Gel Fraction

Gel fraction was highest for C1 Hydrogel and lowest for H Hydrogel. Gel fraction rate of C1 Hydrogel was 167.19%, C2 Hydrogel was 39.45%, CH Hydrogel was 42.17%, H Hydrogel was 39.45%. For the control gel fraction was found 184.14%.

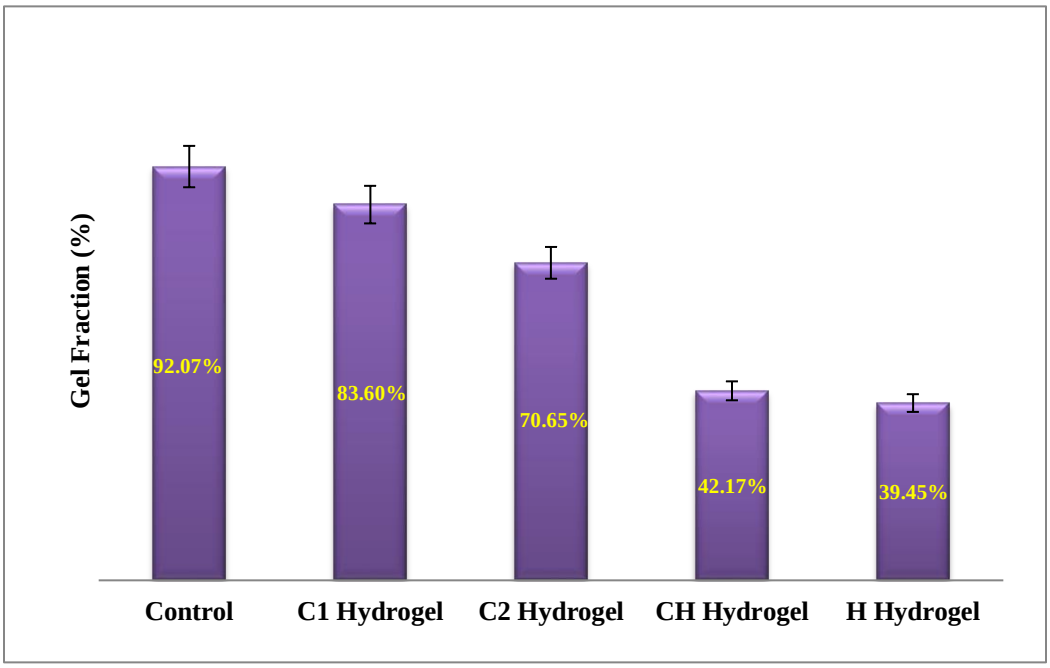

Figure 4.4: Gel Fraction of Hydrogels.

It shows the cross linking behavior of the hydrogel. According to data, it has seen that highest gel fraction was observed for control (only Gelatin and PVA) 92.07%. We also observed that the gel fraction gradually decreased from (Control) to 83.60% & 70.65% for C1 & C2 hydrogel respectively with increasing concentrations of ceramide. It can be also seen that the gel fraction reached to lowest when honey was loaded into a hydrogel (H Hydrogel) at 39.45%. But the addition of ceramide with honey helped to improve the gel fraction (CH Hydrogel: 42.17 %.).

The observed result of gel fraction in these studies for investigating all hydrogel dressing showed proper cross linking.

As per reported by Kim et al 2008 & Ajji et al 2005, as the gel fraction decreased, the flexibility of hydrogel also decreased **Kim, J. O., Park, J. K., Kim, J. H., Jin, S. G., Yong, C. S., Li, D. X., Choi, J. Y., Woo, J. S., Yoo, B. K., Lyoo, W. S., Kim, J. A. and Choi, H. G. (2008), Ajji, Z., Othman, I. and Rosiak, J. M. (2005)**. In this study, we found CH and H were less flexible hydrogel dressings with weaker strength. On the other hand, Control, C1 & C2 hydrogel was found to be more flexible and have higher strength (**Figure 4.4**). We have also observed that the ceramide hydrogel with honey (CH hydrogel) gave a lower gel fraction than did the ceramide hydrogel without honey (C1 & C2 Hydrogel). Honey reduced the Cross-linking interaction between PVA and Gelatin. It may be due to the high density of honey **Tomasik, P. (2003)**. On the other hand, a hydrogel with a higher ceramide concentration gave a lower gel fraction and a lower one gave a higher gel fraction. The effect of ceramide and honey may be utilized for the gel fraction control and regulation. In addition to playing as a burn wound healer and having a good WVT rate, optimum moisture retention capability ceramide and honey also reduces the gel fraction capacity (cross linking reaction) and slows the processing of the gelation.

4.4 Porosity

The porosity of control was 70%, after the incorporation of 5% & 2.5% ceramide and honey it was reduced to 50%, 55.56%, and 58% for C1, C2, and H hydrogel respectively given in **Figure 4.5**. The reduction of porosity for C1, C2, and H- hydrogel due to the incorporation of ceramide into PVA & Gelatin and high density of honey range between 1.38 to 1.45 kg/l at 200C **Tomasik, P. (2003)**. On the other hand, after the addition of ceramide the porosity reduced to

50% and 55.56%, it's due to the incorporation and interaction of ceramide with PVA and Gelatin. Higher ceramide concentrations lower porosity. The lowest porosity was found for CH 32.26% due to both dense honey and ceramide incorporation into the hydrogel dressings.

For an ideal dressing, porosity is an important factor for cell migration, proliferation, and nutrition supply.

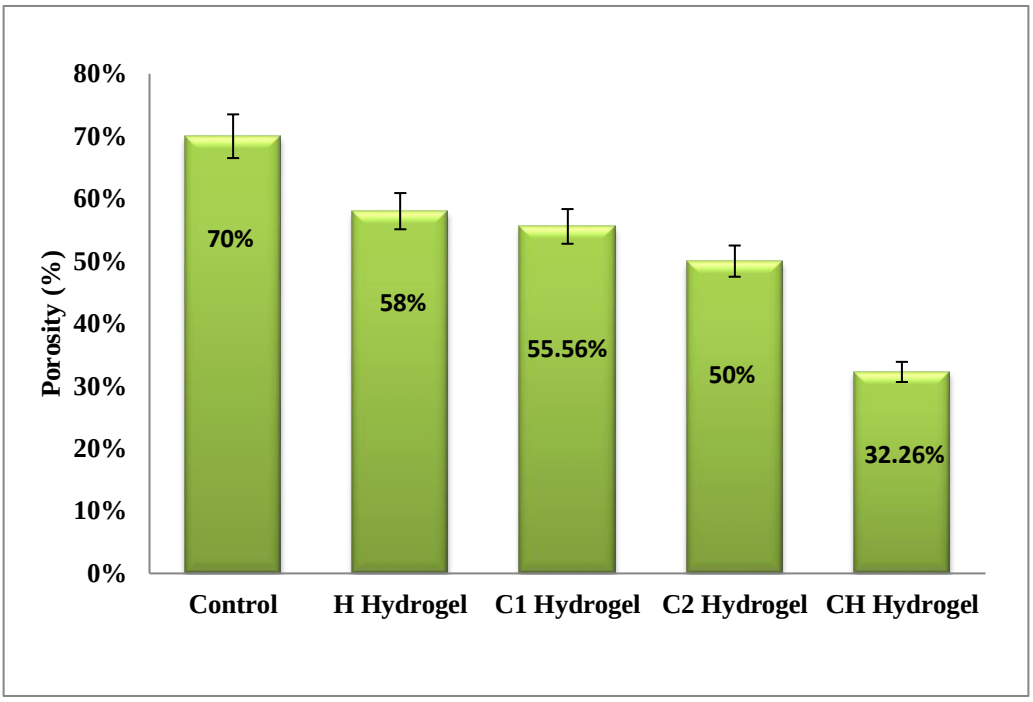

Figure 4.5: Porosity of Hydrogels.

On the other hand, the dense surface is also important for dressing material because it does not allow bacteria to penetrate, which can responsible for microbial infection to burn wounds. So from these studies, we found CH hydrogel with 32.26% porosity, which was not too porous or too much dense dressing material. It was better for cell migration, proliferation, and nutrition supplies as well as a good barrier against bacteria and helps to absorb excess moisture and exudates.

4.5 Swelling Behavior

Among all prepared hydrogels CH hydrogel showed the best swelling result in 5% DA, 0.9% NaCl, and Blood solution (with anticoagulant heparin) constantly than other hydrogels. In 5% DA solution the swelling ratio for CH hydrogel has been noted at 111.63% & 133.83% after 1.5 hr & 3 hr respectively. In 0.9% NaCl solution the swelling ratio was noted at 51.62% & 78.33% after 1.5 & 3 hr respectively. In water & blood (**Figure 4.6)** it was 103.27% & 150.52 %, and 55.11% & 66.21% after 1.5 hr & 3 hr respectively. The swelling ratio of other hydrogels (C1, C2, H & Control) has shown in **Figure 4.7**.

Compared with the control all other hydrogel solutions also showed a good swelling result in all physiological solution. The result of prepared hydrogels to be imposed the hydrophilic nature of hydrogel membrane.

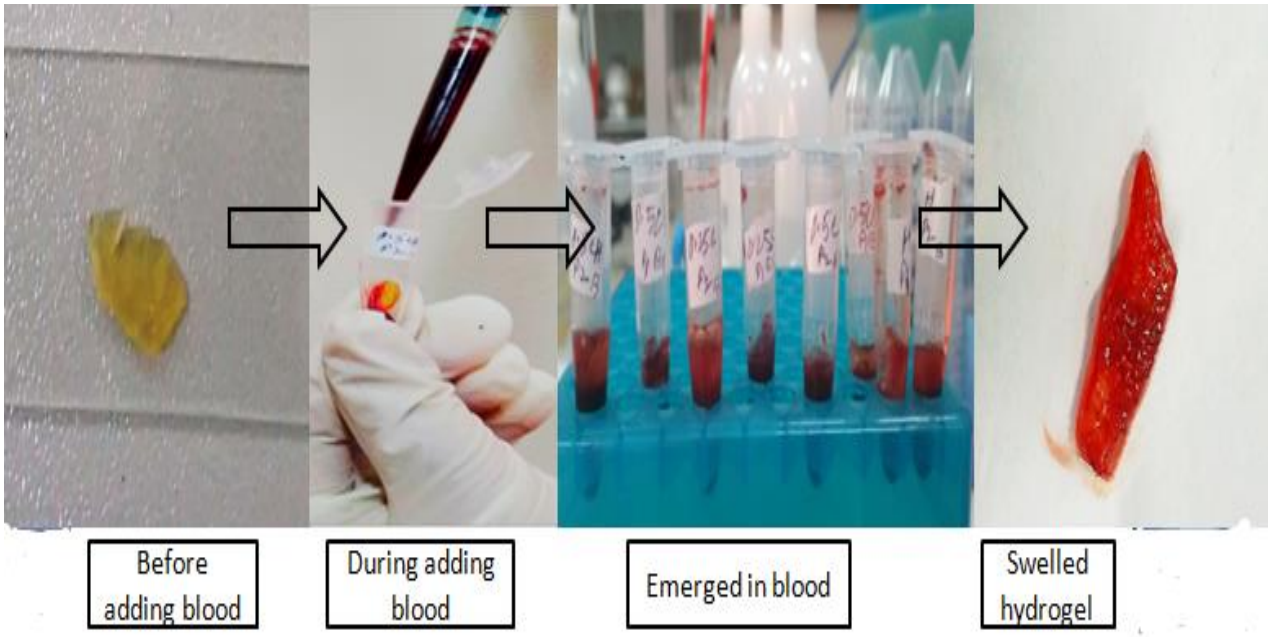

Figure 4.6: Hydrogel Swelled in Blood after Swelling Behavior Test.

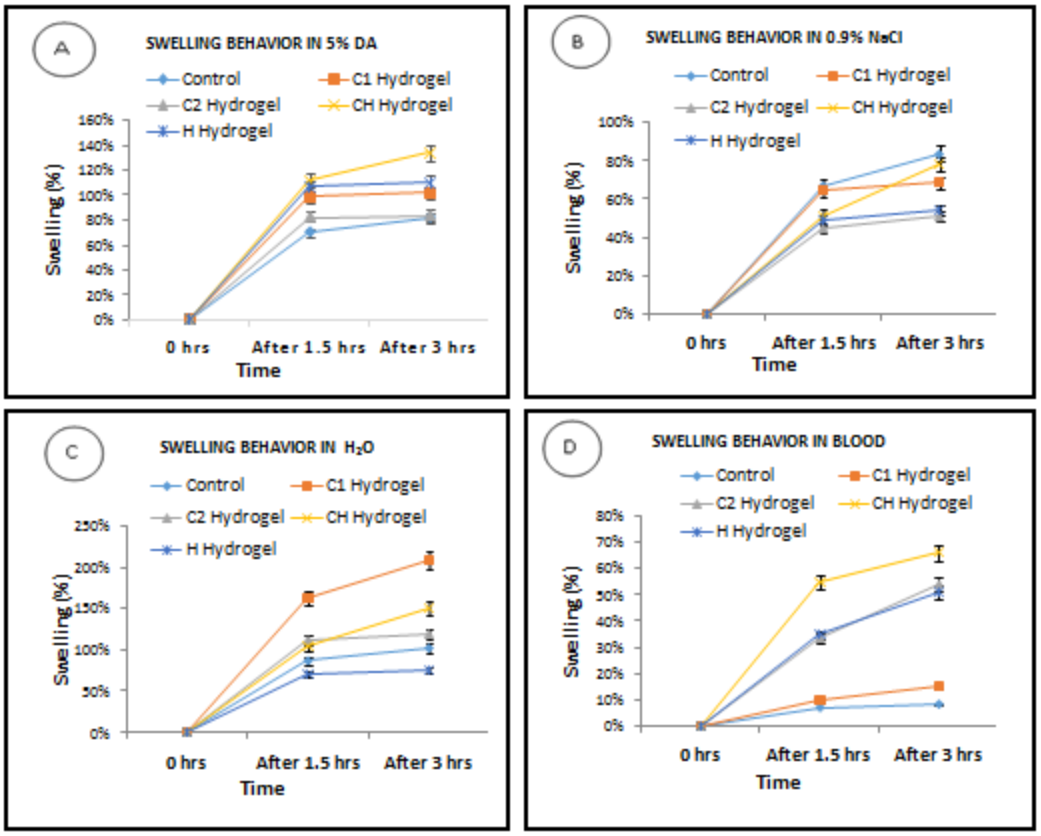

Figure 4.7: Swelling Behavior of Hydrogels in Different Physiological Solutions.

(A)- Swelling Behavior in 5% DA **(B)** - Swelling Behavior in 0.9% NaCl

(C)- Swelling Behavior in Water **(D)** - Swelling Behavior in Blood

4.6 Evaluation of Antimicrobial Activity

Antimicrobial activity of C2, CH, and H hydrogel was investigated against *Staphylococcus aureus* (gram +ve) and *Escherichia coli* (gram -ve) bacteria, and *Candida albicans* fungi (**Figure 4.8**). There was no zone of inhibition observed against *Escherichia coli* bacteria and *Candida albicans* fungi. The zone of inhibition was only found against *Staphylococcus aureus* by CH Hydrogel and H hydrogel, it may be due to the presence of honey in the hydrogel (**Table 4.1**).

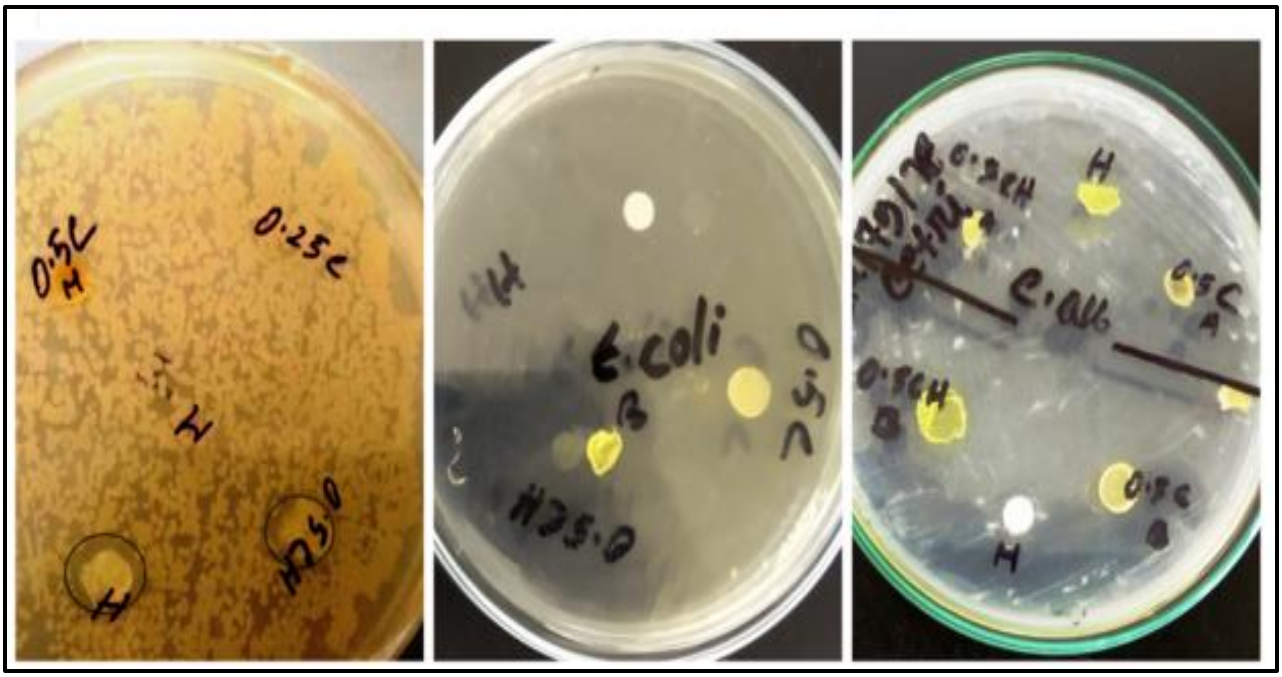

Figure 4.8: Antimicrobial Activity of Hydrogels Against *Staphylococcus aureus, Escherichia coli,* and *Candida albicans.*

Discussion on the result: Honey has a strong antibacterial activity against bacteria **White, Jr., Jonathan, W., Mary, H.S. and Abner, I. S. (1963)**. On the other hand, no zone of inhibition was found for these hydrogel against *Escherichia coli* and *Candida albicans* it may be due to less volume of honey in the hydrogel couldn't break the characteristics cell wall of gram negative bacteria and fungi. Gram negative bacteria *Escherichia coli* have a largely impermeable cell wall which contains a cytoplasmic membrane, a thin peptidoglycan layer and an additional outer membrane. The additional outer membrane composed by lipopolysaccharides and phospholipids which face the external environment. In case of fungal cell wall, it's composed of glucans, chitin and glycoproteins.

Table 4.1: Zone of inhibition of C2 Hydrogel, CH Hydrogel & H hydrogel

Hydrogel Formula	**Measurement of Zone of Inhibition (mm)**		
	Staphylococcus aureus	***Escherichia coli***	***Candida albicans***
C2	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
CH	5.10 ± 0.05	0.00 ± 0.00	0.00 ± 0.00
H	6.00 ± 0.00	0.00 ± 0.00	0.0 ± 0.00

4.7 SEM Analysis

The surface morphology of the control (without ceramide and honey) and ceramide and honey-loaded CH Hydrogel were experimented by SEM as displayed in **Figure 4.9** and **Figure 4.10**.

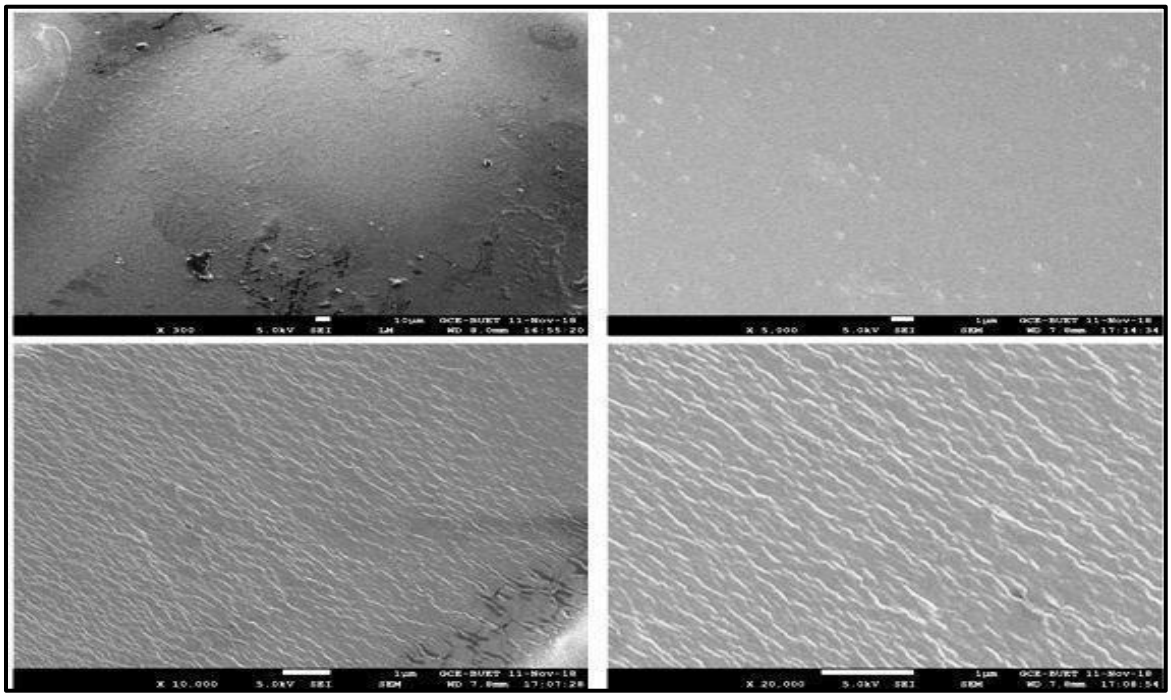

Figure 4.9: SEM Images of Control Hydrogel without Ceramide and Honey (X 500 to X 20,000).

It was noticed that the CH hydrogel surface was composed of both dense and porous. At very large magnifications in CH hydrogel, there have a well-marked presence of dense surface and porous surface **Ashrafuzzaman M. et al. (2021)**. Both dense and porous surfaces will be beneficial for hydrogel dressing and finally for wound healing. The dense surface did not permit the entrance of bacteria into the wound area. On the other hand, the porous surface did allow the cell migration, nutrition supply, and proliferation and helped to absorb excess moisture and serum exudates from the wound area. The presence of porous structure also helps in influencing the degree of swelling.

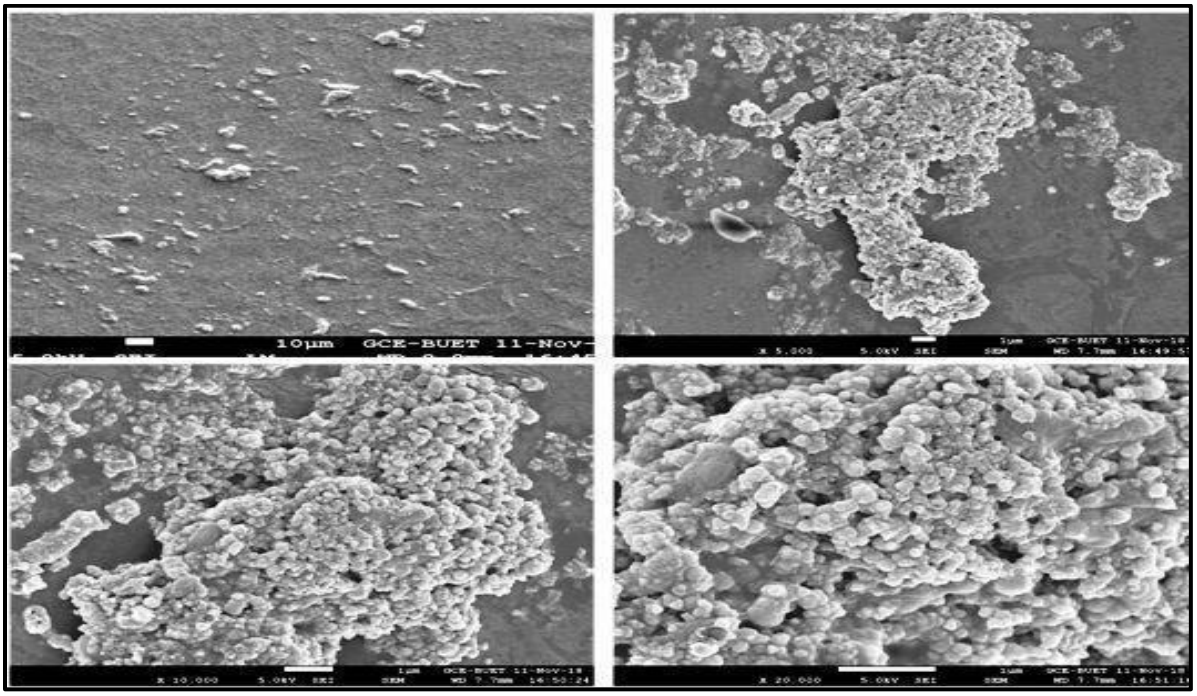

Figure 4.10: SEM Images of CH Hydrogel with Ceramide and Honey (X 500 to X 20,000).

4.8 UV Spectroscopy

The prepared CH Hydrogel was characterized by UV visible spectroscopy. **Figure 4.11** depicts the UV spectrum of CH hydrogel showing a characteristic absorption band at 260 nm and 295 nm.

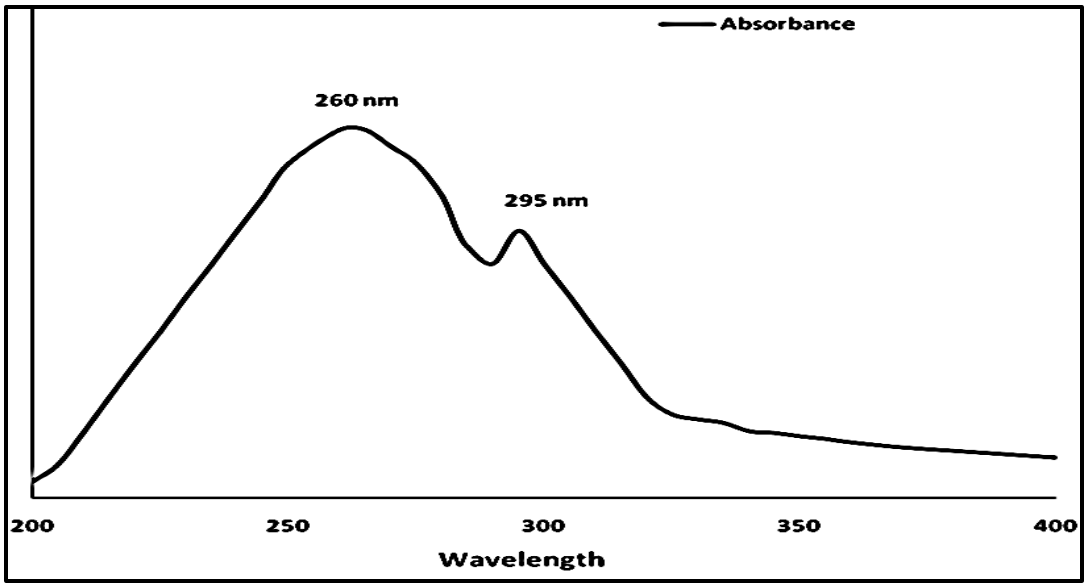

Figure 4.11: UV Spectroscopy of CH Hydrogel.

The absorption band 260 nm indicates the presence of ceramide in the solution **Shoyama, Y., Okabe, H., Kishimoto, Y. and Costello, C. (1978), Couch, L. H., Churchwell, M. I., Doerge, D. R., Tolleson, W. H. and Howard P. C. (1997)** and the absorption band 295 nm indicates the presence of honey in the solution **Kanimozhi, S., Kathiresan, G., Kathalingam, A., Kim, H. S. and Doss, M. N. R. (2020)**. Both absorption bands are slightly different from previous studies; it might be due to the source variations.

4.9 FTIR Analysis

The spectrum of C2 Hydrogel presented in **Figure 4.12** displays the characteristics of absorptions bands: 3094.72 cm^{-1} characterized the stretching mode of C-H and O-H (Carboxylic acid); 2927.94 cm^{-1} characterized the stretching mode of O-H (Carboxylic acid); 1649.14 cm^{-1} characterized the stretching mode of C=C and C=O (Amide), 1541.12 cm^{-1} characterized the stretching mode of C=C and N-H Bending in secondary amine, 1454.33 cm^{-1} characterized N-H

Bending in secondary amine, 1039.63 cm^{-1} characterized the stretching mode of C-O and 852.54 cm^{-1} characterized the bending mode of =C-H group.

The spectrum of CH Hydrogel given in **Figure 4.13** displays the characteristics absorptions bands: 3380.66 cm^{-1} represented to the stretching mode of C-H, N-H, and O-H (Carboxylic Acid), 2933.73 cm^{-1} corresponded the stretching mode O-H: (Carboxylic Acid), 1649.14 cm^{-1} corresponded the stretching mode C=C and C=O (Amide & Aldehyde); 1544.98 cm^{-1} corresponded the stretching mode of C=C (Aromatic Ring), N-H bending in secondary amine and -NO2: asymmetric stretching mode of (Aliphatic & Aromatic); 1411.88 cm^{-1} corresponded the bending mode of C-O-H; 1336.67 cm^{-1} corresponded the stretching mode of C-F and symmetric mode of C-O-H, -NO2; 1242.16 cm^{-1} corresponded the stretching mode of C-F, C-O (Alcohol, Ether, Carboxylic Acid) and bending mode C-O-H; 918.12 cm^{-1} corresponded the out of plane bending Mode of C-H.

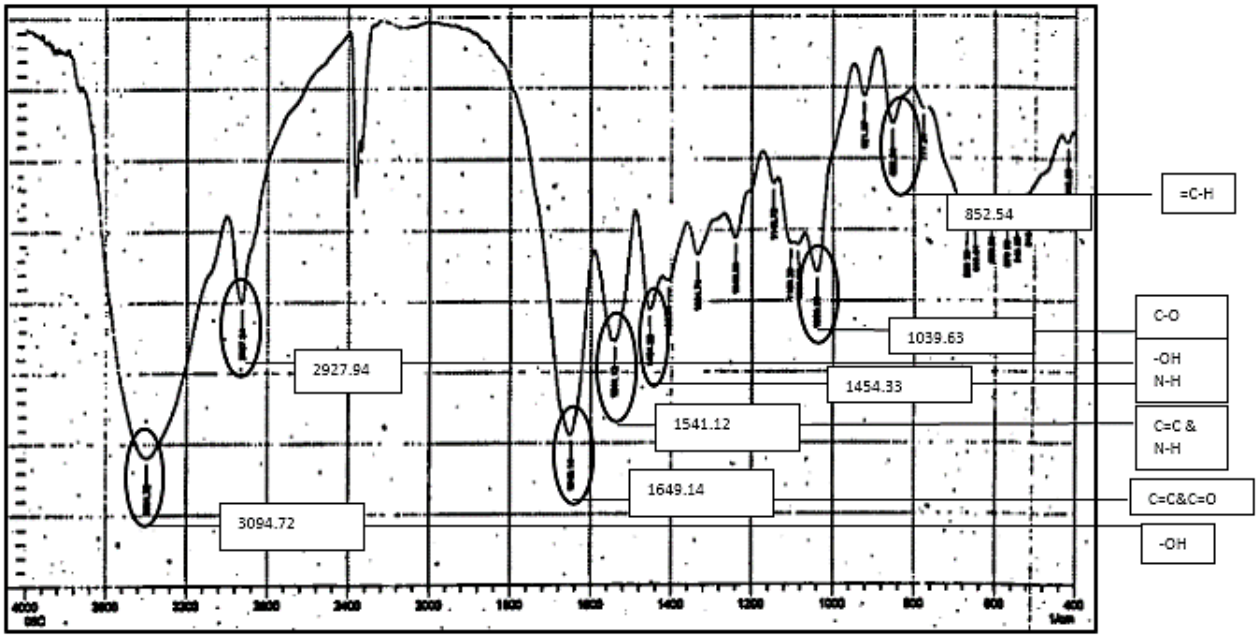

Figure 4.12: FTIR Spectra of Ceramide Loaded Hydrogel (C2 Hydrogel).

Discussion on the result: In our current research we have investigated FTIR studies to confirm the hydroxyl group present in hydrogel dressings, which is the reason for the water-restoring capability of the hydrogel. Our prepared hydrogel membrane showed the free hydroxyl groups present in the membrane. In the case of C2 hydrogel dressing, **Table 2**, the peak at 3394.72 cm^{-1} indicated the presence of free hydroxyl groups, and the peak at 2927.94 cm^{-1} showed the C-H stretching. The spectra of the dressing membrane also showed a peak at 1649.14 cm^{-1}, 1541.12 cm^{-1}, and 1454.33 cm^{-1} implying the presence of an amide bond of ceramide between sphingosine and fatty acid. The membrane also showed a peak at 1039.63 cm^{-1} refers to CO stretching vibrations of the aldehyde group of glutaraldehyde and a peak at 852.54 cm^{-1} refers to CH2 stretching.

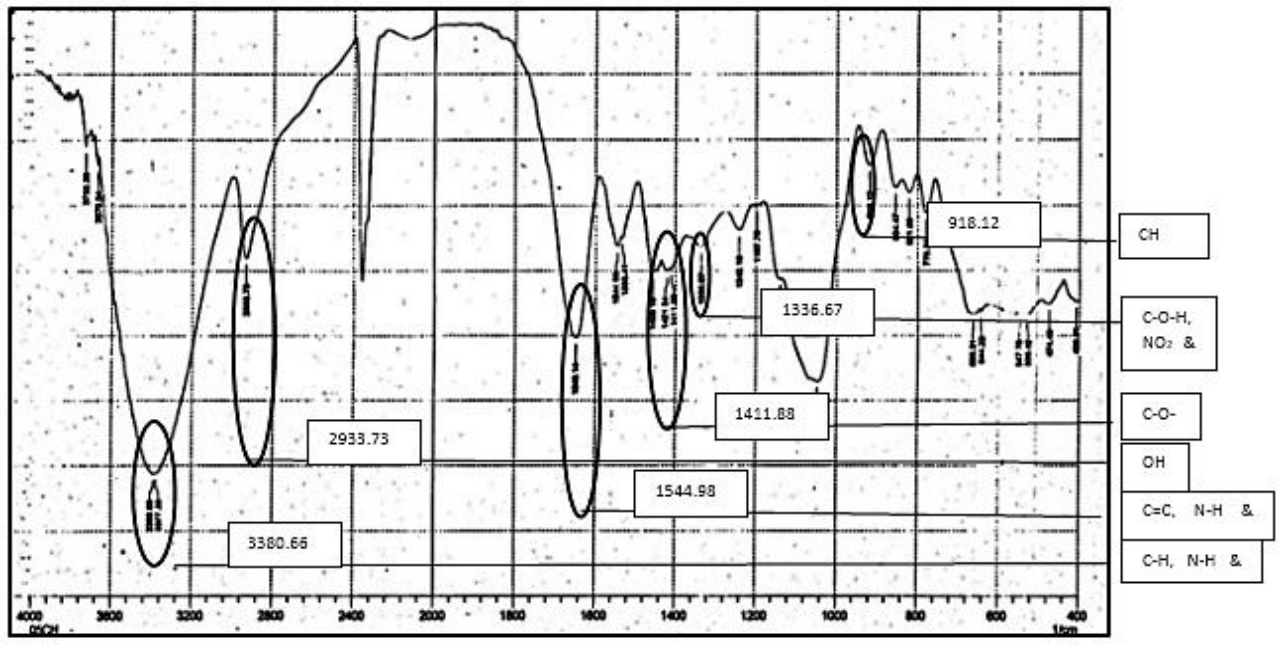

Figure 4.13: FTIR Spectra of Ceramide and Honey-Loaded Hydrogel (CH Hydrogel).

In the case of CH hydrogel dressing, Table 3, the peak at 3380.66 cm^{-1} conveyed the presence of free hydroxyl groups and the peak at 2933.77 cm^{-1} represented the C-H stretching. The

hydrogel showed a peak at 1649.14 cm^{-1}, 1544.98 cm^{-1} and 1411.88 cm^{-1} implying the presence of an amide bond of ceramide between sphingosine and fatty acid. It also showed a peak of fructose and glucose at 1242.16 cm^{-1} and 1336.67 cm^{-1} that represents the CO stretching in the -COH group and C-C stretching in carbohydrates.

4.10 In- Vivo Burn Healing Experiment

The treatment was applied for 15 days. The essential process for burn healing is burn edge contraction which leads to wound closure. The bun wound edge contraction was expressed as an increase in the healing rate. So the determination of the burn wound area was the main criterion for observing wound healing progression. The determination of the burn wound area is presented in **Table 4.2** in millimeter scale and displayed in **Figure 4.14** and **Figure 4.15**.

Table 4.2: Burn Wound Measurement by Diameter in Millimeter (mm) Scale and Reduction from the Day 3 to Day 15

Time Interval (Days)	Burn Wound Measurement by Diameter (mm)		
	C2 Hydrogel	**CH Hydrogel**	**Control**
3	8.30 ± 0.30	8.50 ± 0.32	9.02 ± 0.52
7	5.10 ± 0.51	5.20 ± 0.82	6.03 ± 0.72
10	3.05 ± 0.61	3.20 ± 0.35	6.00 ± 0.42
15	1.07± 0.42	1.30± 0.23	4.02± 0.32

Both C2 and CH hydrogel showed regular burn wound edge contraction during the application of treatment (**Figure 4.14**). The maximum burn diameter contraction was recorded for CH hydrogel. This may be due to the presence of both honey and ceramide in the hydrogel. Previous studies in mice models showed honey hydrogel quickened wound repairing process by increasing re-epithelialization and lowering the inflammatory response **Zohdi, R. M. et al. (2012)**. The result of C2 hydrogel was also significant. The presence of ceramide may play a vital role in wound healing. Ceramide loaded in hydrophilic ointment and hydrocolloid dressing showed rapid wound healing in mice model **Kerscher, M., Korting, H. C., and Schiffer-Korting, M. (1991), Tsuchiya, S., Ichioka, S., Sekiya, N., Tajima, S., Iwasaki, T. and Numata, S. (2012)**. Both C2 and CH hydrogel showed optimum results for wound healing when moisture retention capacity and water vapor transmission rates (WVTR) were investigated. An ideal dressing should have good moisture retention capability and WVTR for wound healing.

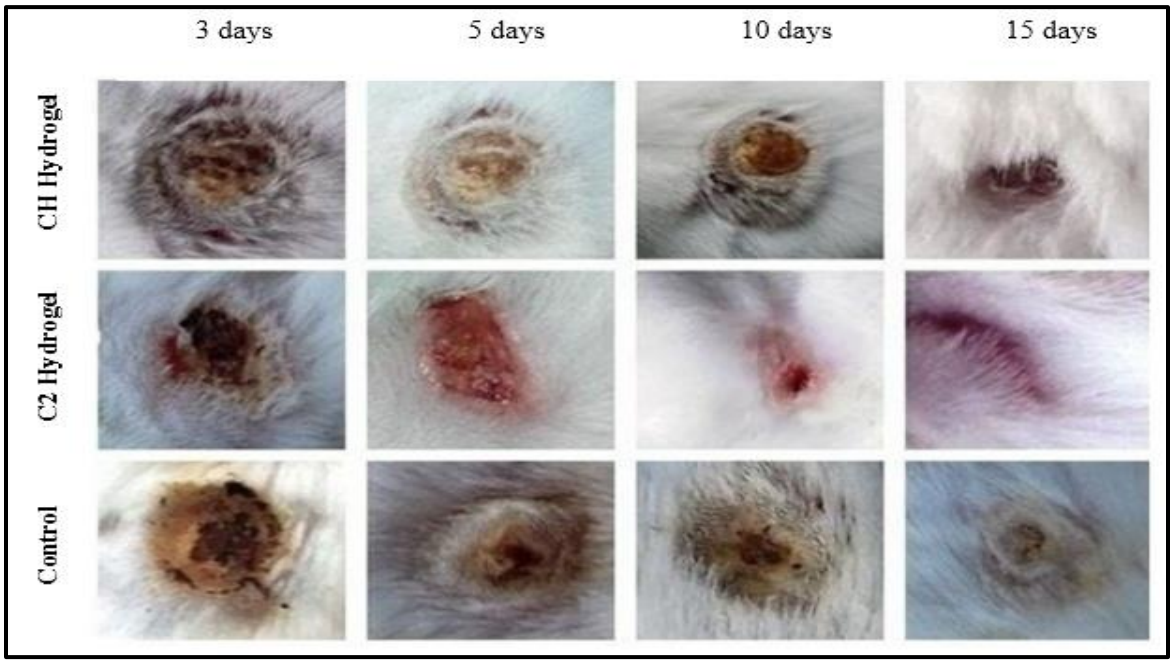

Figure 4.14: Wound Healing Evaluation in Mice Model after 3, 7, 10 and 15 Days for Control (Only PVA & Gelatin), C2 & CH Hydrogel.

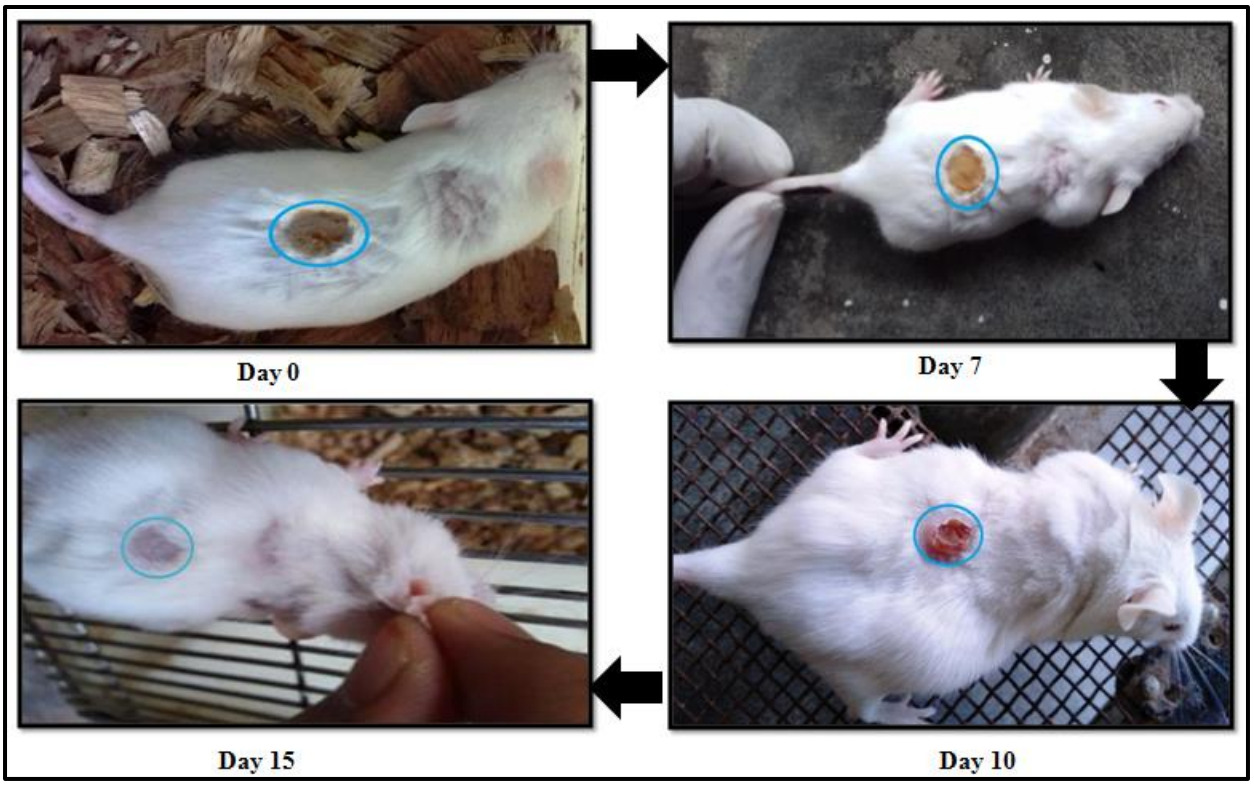

Figure 4.15: Wound Healing in Mice Model.

4.11 Histological analysis

In **Figure 4.16 (A)**, untreated skin after 15 days of burn, we found only there were the presence of Edema, Fibrinoid Necrosis, and Serum Exudates.

In **Figure 4.17 (B)**, Ceramide & honey loaded hydrogel treated skin, were already formed Keratinized Layer, Epidermis Layer, Dermal Papillae, Sebaceous Gland, Hair Follicles, Sweat Gland, Arrector Pili Muscle, and Adipose Tissue.

In **Figure 4.18 (C)**, Ceramide loaded hydrogel treated skin, where already formed Keratinized Layer, Epidermal Layer, Dermal Papillae, Sebaceous Gland, Arrector Pili Muscle, and Adipose Tissue.

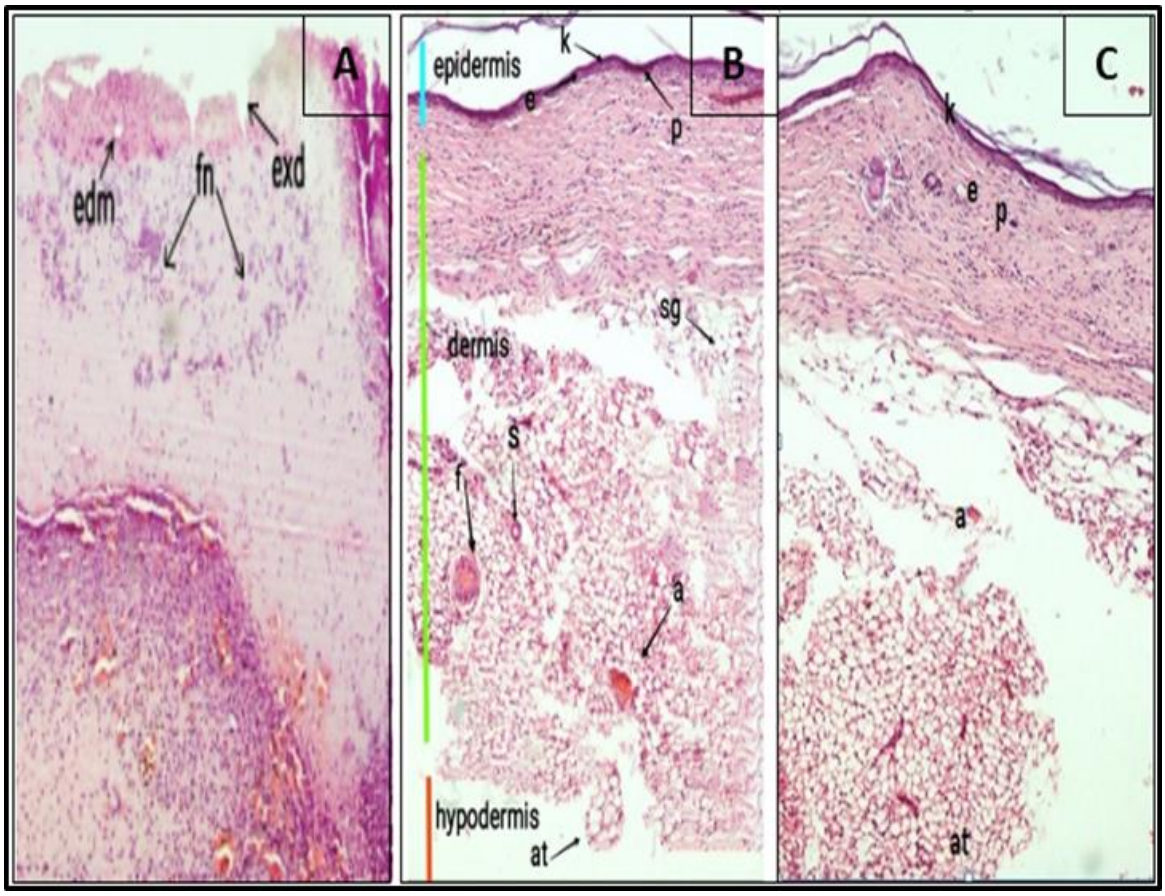

Figure 4.16: **A**: Image of Untreated Skin after 15 Days, there are presence of edm (Edema), fn (Fibrinoid Necrosis) and exd (Serum Exudates); **B**: Image of a Ceramide & Honey Loaded Hydrogel Treated Skin, where already Formed k (Keratinized Layer), e (Epidermal Layer), p (Dermal Papillae), sg (Sebaceous Gland), f (Hair Follicles), s (Sweat Gland), a (Arrector Pili Muscle) and at (Adipose Tissue); **C**: Image of a Ceramide & Honey Loaded Hydrogel Treated Skin, where already Formed k (Keratinized Layer), e (Epidermal Layer), p (Dermal Papillae), sg (Sebaceous Gland), a (Arrector Pili Muscle) and at (Adipose Tissue).

Discussion on the result: After 15 days we observed the presence of keratinized layer, epidermal layer, dermal papillae, sebaceous gland, hair follicles, sweat gland, arrector pili muscle, and adipose tissue in both **Figure 4.17** and **Figure 4.18** it may be due to the presence of ceramide and into both media, which may regulate and influence the cell proliferation, differentiation and

apoptosis of epidermal cells to the wounded area **Geilen, C. G., Wieder, T. and Orfanos, C. E. (1997)**. It is known that the ceramide selectively disallow the prolapse of the protein kinase C isoform PKCα thus affecting cell growth **Jones, M. J. and Murray, A. W. (1995)**. On the other hand, honey increases the healing rate by stimulating leukocytes to cytokines and growth factors release that activate tissue repair and by stimulating the keratinocytes transcription of genes for TNF-a, IL-1ß and TGF-ß **Molan, P.C. (2011), Boekema, B. K. H. L., Pool, L., Ulrich, M. M. W., (2012), Abuharfeil, N., Al-Oran, R. and Abo-Shehada, M. (1999), Majtan, J., Kumar, P., Majtan, T. et al. (2009)**. In **Figure 4.16**, we observed the presence of edema, fibrinoid necrosis & serum exudates, because the skin was in an untreated condition.

CHAPTER 5

CONCLUSIONS

5.1 Conclusion

In this investigation ceramide loaded hydrogel and ceramide with honey loaded hydrogel have been synthesized. Physiochemical and biological properties like water vapor transmission rate, moisture retention capability, swelling behavior, gel fraction and antimicrobial activity was analyzed and found satisfactory result. Besides, the synthesized hydrogels has been characterized by SEM, UV and FTIR test where the result was indicated the confirmation of ceramide and honey properly incorporated into the hydrogel without any chemical and structural change. Finally in vivo test in mice and histological studies ensure presence of regenerative cell and wound healing after burn.

5.2 Limitations

The sample could have been tested with other existing hydrogel or ointment commercial products for comparing the efficiency of this newly synthesized hydrogel.

5.3 Scope for Future Work

In the future, using this hydrogel, clinical studies can be performed on a small scale on healthy and burn patients and evaluated as a potent candidate for burn treatment.

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