DEVELOPMENT OF CHICKEN EGG WHITE AND MUPIROCIN LOADED HYDROGEL DRESSING MATERIAL FOR INHIBITION OF BACTERIAL GROWTH

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M.Sc. ENGINEERING THESIS

DEPARTMENT OF BIOMEDICAL ENGINEERING
MILITARY INSTITUTE OF SCIENCE AND TECHNOLOGY
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ZANNATUL NAIM (SN. 1016260004)

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Masters of Science in Biomedical Engineering

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By

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DEVELOPMENT OF CHICKEN EGG WHITE AND MUPIROCIN LOADED HYDROGEL DRESSING MATERIAL FOR INHIBITION OF BACTERIAL GROWTH

DECLARATION

I hereby declare that the study reported in this thesis entitled "Development of Chicken Egg White and Mupirocin Loaded Hydrogel Dressing Material for Inhibition of Bacterial Growth" is my original 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 Chicken Egg White and Mupirocin Loaded Hydrogel Dressing Material for Inhibition of Bacterial Growth

The bacterial infection of wound is one of the major challenges in wound care treatment. Topical antibiotic mupirocin is an antibacterial agent widely used to inhibit bacterial growth after wound formation in the human body. Antibacterial activity of egg white with wound healing capability also has been reported in research articles. Therefore, the hydrogel was prepared by incorporating chicken egg white and mupirocin into poly vinyl alcohol (PVA) and gelatin polymer matrix via solution casting method for the purpose of bacterial growth inhibition. The developed hydrogels were characterized by Fourier Transform Infrared Spectroscopy (FTIR) that confirmed the presence of a functional group of the different components in the hydrogel and crosslinking occurred by esterification process between PVA and gelatin by glutaraldehyde. The developed hydrogels were characterized morphologically by Scanning Electron Microscope (SEM), it showed a smooth break surface for neat hydrogel (NH) and chicken egg white loaded hydrogel (EH), smooth and homogenous surface for mupirocin loaded hydrogel (MH) and rough surface for mupirocin with chicken egg white loaded hydrogel (MEH). Moreover, swelling behavior in water, moisture retention capability, folding endurance, water vapor transmission rate (WVTR), pH determination, gel fraction, spreadability, and porosity tests were conducted to reveal the chemical and physical properties of the hydrogel. All the values obtained from these tests were compatible with the published data and to some extent with the commercially available products. Eventually, developed hydrogels were characterized biologically by the disc diffusion method to evaluate bacterial inhibition activity. MH and MEH showed significant inhibition against *Staphylococcus aureus, Escherichia coli,* and *Pseudomonas aeruginosa* bacteria and EH showed moderate inhibition against experimented bacteria. This newly prepared chicken egg white and mupirocin-loaded hydrogel might play an important role in inhibiting the growth of bacteria during the wound healing process. Hence, current outcomes with experimental findings can be used for further investigation in future in vivo tests on mice models.

সারসংক্ষেপ

Development of Chicken Egg White and Mupirocin Loaded Hydrogel Dressing Material for Inhibition of Bacterial Growth

ব্যাকটেরিয়া সংক্রমণ ক্ষত চিকিৎসায় একটি অন্যতম প্রধান অন্তরায়। টপিকাল (topical) অ্যান্টিবায়োটিক মিউপিরোসিন (mupirocin) হল একটি অ্যান্টিব্যাকটেরিয়াল এজেন্ট যা মানবদেহে ক্ষত তৈরির পরে ব্যাকটেরিয়ার বৃদ্ধি রোধ করার জন্য ব্যাপকভাবে ব্যবহৃত হয়। গবেষণায় জানা গেছে যে ডিমের সাদা অংশ ক্ষত নিরাময়সহ অ্যান্টিব্যাকটেরিয়াল কার্যক্ষমতা সম্পন্ন। মুরগির ডিমের সাদা অংশ এবং মিউপিরোসিন ভিত্তিক হাইড্রোজেল ড্রেসিং উপকরণ গুলি ব্যাকটেরিয়া বৃদ্ধি রোধের উদ্দেশ্যে দ্রবণ ঢালাই (solution casting) পদ্ধতি দ্বারা প্রস্তুত করা হয়েছিল। বর্ণালীবীক্ষণ Fourier Transform Infrared Spectroscopy (FTIR) ব্যবহার করে তৈরিকৃত হাইড্রোজেলের কার্যকরী মূলক চিহ্নিতকরা হয়েছিল। Scanning Electron Microscope (SEM) ব্যবহার করে তৈরিকৃত হাইড্রোজেলের বৈশিষ্ট্যপূর্ণ মরফলজি (morphology) চিহ্নিতকরা হয়েছিল। তদুপরি, জলেফোলা আচরণ (swelling behavior), আর্দ্রতা ধরে রাখার ক্ষমতা (moisture retention capability), ভাঁজ সহনশীলতা (folding endurance), জলীয় বাষ্পস্থানান্তরের হার (WVTR), pH নির্ধারণ, ভঙ্গুরতা (gel fraction), প্রসারণযোগ্যতা (spreadability) এবং ছিদ্রের (porosity) উপস্থিতি পরীক্ষা হাইড্রোজেলের রাসায়নিক এবং ভৌত বৈশিষ্ট্য প্রকাশের জন্য গবেষণা করা হয়েছিল। অবশেষে, অ্যান্টি-ব্যাকটেরিয়াল কার্যকলাপ অধ্যয়ন এবং মূল্যায়ন করা হয়েছিল। মিউপিরোসিন ভিত্তিক হাইড্রোজেল (MH), ডিমের সাদা অংশ এবং মিউপিরোসিন ভিত্তিক হাইড্রোজেল (MEH), *Staphylococcus aureus*, *Escherichia coli* এবং *Pseudomonas aeruginosa* ব্যাকটেরিয়ার বিরুদ্ধে তাৎপর্যপূর্ণময় জীবাণূরোধী বৈশিষ্ট্য দেখিয়েছিল এবং ডিমের সাদা অংশ ভিত্তিক হাইড্রোজেল (EH) ব্যাকটেরিয়ার বিরুদ্ধে মাঝারি জীবাণূরোধী বৈশিষ্ট্য দেখিয়েছিল। অন্যান্য বৈশিষ্ট্য যেমন ফোলাভাব, আর্দ্রতা ধরে রাখা, ভাঁজ সহনশীলতা, জলীয় বাষ্পস্থানান্তরের হার, pH নির্ণয়, ভঙ্গুরতা, প্রসারণযোগ্যতা, ছিদ্রের উপস্থিতি পরীক্ষা এবং বর্ণালীবীক্ষণ থেকে প্রাপ্ত ফলাফল হাইড্রোজেলে মিউপিরোসিনের উপস্থিতি নিশ্চিত করতে সহায়ক হিসেবে পাওয়া গেছে এবং ব্যাকটেরিয়ার জন্য ড্রেসিং উপাদান হিসেবে গ্রহণযোগ্য বলে মনে করা হয়েছে। এই নতুন তৈরিকৃত ডিমের সাদা অংশ এবং মিউপিরোসিন ভিত্তিক হাইড্রোজেল ক্ষত নিরাময় প্রক্রিয়ার সময় ব্যাকটেরিয়ার বৃদ্ধি রোধে গুরুত্বপূর্ণ ভূমিকা পালন করতে পারে। সুতরাং, পরীক্ষামূলক ফলাফলের সাথে ইঁদুরের উপর ইন ভিভো (in vivo) পরীক্ষায় ভবিষ্যতের গবেষণার জন্য ব্যবহার করা যেতে পারে।

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LIST OF ABBREVIATIONS

(p)ppGpp	Guanosine Pentaphosphate and Tetraphosphate (magic spot)
CMC	Carboxymethyl cellulose
ECM	Extra Cellular Matrix
FTIR	Fourier Transform Infrared Spectroscopy
PEG	Polyethylene Glycol
PLGA	Poly lactide-co-glycolide
PMNs	Polymorphonuclear Neutrophils
PVA	Poly Vinyl Alcohol
RGD	Arginine-glycine-aspartate
SEM	Scanning Electron Microscope
TSST-1	Toxic Shock Syndrome Toxin-1
WVTR	Water Vapor Transmission Rate

CHAPTER 1
INTRODUCTION

1.1 Background

Wound infection triggers the development of chronicity, and thus delays wound healing. Chronic wounds can lead to high morbidity and mortality. *Staphylococcus aureus* and *Escherichia coli* are the common infectious agents in different wounds like surgical wound infections, acute soft tissue infections, bite wound infections; burn wound infections, diabetic foot ulcer infections, leg, and decubitus ulcer infections, etc (Bowler, P. G., Duerden, B. I. and Armstrong, D. G. 2001). A recent study showed that the leading causes of death from infection were multi-drug resistant organisms such as *Pseudomonas* and *Acinetobacter* (Williams, F. N. et al., 2009).

Conventional control methods for microbial populations in wounds include antiseptics, hyperbaric oxygen therapy, surgical debridement, autolytic and enzymatic debridement, biosurgical debridement, and antibiotics (Bowler, P. G., Duerden, B. I. and Armstrong, D. G. 2001). Topical antibiotic mupirocin possesses antibacterial activity which keeps a safe wound area from different kinds of bacterial infection in the skin like burn wound infection, surgical wound infection etc (Breneman, D. L. 1990). It was shown to have a better effect on infection control (Okur, N. Ü. et al., 2019).

On the other hand, egg white also possesses antibacterial activity with wound healing properties which keeps a safe wound area from bacterial contamination and performs an important position in wound recovery (Mine, Y. and Kovacs-Nolan, J. 2004; Jahani, S. et al., 2019). Egg white contains 158 kinds of proteins including ovalbumin, ovotransferrin, lysozyme, and Ovomucin (Abeyrathne, E. D. N. S., Lee, H. Y., and Ahn, D. U. 2014). These proteins help egg white to exert antibacterial activities (Mine, Y. and Kovacs-Nolan. J. 2004). Egg whites derived ovalbumin protein components have been proven to be promoting mammalian cells in the culture (Ruan, G. P. et al., 2015). It also helps to reduce inflammation and soothe burn wound areas. Egg white has been successfully used as a scaffold in vitro and in vivo that allows migration and growth of human fibroblasts (Tao, L. et al., 2021).

Moreover, the egg white can contribute to suppressing bacterial infection and enhance tissue regeneration.

For wound healing, wound dressing is important part for the treatment. But traditional wound dressings like gauze, lint, plasters, bandages, and cotton wool are not suitable for controlling bacterial infection. They are dry and do not control moisture level as a result they have no wound healing capability; they can adhere to wound bed so they may also induce secondary tissue damage, trigger pain, and trauma when removed (Dhivya, S., Padma, V. V. and Santhin, E. 2015). They are only effective in controlling hemorrhage and some cases in protecting from further external friction. However, different dressing materials are also being used for suppressing bacterial infections in wounds. So far, available dressing materials include transparent film dressing, foam dressing, hydrogels, hydrocolloids, hydro fibers, silicon dressing, etc (Sood, A., Granick, M. S. and Tomaselli, N. L. 2014). Antimicrobial properties impregnating in dressings can be instrumental in healing wounds that are at higher risk for infection (Broussar, K. C. and Powers, J. G. 2013). On the other hand, the selection of appropriate dressing material from the available options remains a great challenge (Landriscina, A., Rosen, J. and Friedman, A. J. 2015). Because they inherit various advantages and disadvantages in wound healing (Sun, L. J. et al., 2019). In this experiment, we chose hydrogel dressing due to the fact that they possess almost all the properties that are required for healing purpose (Rosiak, J. M. and Yoshii, F. 1999; Enas, M. A. 2015). So, hydrogel promotes wound healing better than other dressings because of its amazing characteristics.

Gelatin is used as a gelling agent in medication like soft gelatin capsules, hydrogel sheets for wound healing, etc. It also helps to reduce inflammation and soothe burn wound areas. It can absorb more than five to ten instances of its weight in water (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 include wound dressing fabric due to their excellent binding and filling properties, water retention properties, biocompatibility, biodegradable, non-toxic, swelling properties, curative, non-

carcinogenic and excellent film forming ability, which are found to be beneficial (Kawai, F. and Hu, X. 2009).

This research aims to develop chicken egg white and mupirocin loaded hydrogel as a new dressing material. In this study, therefore, mupirocin, egg white, PVA and gelatin were selected to prepare hydrogel dressing materials. The hydrogel was prepared by incorporating mupirocin and egg white into PVA and gelatin polymer matrix via solution casting method. The properties of the hydrogel, such as swelling behavior in water, moisture retention capability, folding endurance, water vapor transmission rate (WVTR), pH determination, gel fraction, spreadability, porosity, SEM, FTIR and antibacterial activity were investigated.

1.2 Problem Statements

Millions of people go through different kind of wound like surgery, bite (pet and wild animal), burn, diabetic foot ulcer, leg and decubitus ulcer and other wounds who face problems in bacterial infection in wound treatment. As a result, healing of wound has been still challenging process. The bacterial infection of 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 synthesize chicken egg white and mupirocin loaded hydrogel dressing material.
- To characterize the physical and chemical properties of this hydrogel dressing material.
- To evaluate the efficacy of this dressing material for treating bacterial infection of wound.

1.4 Motivation of the Thesis

Bacterial infection is an important problem in the wound. After injury, most of the skin loses its protective barrier, increasing the risk of infection. Infection can delay wound healing, causing pain, scarring and even death.

Mupirocin formulation is a commercial drug for the treatment of wound infections and secondary infections in surgical wounds, burn wounds, diabetes and other wounds (Breneman, D. L. 1990). It comes in both 2% cream and ointment. However, creams and lotions have disadvantages such as oiliness and stickiness, which are not suitable for drug users (Hurler, J.et al., 2013). Wound dressings 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 in disturbing new epidermal tissue and causing pain, non-absorbent cause permitting excess wound exudates accumulation and impermeable for proteins and drugs again semi-permeable for gases. Do not absorb blood or exudates, healing may take a longer time; Opaque layer formation may complicate wound tracking, which is not suitable for dried wounds, and provides the opportunity for bacterial invasion. On the other hand, silver, bismuth, and chlorhexidine-containing dressing materials obtained critics by way of the U.S. Food and Drug Administration (FDA) which includes positive dangers of use and low hazard reduction. But egg white is a natural source containing chemical components like lysozyme, ovalbumin, avidin etc. which are effective against some and negative bacteria including bacteria which are responsible for wound infection (Mine, Y. and Kovacs-Nolan, J. 2004). The bacterial inhibition activity of egg white they have also showed wound healing activity. To minimize these risks and disadvantages, our prime motivation behind this current research is to develop dressing materials like hydrogels containing natural elements like egg white together with mupirocin for the inhibition of bacteria that are found after wound formation.

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: materials and methods, chapter 4: results and discussions, and chapter 5: conclusions and recommendations. The details of the organization of the thesis are as follows:

Chapter 1 includes an introduction to the thesis topic and research background. In addition, the motivation and purpose of the thesis are also included in this chapter. Finally, the chapter concludes with a mention of the structure or organization of the thesis in this book.

Chapter 2 covers the theoretical research necessary for research work. Hydrogels, mupirocin, egg white, biomaterials, bacteria, wound healing techniques, etc. is discussed in detail.

Chapter 3 describes the materials and methods of the research work. This chapter included the name of materials used in the thesis, preparation method of hydrogel dressing materials, and physical and chemical characterization methods of hydrogel dressing (e.g. water vapor transmission rate, moisture retention capability, porosity evaluation, gel fraction, swelling behavior, SEM and FTIR) and finally bacterial inhibition test by disc diffusion method against wound infection creating bacteria.

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 disc diffusion test for bacterial inhibition, SEM and FTIR analysis have been demonstrated with a figure with proper illustration and marking.

Chapter 5 concludes the research study, summarizes the results and conclusions of the research study. In addition, this section makes some recommendations to facilitate future research, limitation also mentioned.

CHAPTER 2
LITERATURE REVIEW

2.1 Wound

A wound is an injury or harm to biological tissue integrity, including skin, mucous membranes, and body tissues. Infections can be caused by many conditions such as burns, decoupage, surgeries, skin rupture other skin infections, or illnesses such as eczema or psoriasis, which can occur in lesions.

2.1.1 Types of Wounds

Wounds can be classified in various ways, depending on the duration and severity of the wound. People are likely to experience a variety of ailments throughout their lives as part of their daily lives or activities. Wounds can range from mild to severe, depending on location, healing time, cause, location, and depth or severity. Right below defined different varieties of wounds has been described (Irfan-Maqsood, M. 2016; Types of wounds, 2020). Descriptions of the different types of wounds are:

It can be divided into open wound and closed wound according to open wound or closed wound. An open wound is a wound in which the tissue/body is exposed and open to the outside of doors surroundings, for example, penetrating wounds. It can be further categorized as abrasion, punctures, penetrations, lacerations, incisions, and gunshot. A closed wound is an injury that occurs without contact with tissues and organs. Pain (e.g. pressure wound) can be used as an example of an open or closed wound, depending on its current stage.

Depending on the healing time wound can be divided into acute of chronic wounds. **Acute wounds** are the ones that cure outside of any complexity in an expected quantity of period. **Chronic wounds** take a comparatively lengthy period to cure with some complications. Chronic wounds may be classified as pressure injuries and diabetic ulcers:

Pressure injuries - additionally referred to as stress injuries, these injuries occur while shear force and/or pressure are applied to the skin. People at higher risk of contracting these chronic diseases are sedentary or unable to walk due to illness, infection, physical disability, or movement of all or part of their body, and therefore have movement restrictions

Diabetic Ulcers - These diseases, mostly seen in the toes, are the result of changes in blood vessels and blood flow throughout the body caused by diabetes. It includes neuropathic, ischemic, and neuroischemic.

Depending on clean or contaminated, wounds are classified as: **Clean wound** is one that has no foreign matter or debris in it whereas **Contaminated wounds or infected wounds** are those that might have some dirt, bacteria, or other foreign material and look like yellowish, redness, soreness. **Colonized wounds**, and resistant diseases (such as bed sores) contain pathogenic microorganisms.

Depending on the origin of the wound, they can be internal or external: **Internal wounds** can be caused by poor systemic function, nerve function, neuropathy or medical illness, or decreased blood, oxygen, or other nutrient supply. **External Wounds** The injury may be caused by an external force or an object that enters or does not penetrate.

i. Non-penetrating Wounds: These wounds result from damage or friction with other surfaces. It includes abrasions, lacerations, bruises, and concussions.

ii. Penetrating Wounds: These are caused by injuries and spread to all layers of the skin. Includes: stab wounds, cuts, surgical wounds, etc.

The United States Centers for Disease Control and Prevention (CDC) has developed a classification system that includes four categories of wound conditions (Herman, T. F. and Bordoni, B. 2022).

A. Class 1 wounds: These are considered clean. They have no infection, no pain, and most of them are closed. If these wounds need to be drained, the drain must be closed properly. In addition, these wounds do not pass into the respiratory system, digestion, pregnancy or urine.

B. Class 2 wounds: These are considered to be clean-contaminated. These lesions are not unusual. Class 2 Injuries to the respiratory tract, intestines, genitals, or urinary tract. However, these organisms enter these areas in a controlled manner.

C. Class 3 wounds: These are considered contamination. These are new wounds, open wounds that may result from interrupted aseptic procedures or from leakage from the

intestinal tract into the wound. Additionally, surgery that causes severe inflammation or no inflammation at all is considered a Class 3 wound.

D. Class 4 wounds: These are considered unclean. These wounds are often caused by improper wound care. Class 4 wounds indicate tissue deactivation, usually due to the presence of bacteria in perforated internal organs or at the surgical site.

2.1.2 Wound Infection

Infection occurs when one or more bacteria in the wound cause an infection against the host's immune system, and the action and proliferation of bacteria in the tissue cause local reactions and systemic responses. Most diseases (both acute and chronic) involve a mixture of both oxygen-dependent bacteria groups and oxygen-independent bacteria groups. There are several types of wound infection (Bowler, P. G., Duerden, B. I., and Armstrong, D. G. 2001).

- Surgical Wound Infections
- Acute Soft Tissue Infections
- Bite Wound Infections
- Burn Wound Infections
- Diabetic Foot Ulcer Infections
- Leg and Decubitus (Pressure) Ulcer Infections

Surgical Wound Infections

The risk of infection is often based on the risk of surgery for microbial infection (Raahave, D. et al., 1986). Clean surgery carries a 1% to 5% risk of infection after surgery, while dirty surgery, which is more susceptible to endogenous bacteria, has a 27% risk of infection, according to Nichols, R. L. (1998).

The CDC, Centers for Disease Control and Prevention guideline for the prevention of surgical site infection has recognized *Staphylococcus aureus*, coagulase-negative staphylococci, *Enterococcus spp.*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Enterobacter spp.* as the most frequently isolated pathogens (Mangram, A. J. et al., 1999).

Acute Soft Tissue Infections

Soft tissue infections include skin abscesses, trauma, and necrotizing infections. Microbiological studies have shown that *Staphylococcus aureus* is the sole pathogen in approximately 25% to 30% of skin abscesses (Brook, I. and Finegold, S. M. 1981) and 50% of injuries of multiple etiologies (Brook, I. and Frazier, E. H. 1998) and 47% of soft tissue necrotizing bacteria have a diverse aerobic-anaerobic flora.

Bite Wound Infections

Infections from human bites have been reported to range from 10% to 50%, from dog bites to 20%, and from cat bites to 30% to 50%, depending on the severity and location of the bite (Griego, R. D. et al., 1995). Brook, I. (1987) reported that 74% of 39 human and animal bite wounds contained a variety of aerobic-anaerobic flora, including *Staphylococcus aureus*, *Peptostreptococcus spp*., and *Bacteroides spp.* is the main difference in the two cases.

As well as and anaerobic bacteria such as *Bacteroides*, *Prevotella*, *Porphyromonas* and *Peptostreptococcus spp*. in human and animal bite wounds.

Burn Wound Infections

Infection is a major complication of burns and is estimated to be responsible for 75% of burn deaths (Revathi, G., Puri, J. and Jain, B. K. 1998).

Burned tissues are infected by bacteria such as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella,* and *Enterococcus* and *Candida spp*. (Revathi, G., Puri, J. and Jain, B. K. 1998). Burns occur when the skin or body is damaged by electricity, heat, radiation, chemicals, or electronic devices. Burns cause changes in vascular permeability, plasma protein extravasations, platelet aggregation, and prolonged fibrinolysis.

These wounds or injuries are devastating accidents and cause disability and mortality worldwide (Kumar, S. et al., 2013).

After burn injuries, in most cases, the skin loses its barrier functions as a result there have potential chance of bacterial infection by microorganism. Microbial contamination may also

delay recovery and growth pain, and the chance of scarring to the skin and can be even deadly. The wet nature, high temperature, and nutrient-wealthy surroundings of a burn wound create a perfect territory for the growth of bacteria. However, 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 a lot greater difficult. *Staphylococcus aureus* and *Pseudomonas aeruginosa*, others may additionally consist of *Streptococcus pyogenes* are the most not unusual pathogens isolated from burn wound locations, 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.

Diabetic Foot Ulcer Infections

Diabetic diseases associated with plantar ulcers are infectious diseases due to the high incidence of mixed microflora and the inability of PMNs to destroy invading bacteria.

As with most infections, *Staphylococcus aureus* is a common isolate in diabetic foot infections, other aerobic bacteria include: *Staphylococcus epidermidis*, *Streptococcus spp.*, *Pseudomonas aeruginosa*, *Enterococcus spp.*, and coliform bacteria (Pathare, N. A. et al., 1998).

Leg and Decubitus (Pressure) Ulcer Infections

Staphylococcus aureus is the most prevalent potential pathogen in leg ulcers (Bowler, P. G. and Davies, B. J. 1999). This work reported a significantly greater frequency of *Peptostreptococcus spp.* and pigmenting and non-pigmenting gram-negative *bacilli* in clinically infected leg ulcers than in no infected leg ulcers.

2.1.3 Development of Wound Infection

The human body is nonsterile. Diseases result from interactions between the host, potential pathogens, and the environment. This occurs when microbes successfully evade the host's defense mechanisms and cause adverse changes in the host itself. The development of the

disease precedes the interplay of complexities that are not fully understood. The following way infection may develop (Herman, T. F. and Bordoni, B. 2022).

- The development of the wound infection depends on the pathogenicity and virulence of the bacteria and the immune system of the host.
- Host/pathogen interactions do not necessarily translate into pathological data and new concepts and definitions need to be introduced.
- Microbiological testing alone is not a reliable method for diagnosing infectious diseases but requires a good and holistic patient assessment.

2.1.4 Factors for the Development of Wound Infection

Various microbial pathogens and hosts may be involved in the progression of a wound to an infected state, for example: type, location, size and expansion of the wound, exogenous degree of disease, the rate of blood perfusion in wound infection, host health and immunity, microbial load, and combined virulence level specified by the respective species.

To remove the development of wound infection, the management and significance of all factors are described below:

Size

This factor evaluates wound size first, such as length, width, and depth. Regular measurement of wound size helps to monitor wound healing.

Location

It finds the location of the wound and makes a note. The location of the wound can help determine the etiology of wounds such as foot ulcers associated with diabetes. Wounds can also be located in places that complicate the healing process, such as joints, pressure sores, or places of pressure point.

Microbial infection

Find out the signs of a local infection or systemic infection.

- **Local**: lesions may be red, hot to the touch, purulent discharge or yellow biofilm, or painful.
- **Systemic**: patients may show signs and symptoms such as fever, fatigue, weakness, heartburn, nausea, vomiting or diarrhea. Infections that are not treated early can delay healing, disrupt the wound healing process, cause further damage or progression of the infection.

Necrosis

Necrotic tissue cannot survive due to reduced blood flow. It will appear as an eschar, which is a hard, black, dry tissue. It may also appear as a moist, black tissue. Necrotic tissue should be removed as it can cause infection and delay healing. Wet necrosis indicates superinfection.

Granulation

Healthy granulation tissue is pink. The abnormal tissue may be red, loose, and patchy, and may bleed. Hypergranulution is also considered abnormal. White or yellow tissue may appear in the chronic wounds. Abnormal tissue is associated with infection and poor wound healing. The white or yellow fibrous tissue in chronic wounds is vascular and takes a long time to heal if not removed.

Slough

Slough is yellow or white dead tissue. It can be loose or tracked. Carrion must be removed to encourage the growth of healthy tissue. Loose slough can be removed with bed discomfort. Adhesive slough is difficult to remove and may require surgery.

2.1.5 Bacteria and Wound Infection

Bacteria are the leading cause of skin and soft tissue infections. Infection due to bacteria is one of the severe problems for different types of wounds. Skin infection is very common when it's wounded by trauma, burns, surgery, or other accidents. The skin and mucous membranes are generally good at protecting against infection. Due to skin damage after a wound, the body loses the effective barrier function (its first-line defense capability). When the first line of defense is interrupted some common bacteria (**Table 2.1**) very easily cause infections in wounded areas. According to recent previous data, very common bacterial species detected from wounded area was: *Staphylococcus aureus* (37%), followed by *Pseudomonas aeruginosa* (17%), *Proteus mirabilis* (10%), *Escherichia coli* (6%) and *Corynebacterium spp.* (5%) (**Fig. 2.1**) (Bessa, L. J. et al., 2015).

The barriers are breached for skin damage due to trauma or mucosal damage due to viral infection; these bacteria may gain access to underlying tissues or the bloodstream and cause infection. Common bacterial pathogens (**Fig. 2.2**) associated with acute and chronic wound infection include *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Streptococcus pyogenes*, *Proteus spp.*, *Streptococcus spp.* and *Enterococcus spp.* Other studies have shown that the high infection rate of wounds by potential bacterial pathogens were *Staphylococcus aureus*, *Escherichia coli,* and *Pseudomonas aeruginosa* (Tom, I. M. et al., 2019).

Table 2.1: Common bacteria causing acute wound infection

Group	Species
Gram negative	*Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Actinetobacter baumannii*, *Escherichia coli*, *Serratia marcescens*
Gram positive	*Streptococcus pyogenes*, *Corynebacterium diphtheria* and *Staphylococcus aureus*

For the current study, three bacterial species were selected to apply our prepared hydrogel dressing to check bacterial inhibition by the disc diffusion method. The selected bacteria were:

1. *Staphylococcus aureus*

2. *Pseudomonas aeruginosa*

3. *Escherichia coli*

Scientific classifications of these three bacteria are displayed in **Table 2.2**, **Table 2.3** and **Table 2.4** respectively.

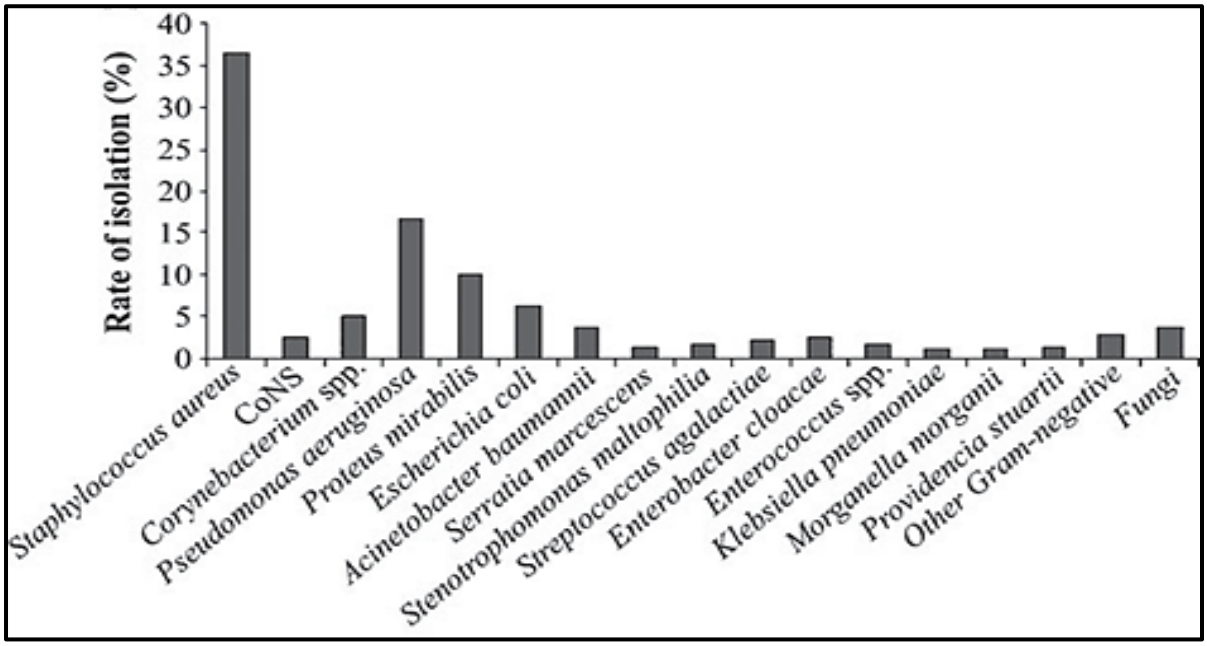

Fig. 2.1: Bacteria isolated from patients with infected wounds (Retrieved from Bessa, L. J. et al., 2015).

Staphylococcus aureus has long been recognized as one of the most significant bacteria causing disease in humans. It is a common cause of skin and soft tissue infections such as abscesses, boils, and cellulitis.

The bacteria are spherical-shaped gram-positive bacteria. These bacteria are commonly available in the upper respiratory tract and skin.

Table 2.2: Scientific classification of *Staphylococcus aureus*

	Staphylococcus aureus
Domain	Bacteria
Phylum	Bacillota
Class	Bacilli
Order	Bacillales
Family	Staphylococcaceae
Genus	*Staphylococcus*
Species	*S. aureus*

Escherichia coli is mostly available in contaminated food and water. The bacterium is also shortly known as *E. coli*. The rod-shaped bacteria are commonly found in the lower intestine of warm-blooded animals. This gram negative bacteria is one of the leading bacteria for wound infection among other bacteria commonly found in wound-infected areas.

Table 2.3: Scientific classification of *Escherichia coli*

	Escherichia coli
Domain	Bacteria
Phylum	Pseudomonadota
Class	Gammaproteobacteria
Order	Enterobacterales
Family	Enterobacteriaceae
Genus	*Escherichia*
Species	*E. coli*

Pseudomonas aeruginosa is a rod-shaped gram negative bacteria. They are responsible for creating disease both in animals (including humans) and in plants. The encapsulated bacteria is responsible for nosocomial infections (hospital-acquired infections) like pneumonia and sepsis.

Table 2.4: Scientific classification of *Pseudomonas aeruginosa*

	Pseudomonas aeruginosa
Domain	Bacteria
Phylum	Pseudomonadota
Class	Gammaproteobacteria
Order	Pseudomonadales
Family	Pseudomonadaceae
Genus	*Pseudomonas*
Species	*P. aeruginosa*

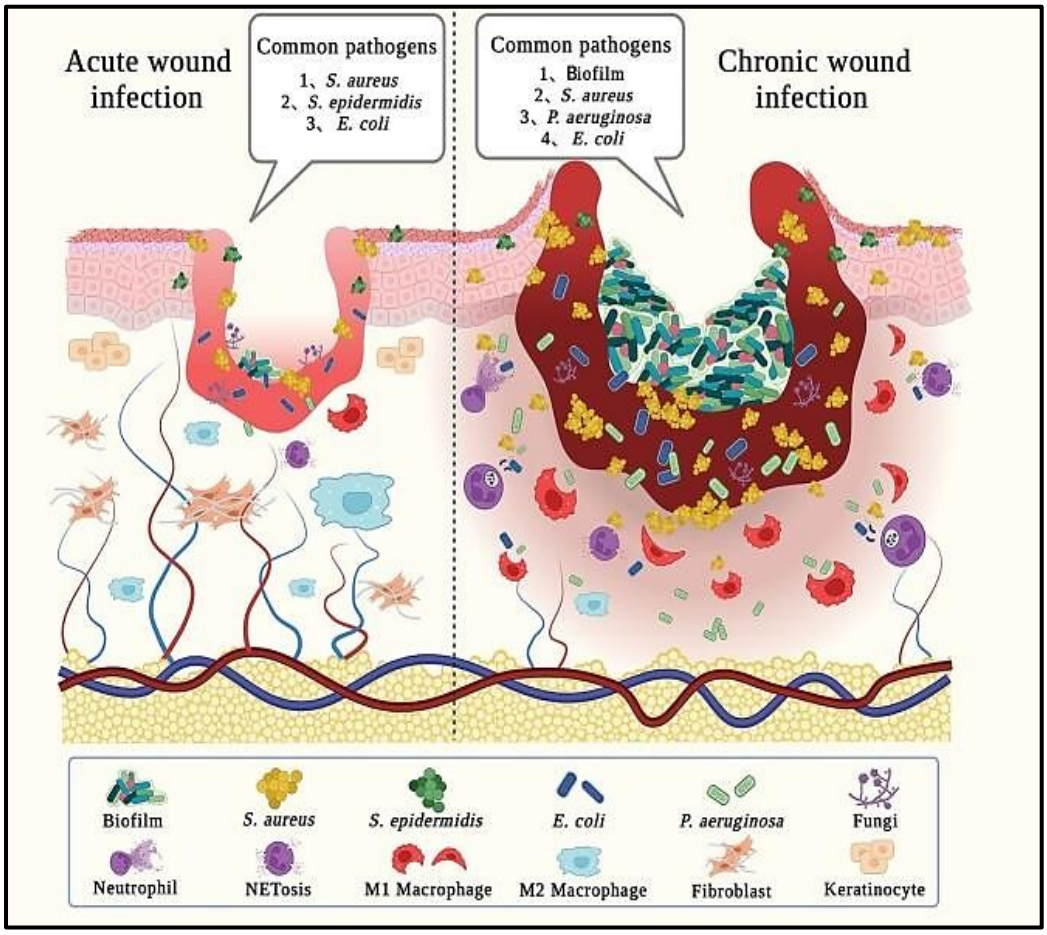

Fig. 2.2: Comparing between acute and chronic wound infection together with common pathogens (Retrieved from Ding, X. et al 2022).

2.2 Wound Healing

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 to skin feature quickly (Martin, P. 1997).

2.2.1 Wound Healing Process

The process includes four tiers: a) Hemostasis b) Inflammation c) proliferation and d) remodeling (Boateng, J. S. et al., 2008). The stages are described in **Table 2.5** and **Fig. 2.3**:

Table 2.5: Four stages of wound healing process

Stages	Description	References
Hemostasis	• The process starts immediately after injury. • Platelets play a role: in forming a clot to seal the damaged site and stop bleeding. • Platelet growth factors are released in this stage. • The process continues for 24 to 48 hours. • Hemostasis includes three main steps: Vasoconstriction, Platelet embolism temporarily plugs the hole in the damaged blood vessel, and Blood coagulation (formation of fibrin clots).	(Davie, E. W., Fujikawa, K. and Kisiel, W. 1991).
Inflammation	• Inflammations begin after 24 hours of injury. • Neutrophils 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. • It can be classified as acute or chronic.	(Hübner, G. et al., 1996).
Proliferation	• Also known as the regenerative phase started on the 3rd day of post-wounding. • Characterized via the presence of fibroblast, red tissue, replacement of dermal tissue, and subdermal tissue in the wound.	(Nissen, N. N. et al., 1998).
Remodeling	• Final level of the wound recovery process. • The new epithelium is formed along with the transition of granulation tissue. • Last more than two years after beginning.	(Liu, D. et al., 2015)

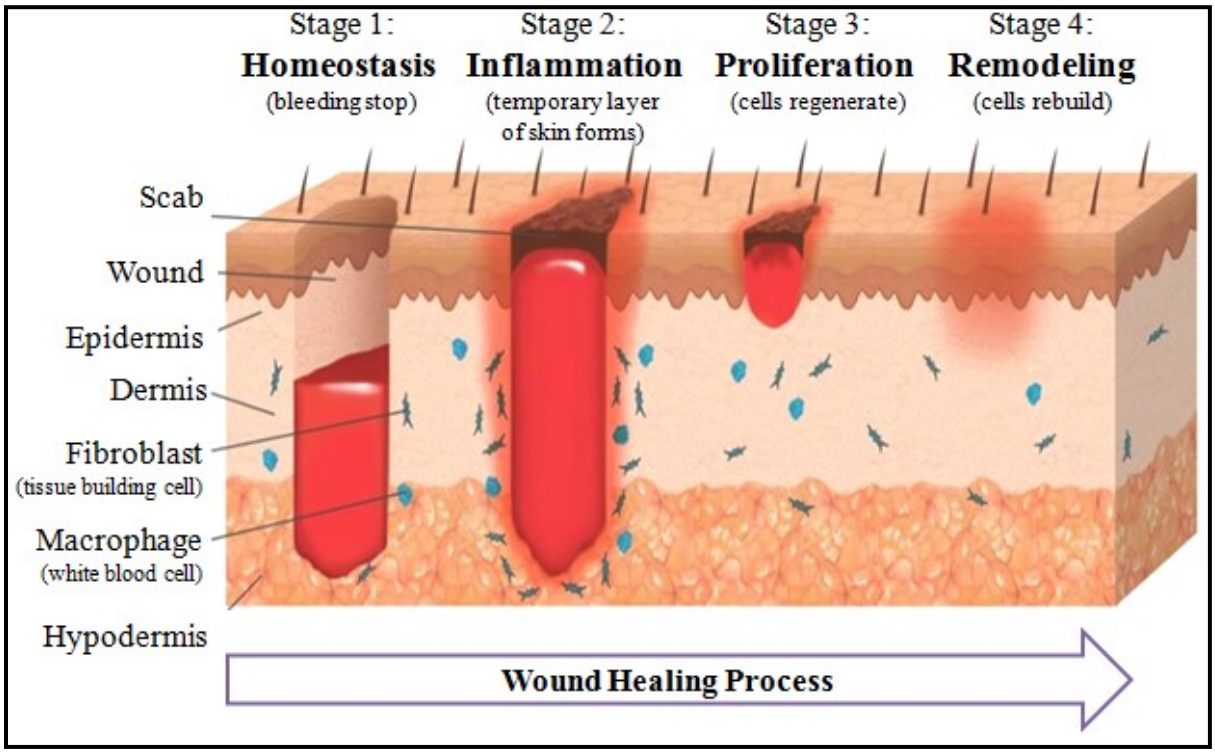

Fig. 2.3: Four stages of wound healing (Retrieved from Deng, X., Gould, M., and Ali, M. A. 2022).

2.2.2 Factors that Delay Wound Healing Process

After injury wound goes through a wound-healing process which take normally four to six weeks, but some factors are responsible for delaying the healing process:

i. Patient Age

Most of all changes in healing ability are age-related. Research shows that people over the age of 60 may experience a delay in recovery due to the physiological changes that occur with advanced age. In addition to many existing diseases, the inflammatory response of the body decreases, angiogenesis slows down, and the epithelialization process slows down. Some skin changes are associated with dry skin due to changes in melanocytes (such as age spots) and decreased sebaceous gland activity.

Decreased collagen synthesis is also associated with slower collagen production during wound healing.

ii. Types of Pain

The severity of the wound affects how quickly the pain is felt. Obviously, larger wounds take longer to heal, but the shape of the wound can affect the healing time. Bacterial wounds generally heal faster than parallel wounds, while circular wounds heal more slowly.

In addition, when there is dead tissue, dryness and foreign matter in the wound, wound healing will slow down.

iii. Infections

Any tear in the skin may permit bacteria, viruses, or parasites to attack the wounded area. Usually, these bacteria are destroyed by leukocytes and other body immune systems. Once an infection occurs it can surface and cause pain or damage that needs to be treated with good wound care and the ability to use antibiotics.

iv. Disease

People with diabetes mellitus or diseases affecting blood vessels can affect the quality of pain. Flow of blood should be smooth for good wound healing and poor supply or flow of blood to the wound surface should be treated. People with continual wounds should see a doctor for a full evaluation to decide on suitable treatment.

v. Malnutrition

In elderly or ill patients, malnutrition can deprive the body of its resources to heal wounds. Malnutrition can occur as the disease increases the person's protein and calorie needs. Also, the wound can release too much protein in one day, especially in severe cases (injury) or pain in the leg. Useless calories may help the body to break down protein to supply energy, increasing the capability of the body to heal.

vi. Dehydration

Dehydration of the wound inhibits cell migration, reduces blood oxygenation, and slows wound healing. Dehydration from a sodium or water deficiency can delay most healing processes. Maximum humans require sixty-four ounces of fluid a day, those trying to relieve

from an injury require more fluids to boost the transport of leukocytes to the injured area to provide oxygen and nutrients. The urine of patients with good hydration is clear and odorless.

vii. Complaint

Bradycardia, hypotension, or cardiac disease may delay healing because the blood carries the substances necessary for the wound-healing process to the tissues. Clogged or narrowed arteries, or heart, kidney, and lung disease can cause problems with the body's ability to deliver vital medical supplies, including leukocytes and enough oxygen supply to damage tissue.

viii. Edema

Tissue may swell after wounding the skin, which is called edema, too much tissue swelling (edema) causes pressure vessels, and as a result flow of blood in the wounded area is very poor. Tissue swelling or edema may be caused by cardiac disease.
Pressure therapy may impactful in returning fluid to the vessels to reduce edema and promote proper healing.

xi. Recurrent Injuries

Recurrent diseases due to the strain or pressure of the region can delay or even stop the healing process. Repetitive injuries usually occur in bedridden patients and can be treated with temporary measures splints or restraints under physician supervision.

x. Patient Behavior

Unfortunately, some patients delay wound healing by making lifestyle choices such as smoking or drinking too much alcohol.
Other patient behaviors that may affect the treatment, such as insomnia, not keeping the affected area clean, poor wound care, poor dressing, not getting the wound wet, and insufficient work.
Counseling patients and delivering careful instruction may help increase adherence to good practice.

2.2.3 Types of Wound Dressing Material

A dressing is a form of bandage or material administered to a wound to allow healing and shield the wound surface from similar injuries. Wound dressing is usually done in direct contact with the protruding wound, such as a bandage used to hold the dressing in place. Many dressing clothes today are self-adhesive.

There several categories of dressing material available for wound healing. A list of some common wound healing dressings has been described with their advantages and disadvantages (Dhivya, S., Padma, V. V. and Santhini, E. 2015).

i) Gauze

They're inexpensive, highly absorbent, and easy to convert to defective and irregular shapes. They're inexpensive, highly absorbent, and easy to convert to irregular size and shape. It causes injuries and waste products that will expose the rest to microorganisms.

ii) Transparent Film Dressing

It can create a humid environment for wound, confirm air exchange, disallow external infection bacteria, and can be easily put on and taken off without causing any pain to the patient. It cannot absorb liquid and is unstable for painful contaminated wounds

iii) Foam Dressing

It has the advantages of good absorption capacity, heat insulation, and strong air replacement ability; requires a lengthy time for non-contaminated wounds. It should be changed daily and may cause skin maceration

iv) Hydrocolloid Dressing

It has high absorbency, creates a wet environment, and prevents the wound from holding bacteria without having a secondary barrier effect. Not suitable for removing infected bacteria, it can hurt the wound when removed, strong adhesive characteristics.

v) Hydro-conductive Dressing

Quickly remove exudates from the product without removing dressing materials. It should be changed daily.

vi) Hydrogel Dressing

Split absorption ability, gas replacement ability, no pain when removed, can reduce the temperature of the wound and moisten the wound environment. Low resistance to infection, often changing bandages needs to wear a secondary bandage.

2.2.4 Characteristics of Ideal Wound Dressing for Wound Healing

An ideal wound dressing fabric/ biomaterial should have the following criteria preserve a wet environment around the wound for healing (Lin, S. Y., Chen, K. S. and Run-Chu, L. 2001; Boateng, J. S. et al., 2008) besides essential elements for an ideal wound dressing displayed in **Fig. 2.4**.

a) Eliminate excess exudates, however do not attain saturation at the wound's outer surface.

b) Shield the wound from microorganisms, infections, or contaminations and reduce the wound surface necrosis.

c) Stop the wound desiccation and stimulate the growth factor.

d) Smooth and relaxed to do away from the skin, should be toxic and allergic-free, non-harmful to living tissue, and elastic.

e) Should be less painful, ache fee during use and removal of the dressing.

f) Can shield the wounded area from repeat trauma and own mechanical protection.

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

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

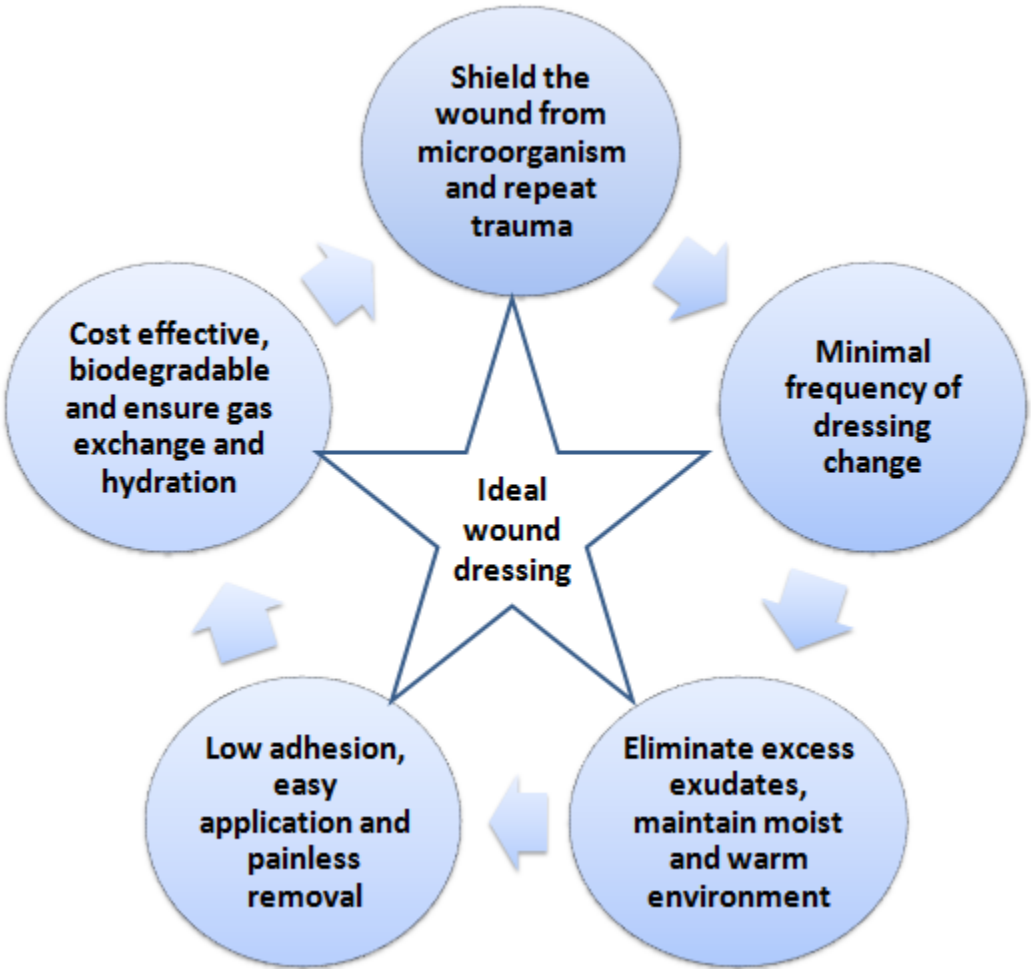

Fig. 2.4: Schematic diagram of the essential elements for an ideal wound dressing.

2.2.5 Hydrogel as a Wound Dressing Material

Hydrogels are hydrophilic dressings with very excessive water content, capable of donate 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 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 debridements. Hydrogels are properly 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, has high exudative capacity, is non-adhesive, easy to remove from the

wound, boost up wound recovery, reduces pain and prevents infection, 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 translucency, semi-permeability to gases and water vapor, poor barrier properties for bacteria, and possibly poor mechanical stability.

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. and Klar, A. S. 2020).

The unique characteristics of hydrogel biomaterials 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 compact network of the hydrogel structure prevents the wound from contamination and stops microorganisms and spread bacteria to the wound. But, hydrogel structure can carry bioactive molecules e.g. antibiotics, prescribed drugs, pharmaceuticals products and other herbal or medicinal product to the wound. These molecules may enter into the hydrogel network during gelation and exchange by absorbing wound exudates during the emission approach after the hydrogel comes in contact with the wound.

The large tissue-like water content of the hydrogel provides the flexibility and elasticity needed to adapt to wounds in many parts of the body region. In this study, we have incorporated mupirocin and egg white into the hydrogel for chemical, physical, biological assessment and bacteria inhibition assessment of hydrogel dressing by disc diffusion method for many kinds of wound recovery.

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

a) Excellent biodegradability, no toxic substances will be produced after degradation.

b) Optimal durability and stability in large environments and during storage.

c) Maximum absorbent capacity (usually equal to swelling) in salt water. The preferred absorbed value (preferred thickness and availability of pore) depends on the administration requirement.

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

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

f) Good photostability, good biocompatibility, good oxygen permeability, moderate pH after water swelling, colorless, odorless, non-toxic, moderate protein adsorption and small cell adhesion.

g) Aqueous surface medium that protects cells and therapeutic agents (peptides, proteins, oligonucleotides, DNA), ease of surface change with particular biomolecules.

h) Soft and tissue-like structures and micro porous structures for additional distribution.

i) Very little friction to the surrounding tissue during implantation.

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

2.2.6 Commercially Available Hydrogel Dressing

Suprasorb® G

- Manufacturer: Lohmann & Rauscher Globa.
- Content: 70% water in acrylic polymer, polyethylene and phenoxyethanol.
- Treats dry wounds, ulcers (lower leg) , burns, first and second degree burns, scalds.

Derma-Gel®

- Manufacturer: Medline Industries, Inc.
- Derma-Gel® Hydrogel Sheet is glycerin based a soft, flexible, semi-occlusive hydrogel dressing that is bacteriostatic and absorbs 5 times its weight in fluid.
- Treatment for blisters, partial and full thickness wounds, leg ulcers, surgical wounds, tears and abrasions, and first and second degree burns.

AquaDerm™

- Manufacturer: DermaRite industries.
- Contains sodium 2-acrylamido-2-methyl-1-propanesulfonate, propylene glycol, polyethylene glycol dimethacrylate.
- Treats burns, minor burns and electrical damage.

Elasto-Gel™ Wound Dressing

- Manufacturer: Southwest Technologies, Inc.
- Glycerin based hydrogel provides moist wound healing, soothing and cool for patient comfort and highly absorbent.
- Treatment for bacteriostatic hydrogel is indicated for used as primary or secondary dressing or for preventative care when used as padding.

DermaGauze™

- Manufacturer: DermaRite industries.

- Hydrogel-impregnated gauze dressing. Acrylate polymers are one of the ingredients of DermaGauze.
- Treatment of partial or long and thick wounds.

Neoheal® Hydrogel

- Manufacturer: Kikgel.
- Hydrogel film consists of electron beam crosslinked PEG, PVP and agar. It consists of 90% water.
- Treatment of wounds, abrasions, burns, bedsores and other chronic conditions.

Simpurity™ Hydrogel

- Manufacturer: Safe n'Simple.
- Absorbent sheets containing polyethylene oxide, polyvinyl alcohol, acrylate, polyurethane, and purified water.
- Treatment for dry wounds, skin burns and dry scabs.

Restore Hydrogel

- Manufacturer: Hollister Incorporated.
- Hydrogel-impregnated gauze pads containing hyaluronic acid promote wound healing through autolytic debridement.
- Treatment for second degree (partial) and third degree (full thickness) wound.

ActivHeal®

- Manufacturer: Advanced Medical Solutions Ltd.
- It is a wound dressing containing 85% water.
- Treatment for ulcers (pressure and leg), diabetic foot ulcers, wound infection, cavity wounds.

DermaSyn®

- Manufacturer: DermaRite industries.

- It's a primary wound dressing composed of alpha-tocopherol.
- Treatment for acute or chronic partial and full thickness wounds.

NU-GEL™

- Manufacturer: Systagenix.
- Contains sodium alginate which effectively debrides necrotic tissue and fibrinous slough.
- Treatment for chronic wound, diabetic foot ulcers, venous leg ulcers and pressure ulcers.

Purilon®

- Manufacturer: Coloplast.
- Contains calcium alginate and sodium carboxymethyl cellulose with purified water.
- Treatment for leg ulcers, pressure ulcers, non-infected diabetic foot ulcers and first and second degree burns.

INTRASITE* Gel

- Manufacturer: Smith and Nephew.
- Consist of Carboxymethyl cellulose and propylene glycol.
- Treatment for pressure ulcers, diabetic foot ulcers, surgical incisions and venous ulcers.

SOLOSITE* Gel

- Manufacturer: Smith and Nephew.
- It contains carboxymethylcellulose (CMC) and glycerin sodium salt containing more than 60% water.
- Treatment of minor injuries, cuts, injuries, skin tears, venous ulcers, surgical incisions, surgical incisions, diabetic foot ulcers, pressure ulcers.

Woun'Dres®

- Manufacturer: Coloplast.
- Contains polymers such as carbomer and collagen, among other components.
- Treat dry wounds.

2.3 Mupirocin

Mupirocin is an antibacterial agent which is applied as a topical administration (e.g. cream, ointment etc.) for the treatment of bacterial skin infection.

It prevents and kills common bacteria (e.g. *Staphylococcus aureus*) which are generally responsible for skin infection due wound or other skin disease like impetigo, folliculitis etc. Physical appearance of mupirocin is presented in **Fig. 2.5**.

Fig. 2.5: Physical appearance of mupirocin.

2.3.1 Role of Mupirocin in Inhibition of Bacteria

Mupirocin is a white crystalline powder, sparingly soluble in water, easily soluble in acetone and dichloromethane. It is a strong acid with a pKa of 4.83, a molecular formula of $C_{26}H_{44}O_9$, and a melting point of about 77-78 ° C and a diffusion coefficient of 2.25. Chemical structure of mupirocin is presented in **Fig. 2.6**.

Previous study reported on mupirocin retardation of wound healing, liposome's in hydrogel (Ishida, M. Nambu, N. and Nagai, T. 1983) and nano-liposomes (Cern, A. et al., 2014) were developed to improve therapeutics (Torchilin, V. and Weissig, V. 2003).

Nevertheless, there are limitations to the approaches, another study where mupirocin nanoparticle-loaded hydrogels were successfully developed which were expected to enhance or improve the antibacterial activity of mupirocin (Kamlungmak, S. et al., 2020). These studies have confirmed the antibacterial properties and mechanism of action of mupirocin, as well as gene analysis.

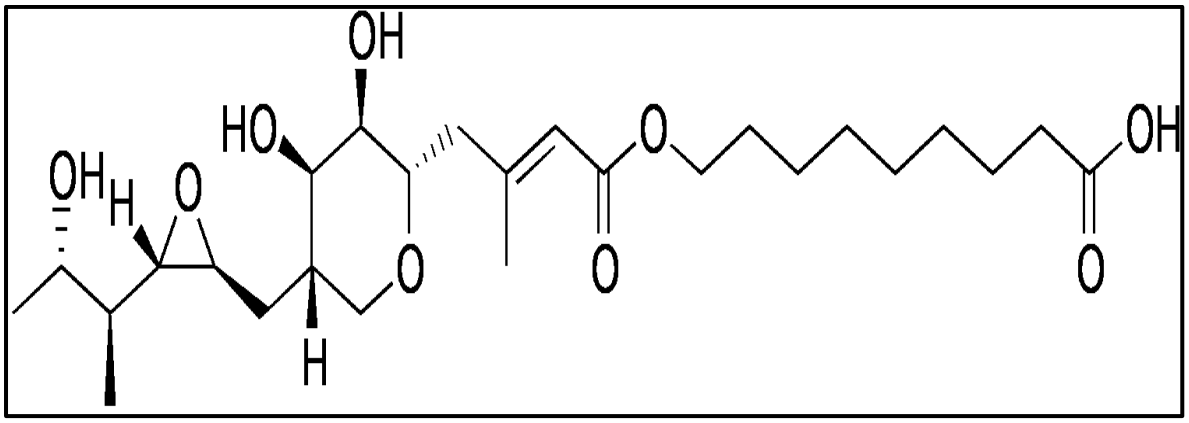

Fig. 2.6: Chemical structure of pseudomonic acid, the principal component of mupirocin (Retrieved from Zhao, C.et al., 2014).

2.3.2 Mechanism of Action

Mupirocin is a mixture of several pseudomonic acids. Mupirocin (pseudomonic acid) is a mono oxycarbonate antibiotic obtained from the fermentation of Pseudomonas fluorescens. Mupirocin inhibits bacterial protein synthesis by reversibly binding and inhibiting bacterial isoleucyl transfer-RNA synthetase and subsequently inhibits the incorporation of isoleucine into bacterial proteins. The mechanism of action is also shown in **Fig. 2.7**.

Isoleucyl-tRNA synthetase, an enzyme that catalyzes the conversion of isoleucine and tRNA to isoleucyl-tRNA. Its mechanism of action is briefly explained as follows:

Isoleucine tRNA synthetase is disallowed by pseudomonic acid in bacteria, resulting in the depletion of isoleucyl tRNA and accumulation of uncharged tRNA reversibly bound to the enzyme.

High consumption of isoleucyl-tRNA leads to disallow of protein synthesis. The free form of tRNA binds to the aminoacyl-tRNA binding site of the ribosome, resulting in the formation of (p)ppGpp, which inhibits RNA synthesis. Co-inhibition of protein synthesis and RNA synthesis leads to bacteriostasis.

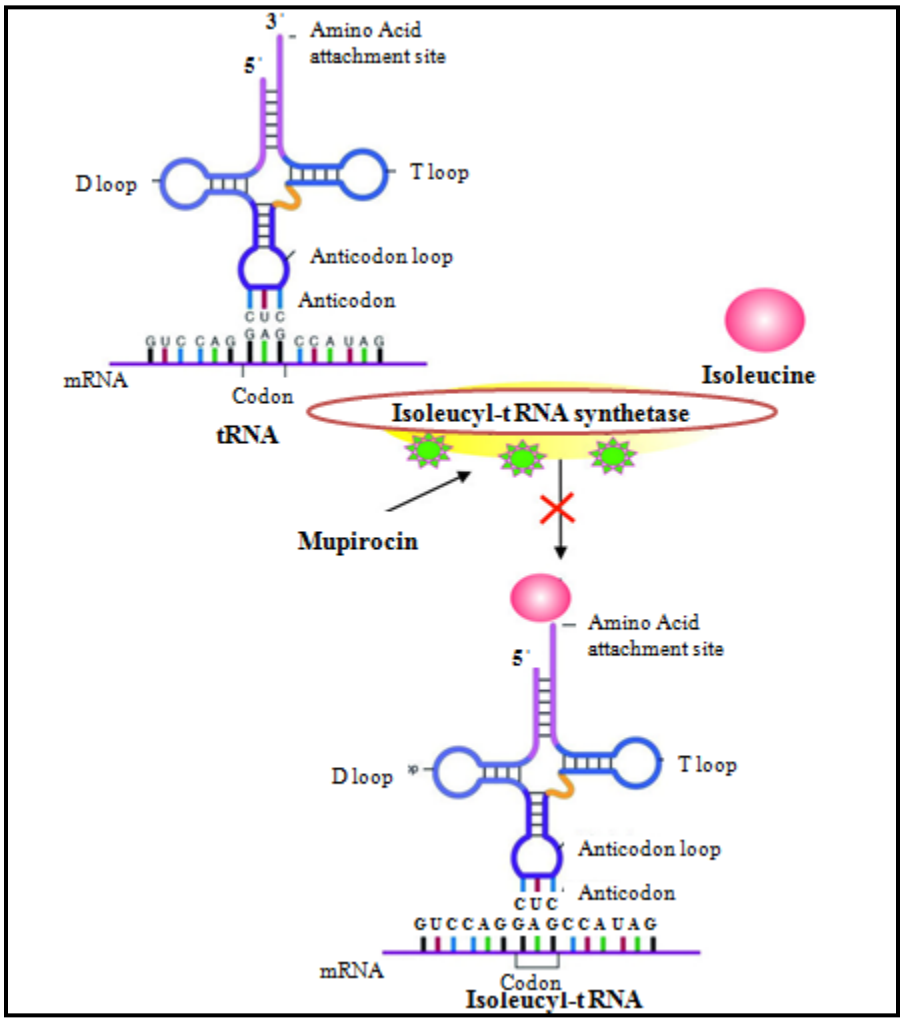

Fig. 2.7: Illustration of mechanism of action of mupirocin (Retrieved from Gangwar, A. et al, 2021).

2.3.3 Use of Mupirocin

- Mupirocin is used to treat skin infections (for example, boils, impetigo or open sores) usually caused by *Staphylococcus aureus* or *Streptococcus pyogenes* infections.
- It may be used against methicillin-resistant *Staphylococcus aureus* (MRSA).
- Mupirocin is ineffective against most anaerobic bacteria, mycobacteria, mycoplasma, chlamydia, yeast, and fungi.
- Preoperative intranasal mupirocin is effective in preventing infection after *Staphylococcus aureus* infection and prophylactic intranasal or catheter site treatment is effective in reducing the risk of catheter site infection in peritoneal dialysis patients (Troeman, D. P. R., Van Hout, D. and Kluytmans, J. A. J. W. 2019).

2.4 Egg White

Chicken Egg white (egg white) is a protein-rich liquid. The main function of these components is to provide nutrition and protect the development of healthy embryos and eggs from invading bacteria. Egg white is a fluid that accumulates in the lumen of the tube from the epithelial cells of the gland and most of the occipital bone (part of the fallopian tube). The egg white around the egg must first be covered by the egg and the shell. Egg white is an aqueous protein solution. It consists of water and its dry matter consists of protein and small amounts of carbohydrates, vitamins and minerals.

The main function of these components is to provide nutrients and protect the developing embryo and healthy eggs from invading bacteria. Egg white surrounds the yolk, which is then covered by the eggshell and shell membrane. Egg whites do not contain microbe-containing nutrients such as iron and are rich in molecules that make them excellent antibacterial properties. Chicken egg white is known to have antibacterial properties. In 1890, Wurtz was the first to demonstrate the bactericidal activity of *Bacillus anthracis*.

Later, in 1909, Laschtschenko confirmed these findings. A heat-stable enzyme is thought to be involved in this process. This bactericidal enzyme was first discovered by Fleming in 1922 and named lysozyme.

Since then, ovalbumin, lysozyme, and other egg white proteins with antibacterial properties have been reported. It has antibacterial properties due to the presence of certain biological substances.

2.4.1 Component of Egg White

The main component of egg white is water, it consists of 84-89 percent water, protein 10-11 percent and 1 percent of fat and carbohydrates, vitamins and minerals.

Vitamins include vitamin B1, vitamin B2, vitamin B6, vitamin b12, folic acid, niacin, biotin, and pantothenic acid; **minerals** include: sodium, chloride, potassium, calcium, phosphorus, iron, magnesium, sulphur, zinc, copper, manganese and iodine; **essential amino acids** include: Isoleucine, leucine, lysine, methionine, cystine, phenylalanine, tyrosine, tryptophan and valine.

The main components of egg white are illustrated in **Fig. 2.8** and all components are described in **Table 2.6**.

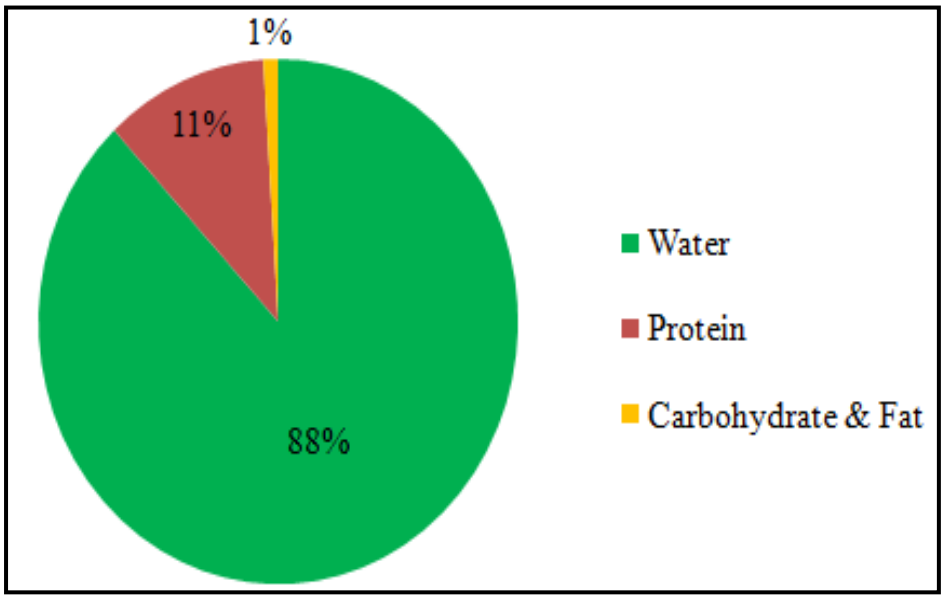

Fig. 2.8: Main component of egg white (Retrieved from Réhault-Godbert, S., Guyot, N. and Nys, Y. 2019).

Table 2.6: Components of egg white

Component (per 100 g)	Egg white	Reference
Water (g)	88.6	
Proteins (g)	10.6	
lipids (g)	0.1	
Carbohydrates (g)	0.8	
Minerals (mg)		
Na(Sodium)	155	
Cl (Chlorine)	175	
K (Potassium)	140	
Ca (Calcium)	8	
P (Phosphorus)	18	
Fe (Iron)	0.1	
Mg (Magnesium)	10	
S (Sulphur)	163	
Zn (Zinc)	0.12	
Cu (Copper)	0.02	
Mn (Manganese)	0.007	
I (Iodine)	0.003	
Vitamins (mg)		(Jalili-Firoozinezhad, S. et al., 2020)
Vitamin B1	10	
Vitamin B2	430	
Vitamin B6	10	
Vitamin B12	0.1	
Folic acid	12	
Niacin	90	
Biotin	7	
Pantothenic acid	250	
Essential amino acids (mg)		
Isoleucine		
Leucine	560	
Lysine	880	
Methionine + Cystine	660	
Phenylalanine + Tyrosine	670	
Threonine	1020	
Tryptophan	470	
Valine	170	

2.4.2 Medicinal Properties of Egg White

Egg white has many medicinal properties, due to having medicinal properties they are used in wound dressings and in other biomedical applications which are illustrated in **Fig. 2.9**.

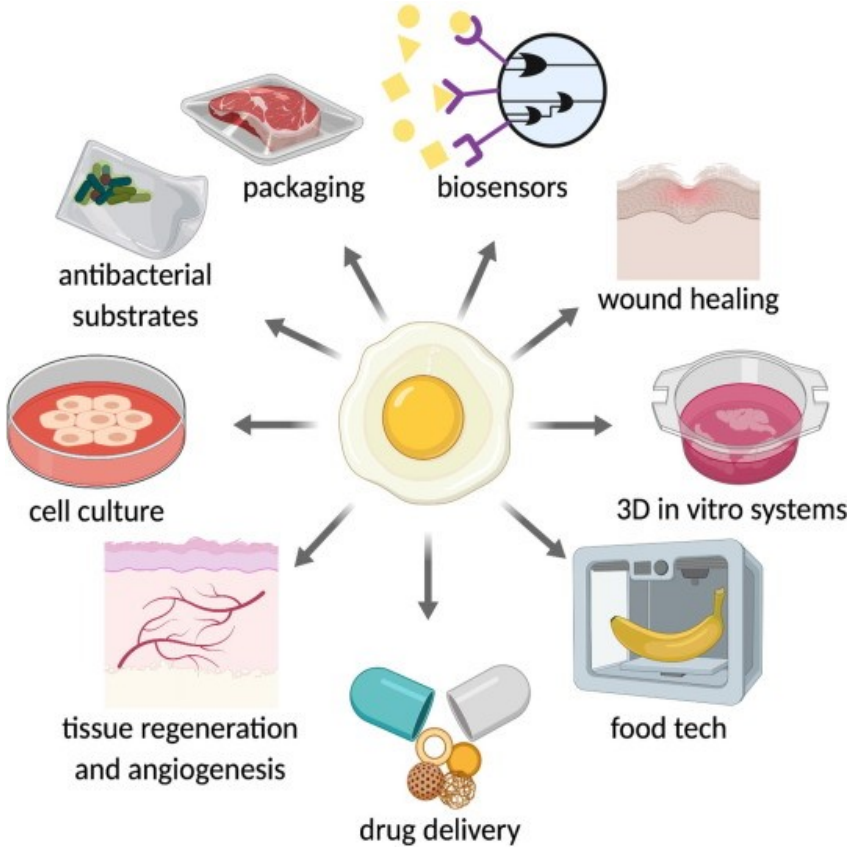

Fig. 2.9: Biomedical application of egg white (Retrieved from Jalili-Firoozinezhad, S. et al., 2020).

Egg whites in particular have three properties that will affect the healing process are described below:

i. Antibacterial Properties: Egg white is an antimicrobial agent against some bacteria which are responsible for creating wound infections.

ii. Wound Healing Properties: Preliminary evidence suggests that egg white can improve wound healing.

iii. Pro- and Anti-inflammatory Properties: Wounds are an inflammatory disease. In terms of inflammation, egg white is both pro-inflammatory (increases inflammation) and anti-inflammatory (reduces them).

2.4.3 Antibacterial Properties of Egg White

Egg white contains different chemical components, among them lysozyme and ovotransferrin play key roles in bacteria inhibition activity. The antibacterial effects of different chemical compounds of egg white are described below and antibacterial properties have been demonstrated in **Fig. 2.10**:

Ovalbumin

Accounting for more than half of total ovalbumin, ovalbumin is a monomeric phosphoglycoprotein widely used as a model to study protein-structure interactions (Li-Chan, E. and Nakai, S. 1989; Ibrahim, H. R. 1997).

Functionally, ovalbumin is important for the gelling, foaming, and emulsifying properties of egg whites.

Lysozyme

Lysozyme has an important role in natural defense mechanisms (Kijowski, J., Lesnierowski, G. and Fabisz-Kijowska, A. 2000). It acts as a mucopeptide N-acetylmuramoyl hydrolase, exerting bacteriolytic activity by hydrolyzing the link between N-acetylmuramic acid and N-acetylglucosamine of peptidoglycan, a structural component of the bacterial cell wall.

It has shown antibacterial activity against bacteria such as *Bacillus stearothermophilus*, *Clostridium tyrobutyricum*, *Clostridium sporogenes* and *Bacillus spp.* (Losso, J. N., Nakai, S. and Charter, E. A. 2000).

Enzymatic processing of lysozyme has been shown to have antibiotic use (Pellegrini, A. et al., 1997; Ibrahim, H. R., Thomas, U. and Pellegrini, A. 2001). Pellegrini, A. et al., (2000) reported that the polypeptide derived from lysozyme not only affects the outside of *Escherichia coli*, but also inhibits DNA and RNA synthesis.

Lysozyme, in addition to its many applications as an antibacterial food, is added to oral health products such as toothpaste, mouthwash and chewing gum, removing bacteria causing bacteria and increasing immunity while preventing infection of the oral mucosa.

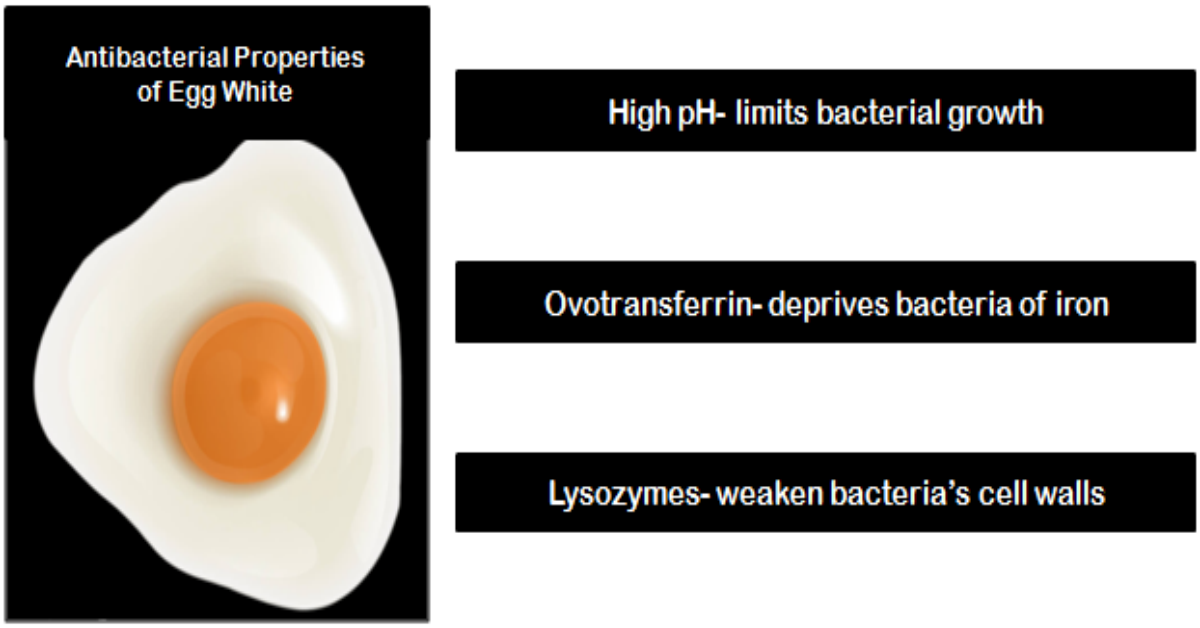

Fig. 2.10: Three main components of egg whites that make them antibacterial (Retrieved from Legros, J. et al., 2021).

Ovotransferrin

Ovotransferrin is a monomeric glycoprotein belonging to the transferrin family, showing antibacterial activity against many bacteria, including *Pseudomonas spp., Escherichia coli, Streptococcus mutans*, *Staphylococcus aureus*, *Bacillus cereus* and *Salmonella enteritidis* (Mine, Y. and Kovacs-Nolan, J. 2004). It has been suggested that ovotransferrin can be used as an antibiotic by passing through the outer membrane and reaching the membrane, causing selective ion permeability and potential dispersion.

The 32-amino acid ovotransferrin peptide OTAP-32 has also been shown to kill gram-negative bacteria through self-energy uptake across the bacterial outer membrane and disruption of the cytoplasmic membrane.

Avidin

Chicken avidin is a tetrameric glycoprotein with antimicrobial properties that has been shown to inhibit the growth of biotin-requiring bacteria and yeasts. The antibacterial activity of avidin is also found in *Escherichia coli*, *Klebsiella pneumoniae*, *Serratia marcescens*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Staphylococcus epidermis* (Mine, Y. and Kovacs-Nolan, J. 2004).

Ovomucin

Egg white ovomucin is a macromolecular and glycosylated glycoprotein containing a peptide-rich subunit and a carbohydrate-rich b subunit. Ovomucin prevents the spread of bacteria by performing physical activity in the egg, such as by controlling the structure and viscosity of the egg white protein. It also shows many chemical uses.

Cystatin

Egg white cystatin belongs of the cystatin 'super family', which are type 2 cystatins that inhibit most cysteine proteases, including ficin, papain, and cathepsins B, C, H and L (Li-Chan, E. and Nakai, S. 1989). It has two disulfide bonds, but no carbohydrates.

Egg white cysteine protease inhibitor has been shown to have antibacterial properties, inhibiting the growth of bacteria that cause infection like group A *streptococci*, *Salmonella typhimurium* and *Porphyromonas gingivalis*. Peptides from cystatins can also inhibit the growth of *Porphyromonas gingivalis* (Mine, Y. and Kovacs-Nolan, J. 2004).

Ovomacroglobulin (ovostatin)

Also known as ovalstatin, ovomacroglobulin is a glycoprotein consisting of four subunits linked in pairs by disulfide bonds. Due to its protease inhibitory effect, the antibacterial activity of ovomacroglobulin against *Serratia marcescens* and *Pseudomonas aeruginosa* has been confirmed in vitro and in vivo, and has been shown to reduce bone resorption and promote good wound healing in a rabbit model with try keratitis. Ovomacroglobulin has also been shown to promote healing in mice by increasing tissue fibroblast growth, collagen deposition, and capillary formation. It has also demonstrated many other biological functions,

including protease inhibition by ovomacroglobulin, inhibition of *Pseudomonas aeruginosa* and *Vibrio vulnificus* sepsis due to inhibition of kininogenic proteases, and inhibition of the inflammatory protease medullasin in vitro.

2.4.4 Wound Healing Properties of Egg White

Although the scientists did not study egg whites role in wound healing topically by reducing inflammation of wounds, they did study how egg whites were affected when people ate eggs. Information from these studies may help us consider whether egg whites have similar benefits. So far we've seen that eating egg whites:

- Causes more inflammation in healthier people and people who have allergic sensitivity to egg whites.
- Reduces pain in obese or diabetic patients.

But don't pull anything more than that. Before we can say anything definitive, we should experiment with the effects of egg white using egg white topically.

2.4.5 Pro- and Anti-inflammatory Properties of Egg White

The wound healing experiment and research on egg whites are not too much. Recent research suggests that egg whites may promote faster wound healing, but more investigation is required to ensure this effect. (Jahani, S. et al., 2019). Another study suggests that egg white may help heal wounds by stimulating skin production (Geng, F., Huang, X. and Ma, M. 2016).

2.5 Gelatin

The matrix of polypeptides which is hydrolyzed from collagen, is 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). The chemical structure and physical appearance of gelatin are shown in **Fig. 2.11** and **Fig. 2.12** respectively.

Improves wound healing by helping to reduce fluid exudation (Tanaka, A., Nagate, T. and Matsuda, H. 2005). Gelatin is considered the best product for regenerative medicine due to its excellent biocompatibility and biodegradability.

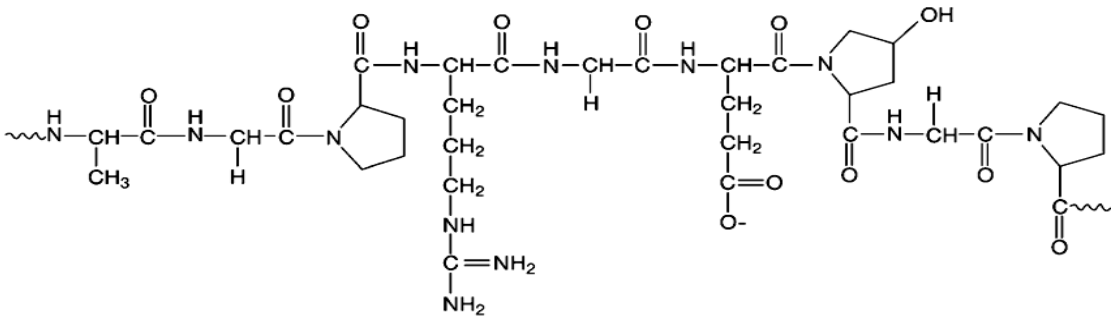

Fig. 2.11: Chemical structure of gelatin (Retrieved from Kommareddy, S., Shenoy, D. B. and Amiji, M. M. 2007).

Gelatin is soluble in water at 37°C, non-immunogenic, and hermaphrodite (Pierce, B. F. et al., 2012). Because of these properties, gelatin-based hydrogels are used in tissue engineering and drug delivery systems. In addition, the mechanical and chemical properties of gelatin can be modified using various crosslinkers such as glutaraldehyde.

Fig. 2.12: Physical appearance of gelatin.

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.

2.6 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 **Fig. 2.13** it has been applied (Kenawy, E. K. et al., 2014).

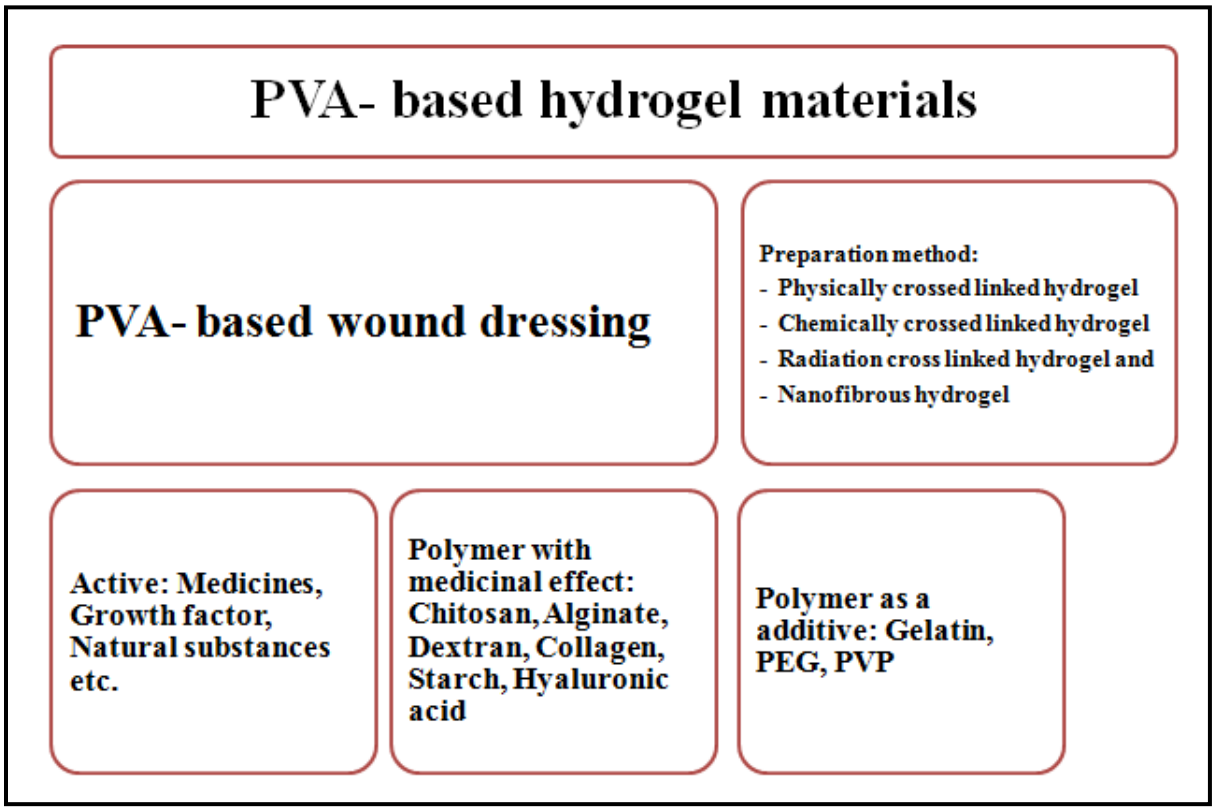

Fig. 2.13: PVA based wound dressing (Retrieved from Jin, S. G. 2022).

PVA is also one of the widely known synthetic polymers that 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. et al., 1994). The physical

appearance and chemical structure of PVA are demonstrated in **Fig. 2.14** and **Fig. 2.15** respectively.

For hydrogel dressing membrane preparations, PVA is one of the most crucial and carried out polymers. It is water soluble, biocompatible, non-toxic, and biodegradable. It possesses a simple structure and chemical amendment is also likewise clean. It is known to show remarkable film-forming capacity and water retention properties (Peppas, N. A. and Merril, E. W. 1977).

Fig. 2.14: Physical appearance of PVA.

The crosslinking agent glutaraldehyde is carried out to gelatin polymer with chemically crosslinked PVA for biomedical research (Pal, K., Banthia, A. K. and Majumder, D. K.2007). Our attempt was to prepare a hydrogel for bacterial inhibition by esterifying -OH group of PVA with the -COOH group of Gelatin where mupirocin and egg white had been loaded. In this study, PVA/glycerin is used as a supplement for other materials due to its skin healing properties, biocompatibility, curative dermal benefits and swelling properties (Fluhr, J. W., Darlenski R. and Surber, C. 2008).

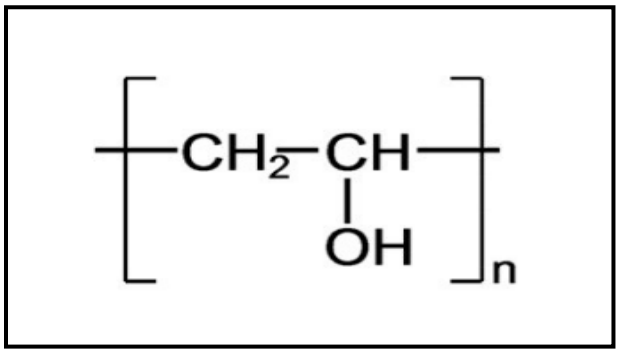

Fig. 2.15: Chemical structure of PVA (Retrieved from Gaaz, T. S. et al., 2015).

CHAPTER 3
MATERIALSAND METHODS

3.1 Materials

Mupirocin was purchased from Sigma Aldrich, Germany. Polyvinyl alcohol (PVA), glutaraldehyde (GA), type B gelatin, and glycerin were bought from Sigma Aldrich, Germany. Throughout the experiments, DW (distilled water) and molecular biology-grade water were used

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. SEM of hydrogel has been experimented with in the Biomedical Engineering Department of BUET. 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
- Measuring scale
- Spatula and other spoon
- Beaker
- Petri-dish
- Micro-pipette
- Standard Weight Bar
- SEM (Scanning Electron Microscope)
- FTIR (Fourier Transform Infrared Spectroscopy)
- Chemical Hood
- pH Meter

3.3 Preparation of Hydrogel

The membrane of the hydrogel dressing was prepared by casting from solution called solution casting method described in Hasan et al with slight modification (Hassan, A. et al., 2017). Briefly, a 10% gelatin solution was prepared by following the ratio of 1.25:1:12.5 for gelatin, glycerin and water by using a hotplate magnetic stirrer with constant stirring. Then the 1% chicken egg solution was dissolved into the gelatin solution. 2% mupirocin were added in 10% PVA solution. Pour the PVA-Mupirocin solution into the gelatin and egg mixture, stirring constantly. Prepare the crosslinker solution by adding 0.5 ml of GA and 0.05 ml of HCl to 10 ml of ethanol and add to the mixture with constant stirring. The solution is poured into a petri dish and the resulting mixture is left at room temperature for 48 hours to form a hydrogel. Keep heating and mixing throughout the process. The temperature was maintained between 50°C to 60°C along the total route.

Four hydrogels dressing materials had been prepared for our experiment, which are:

a) Mupirocin loaded Hydrogel as MH

b) Mupirocin and Chicken Egg White Loaded Hydrogel as MEH

c) Chicken Egg White Loaded Hydrogel as EH

d) Neat Hydrogel as NH

Table 3.1: Composition of prepared hydrogels formula (%)

Hydrogel formula	**Mupirocin content**	**Egg white content**	**PVA content**	**Gelatin content**
MH	2	-	10	10
MEH	2	1	10	10
EH	-	1	10	10
NH	-	-	10	10

3.3.1 MH Dressing Material

To prepare 2% mupirocin hydrogel, 0.2 g mupirocin was added into 10ml distilled water and followed the above process. The prepared hydrogel dressing material was name MH has been presented in **Fig. 3.1**:

Fig. 3.1: MH dressing material (containing 0.2% mupirocin).

3.3.2 MEH Dressing Material

To prepare 2% mupirocin and 1% chicken egg white hydrogel, 0.2 g mupirocin was added into 10ml distilled water, and 1 ml chicken egg white was added into 100 ml distilled water then followed the above process. The prepared hydrogel was named MEH has been presented in **Fig. 3.2**:

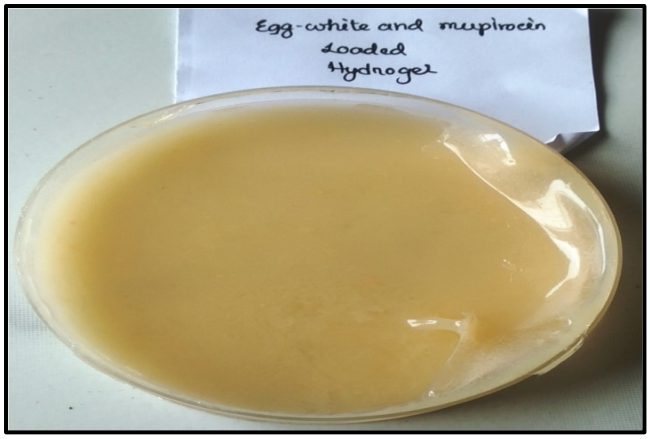

Fig. 3.2: MEH dressing material (2% mupirocin and 1% chicken egg white).

3.3.3 EH Dressing Material

To prepare 1% chicken egg white hydrogel dressing material, 1 ml chicken egg white was added into 100 ml distilled water and followed the above process. The prepared hydrogel was named EH has been presented in **Fig. 3.3**:

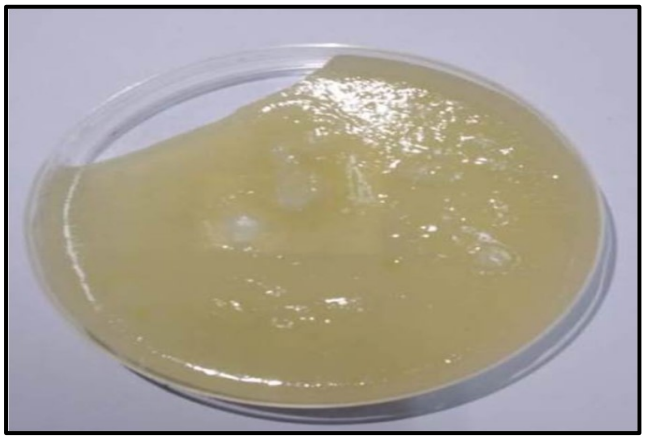

Fig. 3.3: EH Dressing Material (containing 1% chicken egg white)

3.3.4 NH Dressing Material

(No Mupirocin & chicken egg white, only PVA and Gelatin): To prepare control hydrogel, neither mupirocin nor chicken egg white was added, only following the above process without adding mupirocin and chicken egg white. The prepared hydrogel named Neat Hydrogel has been presented in **Fig. 3.4**:

Fig. 3.4: NH Dressing Material (contain only PVA & Gelatin)

3.4 Characterization

The prepared hydrogel was characterized by swelling behavior, moisture retention capability, pH determination, gel fraction, spreadability measurement, porosity, drug compatibility study: FTIR analysis, SEM analysis, and evaluation of bacterial inhibition activity. All are described below:

3.4.1 Swelling Behavior

Measure the swelling behavior of the hydrogel dressing film by wetting the hydrogel in Water. The swelling was measured by cutting the hydrogel dressing membrane and weighing it. Then, the hydrogel membrane was soaked in water for 2 and 4 hours. After a certain period of time, the hydrogel membrane was removed from the water, and the hydrogel surface was dried with filter paper to wipe off water droplets and weighed again. Experiments were performed at room temperature. Calculate the swelling behavior using the formula below (Pal, K., Banthia, A. K. and Majumdar, D. K., 2007).

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

Where,

W_s= weight of swelled membrane and W_d= weight of the dry membrane.

3.4.2 Moisture Retention Capability

To measure water retention, the hydrogel dressing membrane was cut into equal pieces and weighed, and placed in an oven at 45 °C for 5 h. Calculate the water holding capacity using the formula below (Roy, N. et al., 2011):

$$Moisture\ retention\ capability\ (\%) = \frac{W_f}{W_i} x\ 100$$

Where,

W_f= weight before placing in an oven and W_t= weight after 5h

3.4.3 Folding Endurance

The fold resistance of the hydrogel was determined by repeatedly folding in the same position until one patch broke, or bending up to 300 times without breaking (Patel, V. M., Prajapati, B. G. and Patel, M. M. 2007). Average values were recorded. The number of times the film can be folded in the same position without tearing determines the value of the folding durability

3.4.4 Water Vapor Transmission Rate (WVTR)

To measure WVTR, take a 1.14 cm mouth test tube and put 10 ml of molecular biology grade water in it. The open tubes were wrapped with a hydrogel film and sealed with teflon tape. Measure weight of the tubes and put them in the oven at 35 °C for one day (24 hours). After 24 hours, the tubes were removed from the oven and weighed again. Calculate the water vapor transmission rate (WVTR) using the formula below (Boonkaew, T.A.B. et al., 2014):

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

Where,

A= area of the round mouth of the test tube; W_i= initial weight of test tube and W_f=final weight of test tube

3.4.5 pH Determination

Weigh out 1 g of each hydrogel and mix with 25 ml of distilled water. The pH of the mixture was measured using a calibrated pH meter (Orion Research, Inc., USA) with buffer solution 7.0 before each use. Experiments were performed in triplicate and mean values were calculated.

3.4.6 Gel Fraction

The prepared hydrogel film is cut into uniform pieces and then placed in a vacuum oven to wait for constant weight. The hydrogel film was then weighed. Then the hydrogel membrane was kept in distilled water for 4 days. The film of this hydrogel wrap was removed from the

water after 4 days and placed back in the oven to achieve a constant weight. Calculate the gel fraction using the following formula below (Hago, E. E. and Li, X.2013):

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

Where,

W_i= weight after placing in a vacuum oven and W_f= weight when the wet samples were dried in a vacuum oven.

3.4.7 Spreadability Measurement

The hydrogel was determined by pressing 0.5 g of the hydrogel onto two horizontal plates (20 x 20 cm), then put a sample weight of 10 g (standard weight bar) on the top plate and left for approximately 5 minutes, so that it would not spread any further was expected (Reham, F. E. et al., 2015). The diameter of the extended circle is measured in millimeters and the spreadability value is obtained by comparing it. The results obtained are the average of three determinations.

3.4.8 Porosity Evaluation

Following the liquid displacement method, the porosity (P) was calculated by using the formula below (Dhasmana, A., Singh, L., Roy, P., Mishra, N. C. 2018):

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

Where,

S_1= initial volume;

S_2= volume after the sample soaking;

S_3= volume after removal of sample and

P= porosity

3.4.9 Drug Compatibility Study: FTIR Analysis

FTIR (Shimadzu, IR Prestige-21 PC) was used to measure the main groups, chemical structures, elements, and structural changes in the hydrogel. The FTIR value of the hydrogel film varies between 4000 cm^{-1} to 400 cm^{-1}.

For hydrogel dressings (NH, MH, MEH, and EH), properly dry in an oven for 48 hours to pulverize. Then, pellets containing 1/10 KBr (infrared level) with powder hydrogel were prepared by pressing and the pellets were put into the FTIR machine for testing. All tests were performed at room temperature.

3.4.10 SEM Analysis

Hydrogels were freeze-dried before being examined by SEM. The experiments were carried out using FESEM (Field Emission Scanning Electron Microscopy) (Model: ZEISIS, Germany) on the surface of the hydrogel film. Before testing, samples were covered with a thin layer of gold. Use a spray coater to create this process.

A second generator with 5 kV acceleration was used to observe the hydrogel surface. Analysis of surface patterns of hydrogel dressing films by SEM.

3.4.11 Evaluation of Bacterial Inhibition Activity

Determination of the antibacterial activity of hydrogels against *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* followed by disc diffusion method (Bauer, A. W. et al.,1966; Diem, L. N. et al., 2023).

PVA-gelatin hydrogel (NH) was used as a control. Test patterns are MH, MEH, and EH. The samples were placed on MacConkey agar medium inoculated with *Escherichia coli* and MHA agar medium inoculated with *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The zone of inhibition was calculated after 24 hrs of incubation at 37°C temperature. After incubation, antibacterial activities were evaluated according to the developed area of inhibition.

CHAPTER 4
RESULTS AND DISCUSSIONS

4.1 Swelling Behavior

The human body's blood contains as much as 90% of the water, and the astounding swelling properties of the hydrogels make a gel absorb an enormous amount of water in the blood to assist in stopping bleeding. The magnitudes of swelling ability act as a determining factor to release drug that may be encapsulated in the hydrogel and to absorb the exudates at the wound site (Satish, A. et al., 2019).

The swelling ratio for Neat hydrogel (NH) has been noted at 260% and 350%, for MH it has been noted at 130% and 180%, for EH it has been noted at 120% and 170% and for MEH it has been noted at 110% and 160% after 2 hr and 4 hr respectively. The swelling ratio of hydrogel dressing materials (MH, MEH, EH, and NH) is shown in **Fig. 4.1**.

The swelling index in the NH is quite high compared to other formations. In the neat hydrogel, the components are only PVA and Gelatin. The mechanism that causes this hydrogel polymer swelling in aqueous media can be described in two different ways: Firstly, the water solubility of gelatin, which increases the water affinity in film and its high amount in the film matrix (Ghaderi, J. et al., 2019). Secondly, swelling may also occur due to repulsion among the adjacent amines in gelatin molecules that become positively charged in water and could result in an increased swelling ratio (Pal, K. et al., 2007).

In water, compared with the NH all other hydrogel dressing materials also showed a good swelling result. The swelling ratio was reduced with present of Mupirocin in MH then egg white in EH and finally both in MEH.

It might very well be that both mupirocin and egg white interfere with the water affinity for gelatin and they also interfere gelatins to become positively charged that makes the decreased swelling ratio.

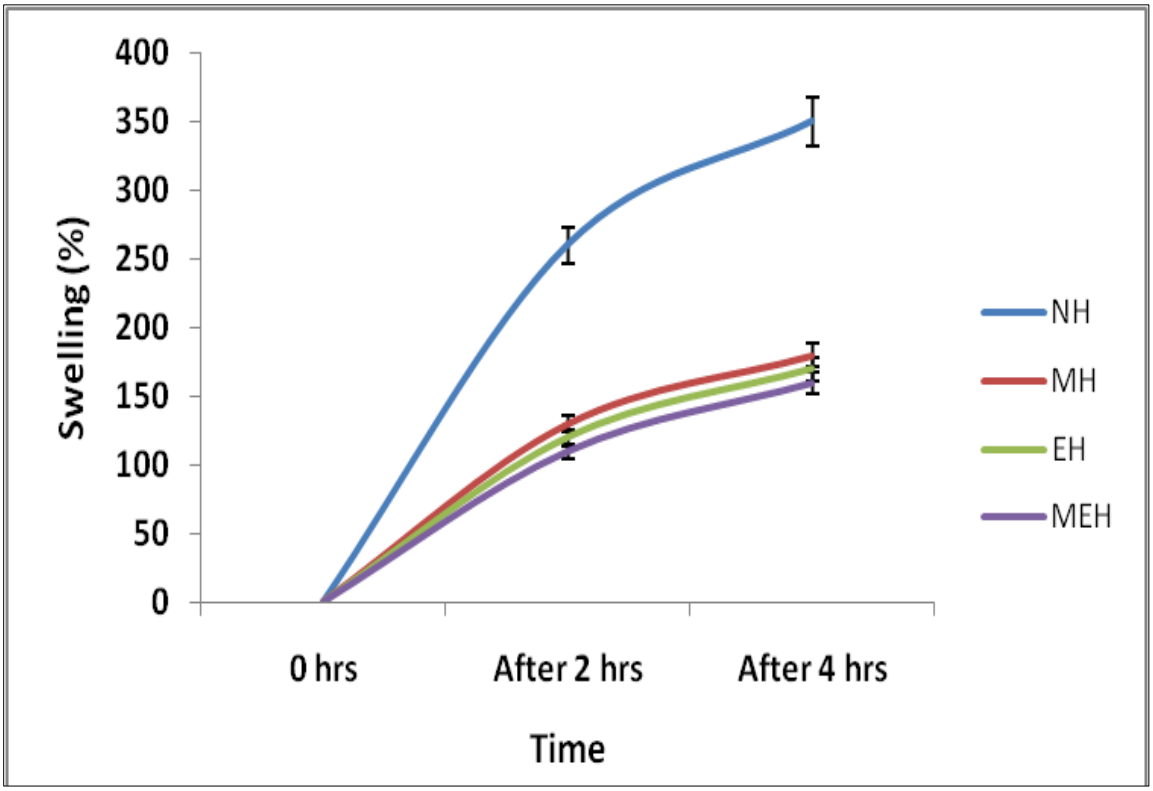

Fig. 4.1: Swelling behavior of hydrogels in water.

The porous surface of hydrogel has a big surface area that allows absorbing water rapidly and widely. NH has a more porous surface than another prepared hydrogel, so the swelling ratio was highest among all hydrogels.

The lowest swelling ratio was reported for MEH, because the addition of both mupirocin and egg white reduced the porous surface of hydrogels and made the tighter structure formation.

4.2. Moisture Retention Capability

The highest moisture rate was recorded for MEH at 40.68%, then MH at 30.93%, then NH at 22.16%, and the lowest recorded for EH at 16%. The moisture retention rate of hydrogels and image after lost moisture from hydrogel are shown in **Fig. 4.2** and **Fig. 4.3** respectively.

Excessive water loss from the wound can cause the body to shrink, resulting in decreased body temperature, and increased cell metabolism finally leading to cell death (the main

component of the body is water so the cell environment is also moist) (Hoffman, A. S. 2002; Bryan, J. 2004).

On the other hand, excess moisture can increase the risk of bacteria and bad odor. Therefore, the best dressing needs to have a good moisture content of the wound, which is very important for wound healing. In this study, we found that the moisturizing ability of EH is too less. (Chicken egg white hydrogel) which was only 16%. After the addition of mupirocin, the moisture retention capacity increased to 30.93% for MH but addition of both mupirocin and egg white the rate was 40.68% noted for MEH in **Fig. 4.2**.

It might be the antibiotic mupirocin somehow increases the water retention capacity of the hydrogel since mupirocin added hydrogels are showing the highest water retention capacity.

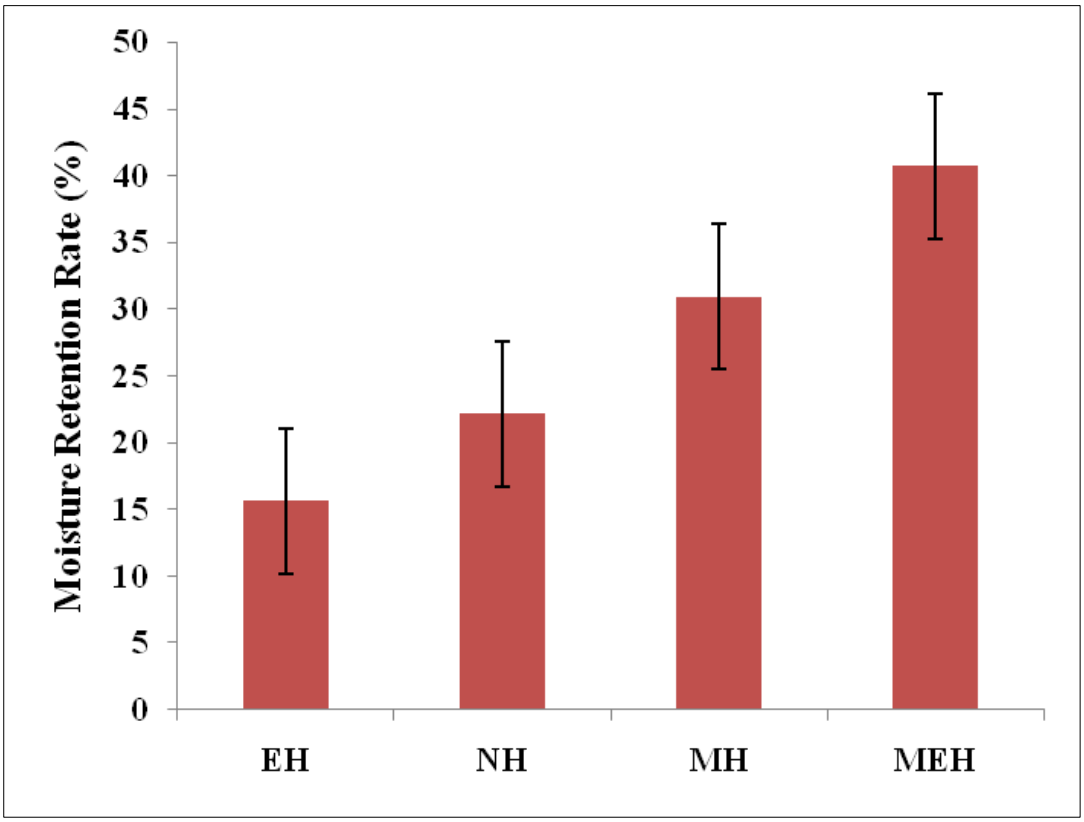

Fig. 4.2: Moisture retention capability of prepared hydrogels.

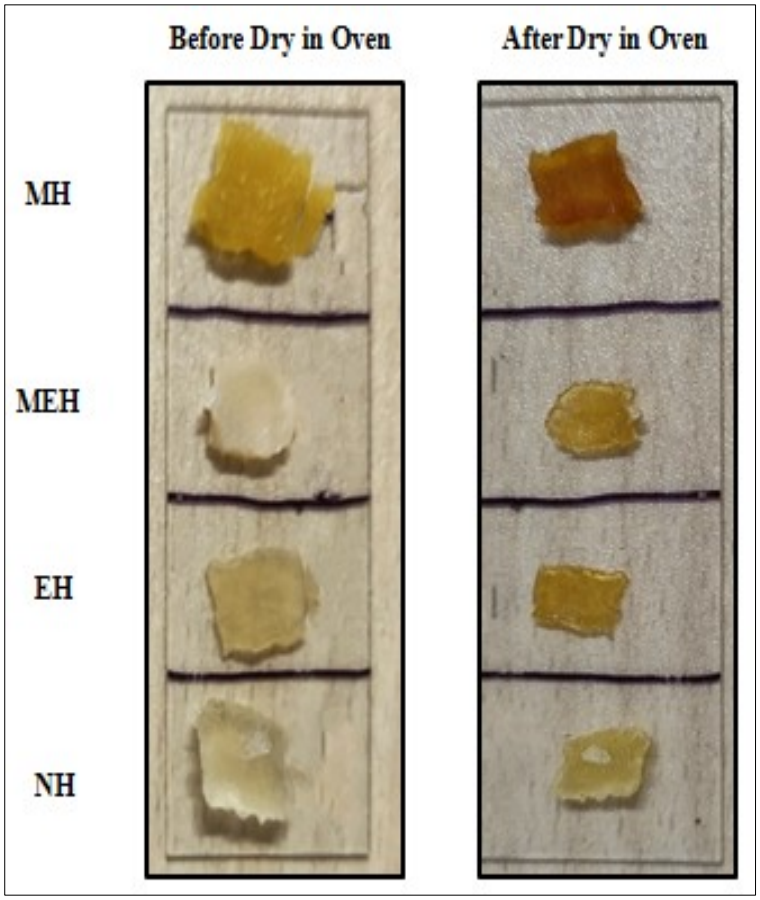

Fig. 4.3: Moisture retention capability of hydrogel (left: before dry, right: after dry).

4.3. Folding Endurance

The test was performed to find the flexibility of hydrogel which is needed to handle the dressing easily and comfortably for secured application of hydrogel on the wound. More than 300 times folding endurance was recorded for NH and MEH, whereas more than 250 times for MH and more than 200 for EH were observed. No films broke when they were folded around 200 times in the same position and with the same strength. Therefore, we can enunciate that the hydrogels showed good physical condition. **Table 4.1** revealed the folding endurance of all prepared hydrogels.

Table 4.1: pH, folding endurance, and spreadability of prepared hydrogels

Formula	Folding endurance	pH	Spreadability (mm)	Egg white content (ml)	Mupirocin content (g)	General appearance
NH	>300	5.3	5	0	0	Transparent
MH	>250	5.6	6	0	0.2	Transparent
MEH	>300	6.5	8	1	0.2	Transparent
EH	>200	6.6	4	1	0	Transparent

4.4 Water Vapor Transmission Rate

Optimal WVTR is the main tool of effective and efficient dressings to prevent dehydration and slow accumulation of exudates in the wound. PVA and gelatin are hydrophilic polymers. As a result, they can absorb more water, but the crosslinking of the gelatin chains reduces the absorbent capacity (Wang, L., Xue, J. and Zhang, Y. 2019) because crosslinking reduces the free space in the polymer matrix, which creates a thick film structure. it causes a decrease in WVTR by affecting the diffusion between water molecules (Dammak, I., Lourenço, R. V. and Sobral, P. J. A. 2019).

Therefore, WVTR in fabricated hydrogels was measured in this study. In **Fig. 4.4**, the WVTR was maximum for NH, which is 98.35 $gm^{-2}h^{-1}$, on the other hand, the lowest rate was noted for MEH, which is 47.76 $gm^{-2}h^{-1}$ among prepared hydrogel dressing. So, the presence of mupirocin and egg white caused the reduction in the WVTR. Mupirocin incorporated hydrogel (MH) showed 55.28 $gm^{-2}h^{-1}$ WVTR. Whereas egg white containing hydrogel (EH) 50.34 $gm^{-2}h^{-1}$ WVTR. Analysis shows that it is mainly mupirocin that reduces the WVTR in the hydrogel. However, egg white also showed some effect in this reduction.

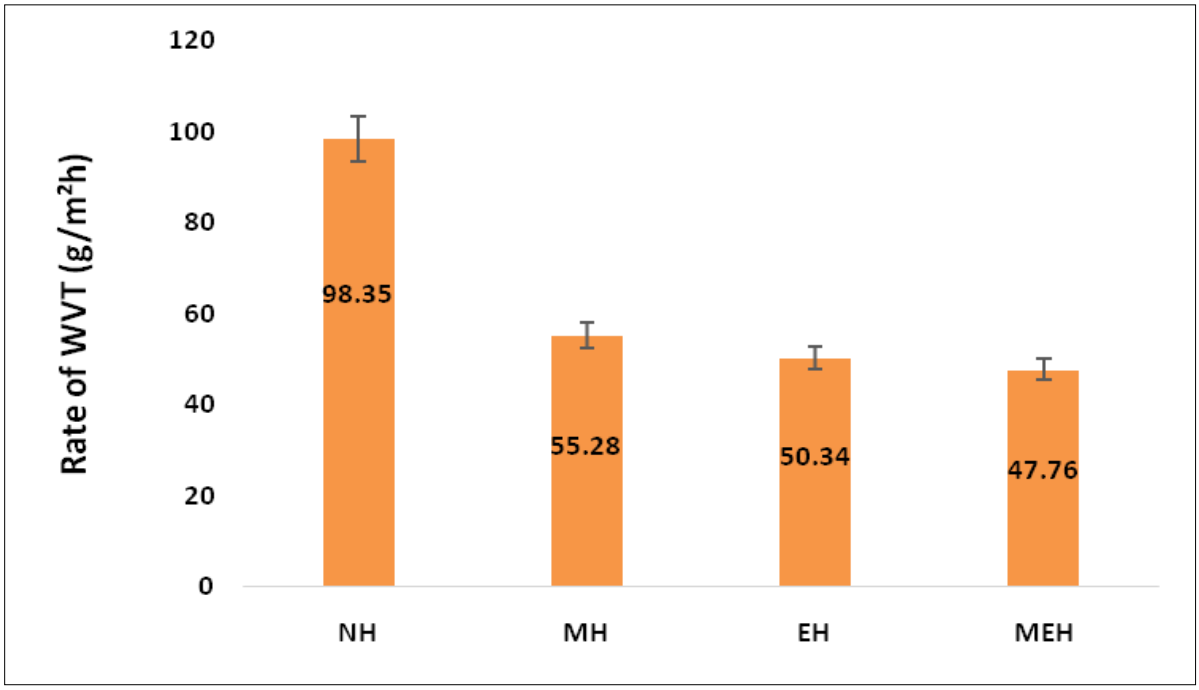

Fig. 4.4: WVTR of prepared hydrogels

Other published articles mentioned that a WVTR value that is too high may cause the wound to dry quickly, leading to scarring, while a WVTR value that is too low may cause exudate to accumulate, increasing the risk of infection and delaying treatment. (Killion, J. A. et al., 2011). Therefore, for an ideal hydrogel dressing, the WVTR value should not be large or small and the wound moisture should be kept at the best value.

In this study, the WVTR value of MH, MEH & EH is comparable to commercially available Metalline wound (Lohmann & Rauscher International GmbH & Co. KG, Germany) dressings which have been reported to have 53 $gm^{-2}h^{-1}$ WVTR value (Razzak, M. T. et al.,2001). The found values are also compared with WVTR values of 35- 56 $gm^{-2}h^{-1}$ for PVA-Clay nano composite hydrogels used in wound dressings (Kokabi M., Sirousazar M. and Hassan Z. M. 2007). Based on the data mentioned in (Razzak, M. T. et al., 2001), The WVTR of some commercial dressings ranged from 33 (Op site) to 208 (Omidderm) g/m^2h, indicating that the WVTR of the hydrogel produced in this study was of a suitable variety for wound healing. According to (Razzak, M.T. et al., 2001), higher WVTR values lead to drying of the wound

surface, while lower WVTR values result in a higher risk of wound exudate accumulation and disease growth.

4.5 pH Determination

The pH of all hydrogels were within satisfactory limits of 5.6 to 6.6 (**Table 4.1**) and hence no skin irritation would take place if these hydrogels were used in vivo (Helal, D. A. 2012). All values lie in the normal pH of human skin 5.5. These results identified were suitable for topical application owing to the acceptable pH measurements.

Acidic pH creates an unfriendly environment for bacterial growth (Lund, P. A. et al., 2020) and a friendly environment for oxygen release (Mao, N. and Russell, S. J. 2004). So lower pH helps to bacteria inhibition and more oxygen release helps to repair damaged tissue.

4.6. Gel Fraction

The gel fraction represents the crosslinking properties of the hydrogel. Gel fraction was highest for EH, which is 84.12% and lowest for MH, which is 52.35%. Other two formations that are NH and MEH showed Gel fraction that are 82.53% and 83.93%, respectively.

NH, MEH, and EH were observed to show similar gel fraction values whereas MH showed a lower value. It seems the addition of mupirocin interferes with the crosslinking of PVA-Gelatin whereas the addition of egg white somehow recued the crosslinking inhibiting effects of mupirocin. The mechanism by which egg white recued this function is yet to be studied. On the other hand, egg while alone showed no effect when compared to NH hydrogel. This confirms egg white somehow impedes the inhibitory effect of mupirocin. However, gel fraction value of all hydrogel dressing except MH showed proper crosslinking efficiency.

In a study reported by Kim, J. O. et al (2008) and Ajji, Z., Othman, I., Rosiak, J. M. (2005), as the gel fraction decreased, the flexibility of hydrogel also decreased. In this study, MH was found to be less flexible as it showed the lowest value in this experiment (the comparative results are given in **Fig. 4.5**).

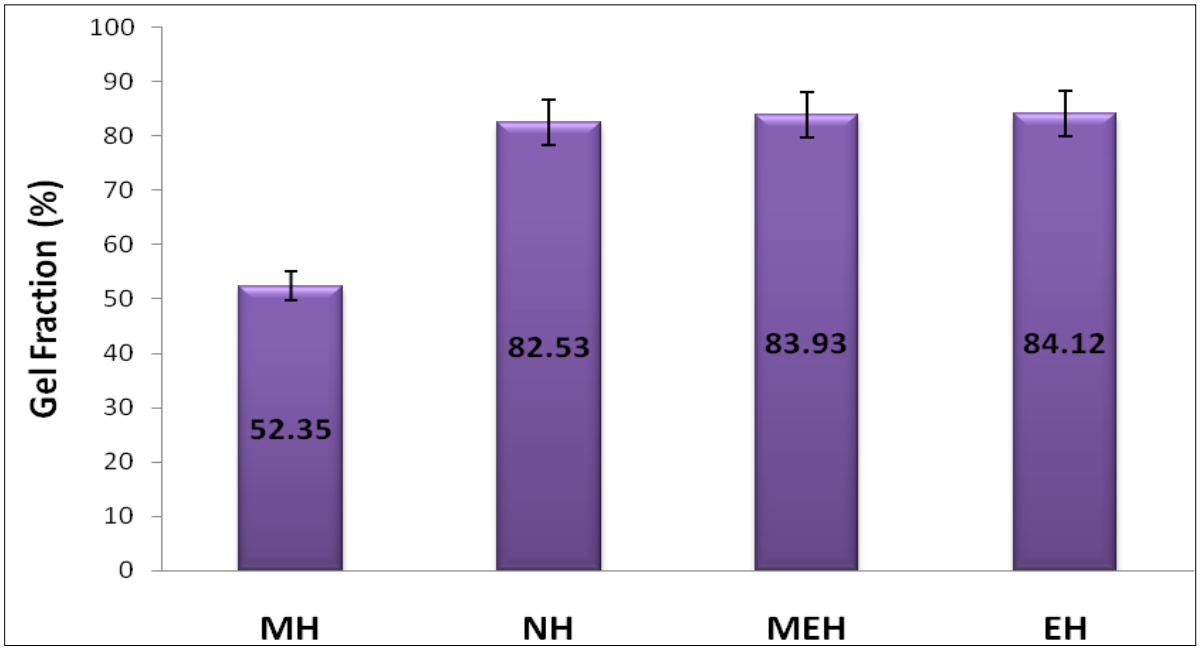

Fig. 4.5: Gel fraction of hydrogels.

4.7. Spreadability Measurement

Spreadability is important for patient compliance and helps to spread the gel evenly across the skin. The highest spreadability of formulation was found to be 8 mm which was MEH as shown in **Table 4.1**. Spreadability for NH, MH, and EH was 5 mm, 6 mm, and 4 mm respectively.

The spreadability of hydrogel is important for homogenous administration of the hydrogel dressing on the skin. So, the prepared gel must meet the best conditions for good spreading and drug use which are important factors for patient compliance. Good spreading is one of the criteria for the gel to achieve the desired effect.

4.8 Porosity

Hydrogels for wounds should be permeable to water vapor, gases, and small protein molecules, but not microorganisms (Weller, C. and Sussman, G. 2006). Moreover, the porous network may allow for the solvent to flow simultaneously by convection and diffusion,

finally reducing the swelling time and enhancing solvent uptake (Ulbricht, M. 2006; Ceylan, D. et al., 2006; Sannino, A. et al., 2003 and 2006; Pradny, M. et al., 2005; Kato, N. et al., 2004). The degree of porosity determines mechanical properties, with the stiffness of the scaffold decreasing as porosity increases (Gerecht, S. et al., 2007). The porosity, pore architecture, and pore interconnectivity play a pivotal role in cell survival, proliferation, and migration to fabricate functional hydrogel and secrete ECM (Mandal, B. and Kundu, S. 2009; Lien, S. M., Ko, L. Y. and Huang, T. J. 2009). Another article also showed that pore interconnectivity allows for cell in growth, vascularization, and nutrient diffusion for cell survival (Griffon, D. J. et al., 2006; Kim, H. J. et al., 2005; Roy, T. D. et al., 2003). The extent of ECM secretion also increases by increasing the pore size (Lien, S. M., Ko, L. Y. and Huang, T. J. 2009).

Furthermore, the presence of an interconnected porosity is also strongly required for biomedical purposes because of the need to promote and guide cell infiltration in three dimensions and because of the improvement of the sequestration and release of bioactive moieties (Drury, J. L. et al., 2003; Sannino, A. et al., 2006).

The porosity of the control gel NH was 78%. After the incorporation of 1% egg white, it was reduced to 60% for EH, after 2% mupirocin incorporation it was 57.14% for MH and incorporation of both Mupirocin and egg white it was calculated 54.55% for MEH shown in **Fig. 4.6**. The porosity for EH, MH and MEH gradually decrease due to the incorporation of egg white, mupirocin and both together Mupirocin and egg white into PVA and Gelatin respectively. This is because of the incorporation and physical interaction of mupirocin and egg white with PVA and gelatin. An increase in the degree of swelling has been also previously reported for hydrogels as porosity augments (Liu, Q. et al., 2001; Wu, Y. H. 2010).

This is the trend found in the swelling test (**Fig. 4.1**) where the control hydrogel showed the highest swelling behavior as the control hydrogel showed the highest porosity as well. The other three hydrogels showed similar patterns. The density of mupirocin and chicken egg white almost similar 1.2g/cm^3 and 1.03g/cm^3 respectively. For optimal dressing, porosity is important for cell migration, growth and nutrition.

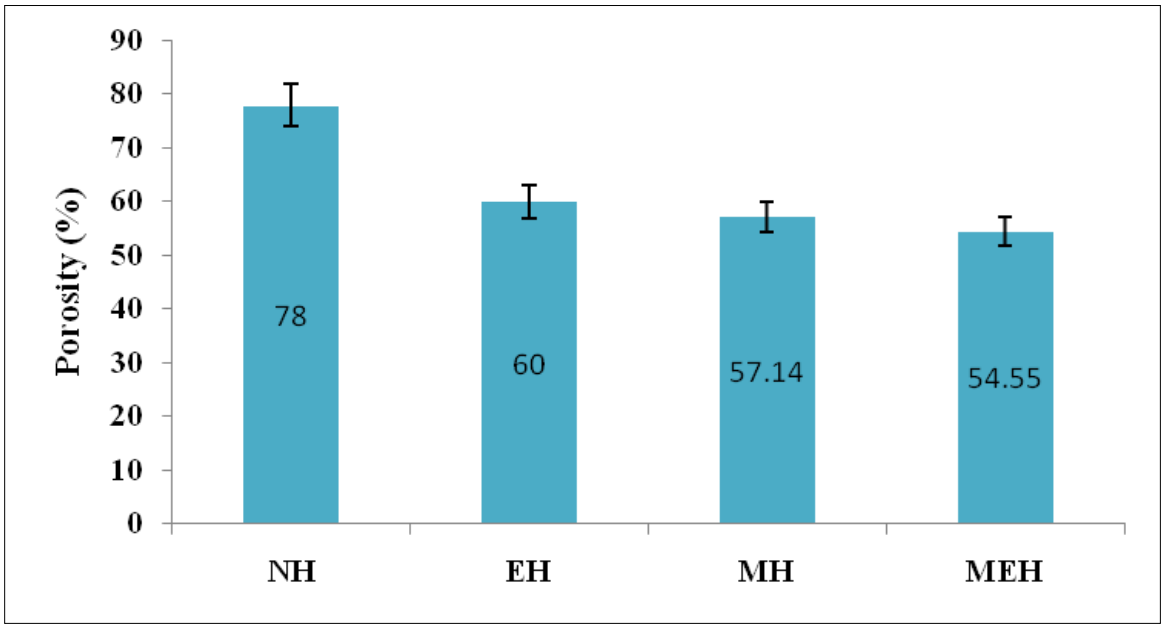

Fig. 4.6: Porosity of hydrogels

On the other hand, density is also important for dressing materials, as it does not allow the penetration of bacteria that may cause microbial infection of the burn wound. Therefore, from these studies, we found that MEH has a porosity of 54.55%, which is neither very porous nor dense compared to pure hydrogel (NH) and other hydrogel dressing materials (EH and MH). It is necessary for cell movement, growth, and nutrition and is also good for bacteria inhibition, helping to absorb excess water and exudates.

4.9 Drug Compatibility Study: FTIR Analysis

The spectrum of mupirocin-loaded hydrogel dressing material (MH) presented in **Fig. 4.7** displays the characteristics of absorption bands: 3012.142 cm^{-1} characterized the stretching mode O-H group, 1645.947.94 cm^{-1} characterized the stretching mode of C=C and C=O; 1559.13 cm^{-1} characterized the stretching mode of $-NO_2$, 1419.352 cm^{-1}characterized C-O-H bending, 1045.229 cm^{-1} characterized the stretching mode of C-O and C-F group.

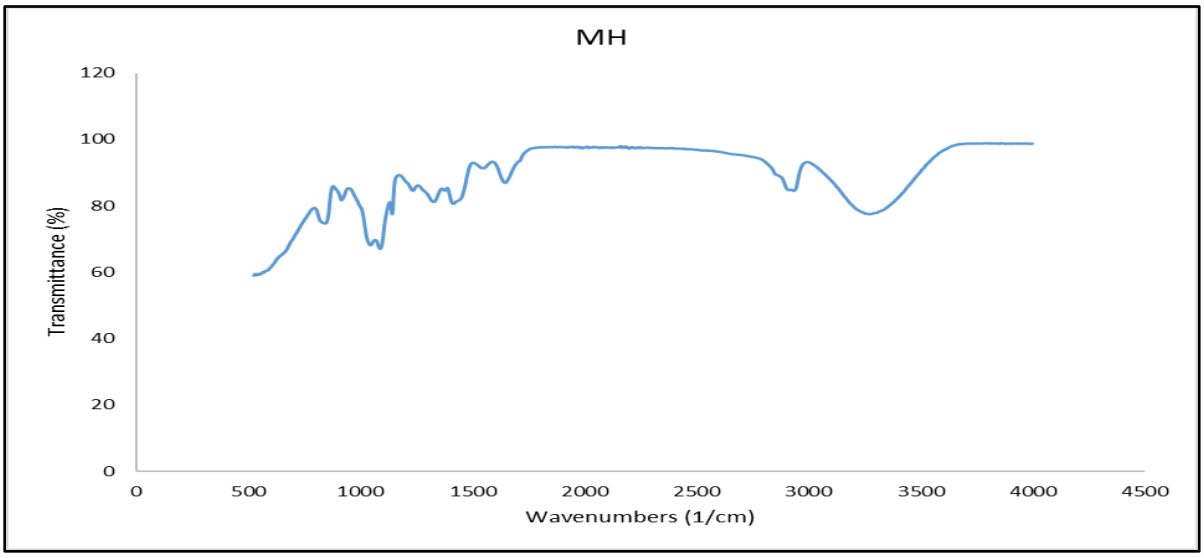

Fig. 4.7: FTIR spectra of mupirocin hydrogel dressing material (MH).

The spectrum of egg white loaded hydrogel (EH) given in **Fig. 4.8** displays the characteristics absorption bands: 3344.444 cm^{-1}represented to the stretching mode of O-H, 1635.822 cm^{-1} corresponded the stretching mode C=C, C=O and N-H; 1165.276 cm^{-1} corresponded the stretching mode of C-F, C=O and C-O; 1046.193 cm^{-1} corresponded the stretching mode of C-F and C-O.

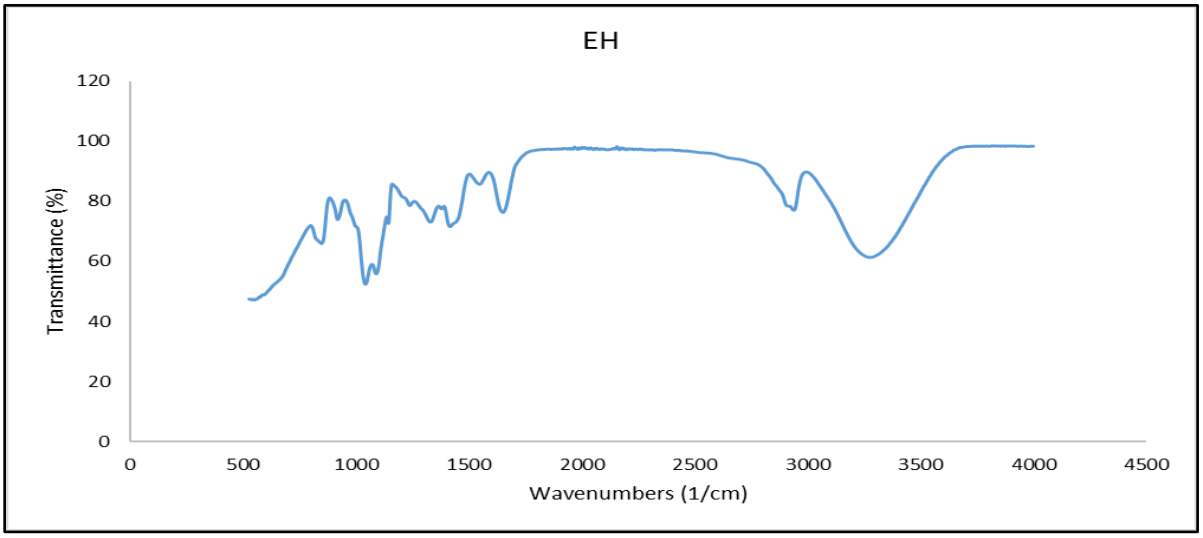

Fig. 4.8: FTIR spectra of chicken egg white-loaded hydrogel (EH).

The spectrum of mupirocin and egg white loaded hydrogel (MEH) given in **Fig. 4.9** displays the characteristics absorption bands: 3334.32 cm^{-1}represented to the stretching mode of O-H, 1635.933 cm^{-1} corresponded the stretching mode C=C, C=O and N-H; 1416.459 cm^{-1} corresponded the stretching mode of C-O-H; 1091.512 cm^{-1} corresponded the stretching mode of C-F and C-O.

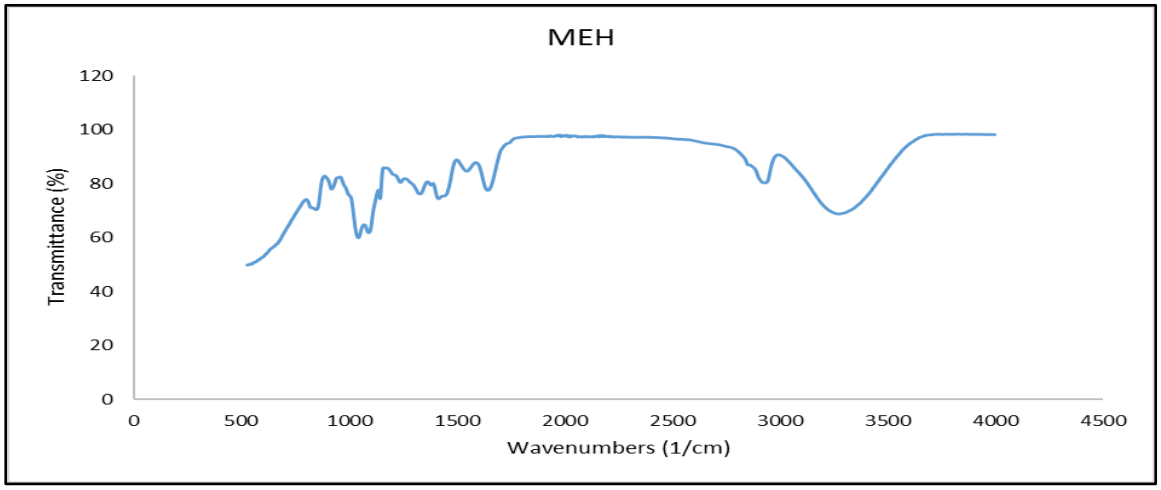

Fig. 4.9: FTIR spectra of mupirocin and chicken egg white-loaded hydrogel (MEH).

The spectrum of neat hydrogel (NH) given in **Fig. 4.10** displays the characteristics absorptions bands: 3323.713 cm^{-1}represented the stretching mode of O-H, 1642.572 cm^{-1} corresponded the stretching mode C=C, C=O; 1433.321 cm^{-1} corresponded the stretching mode of C-O-H; 1104.113 cm^{-1} corresponded the stretching mode of C-F, C=O and C-O.

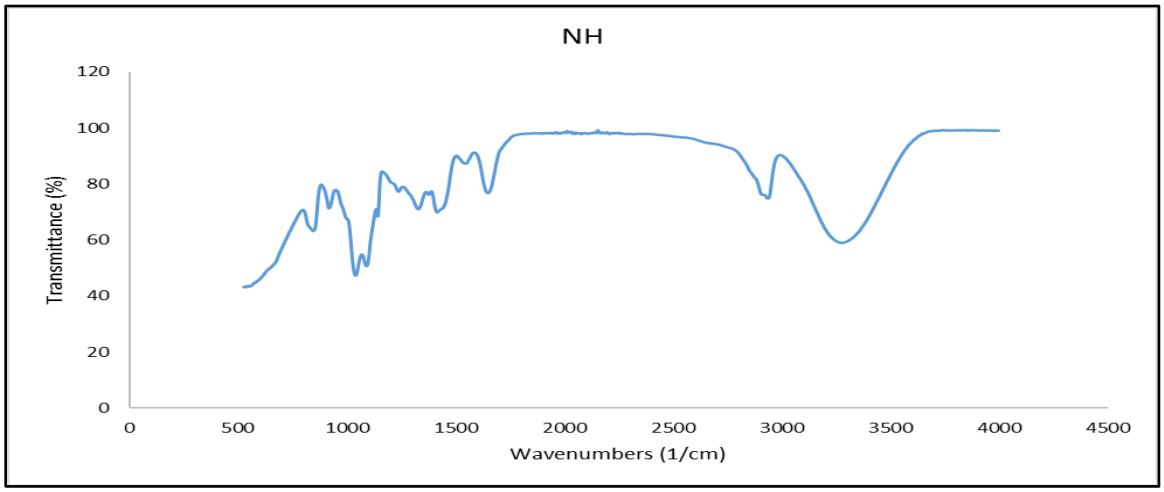

Fig. 4.10: FTIR spectra of neat hydrogel (NH).

All FTIR peak data from prepared hydrogel indicates that the drug is compatible with the polymer used in the formulations as shown in **Table 4.2**.

The compatibility study showed that the major peaks in Fourier transform infrared spectroscopy (FTIR) spectra of the pure drug (mupirocin and egg white) were found to be intact in their physical mixture. Hence there is no interaction between drug and polymer in their physical mixture.

Table 4.2: FTIR spectra of prepared hydrogels dressing materials

Hydrogel	Wavenumber (1/cm)	Functional group
MH	3312.142	O-H
	1645.947	C=C & C=O
	1559.13	$-NO_2$
	1419.352	C-O-H
	1045.229	C-O & C-F
EH	3344.444	O-H
	1635.822	N-H, C=C & C=O
	1165.276	C-O, C=O & C-F
	1046.193	C-O & C-F
MEH	3334.32	O-H
	1635.933	N-H, C=C & C=O
	1416.459	C-O-H
	1091.512	C-O & C-F
NH	3323.713	O-H
	1642.572	C=C & C=O
	1433.321	C-O-H
	1104.113	C-O, C=O & C-F

4.10 SEM Analysis

Figure 4.11 demonstrates the surface morphology of prepared hydrogel by SEM analysis. In this experiment, NH revealed a smooth break surface with some particles which may be gelatin particles remaining insoluble in neat hydrogel. SEM analysis of EH hydrogel showed a homogenous and smooth break surface with irregularly shaped visible particles in the hydrogel. Some particles looked like leaf-shaped some were rectangular and some were squared-shaped.

It also exhibited layered and flaky microstructures but no crack surface was found. MH hydrogel was found to be smooth and homogenous surface with visible dot like spherical shaped particles. The dot like spherical shaped visible particles indicated the incorporation of mupirocin into this hydrogel. MEH hydrogel which composed of mupirocin and egg white displayed a rough surface.

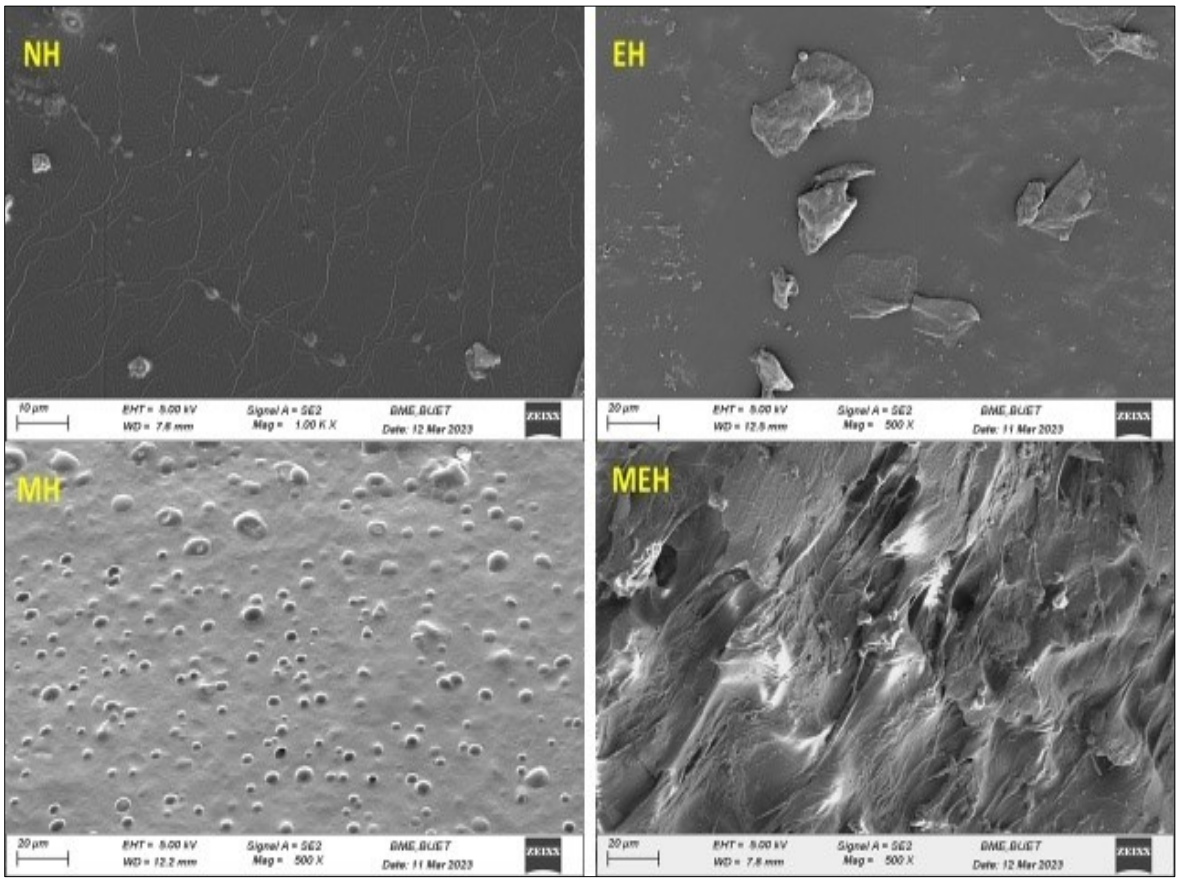

Fig. 4.11: SEM of prepared hydrogels.

4.11 Evaluation of Bacterial Inhibition Activity

The antibacterial activity is a key requirement for a hydrogel if designed to be used as skin dressing material to prevent bacterial infection as a major contributor to delaying the wound healing process (Perumal, S. et al., 2014; Daghdari, S. G. et al.,2017).

Antibacterial activity of MH, MEH, EH and NH hydrogel dressing materials were investigated against *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*, which are the common pathogens associated with acute wounds.

There was zone of inhibition observed against all bacteria by MH, EH and MEH. But no zone of inhibition was found for neat hydrogel (NH). This is because NH did not have either the antibiotic mupirocin or chicken egg white. Inhibition zones against all experimented bacteria like *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* are demonstrated in **Fig. 4.12**, **Fig. 4.13**, **Fig. 4.14**, **Fig. 4.15**, **Fig. 4.16,** and **Fig. 4.17**.

A sample of the zone of inhibition of *Staphylococcus aureus* regarding this study is given in **Fig. 4.12** and its comparative sizes are given in the **Fig. 4.13**.

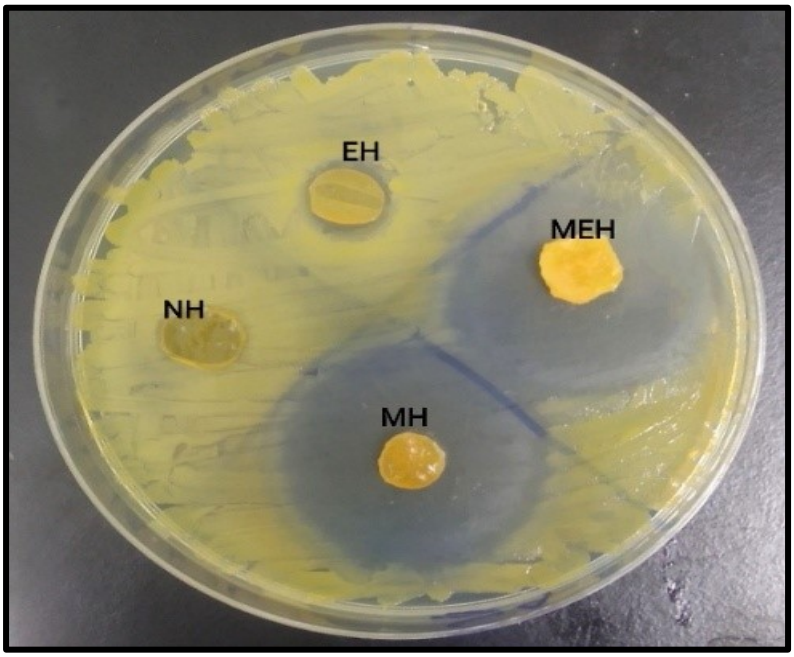

Fig. 4.12: Bacterial growth inhibition of hydrogel against *Staphylococcus aureus*.

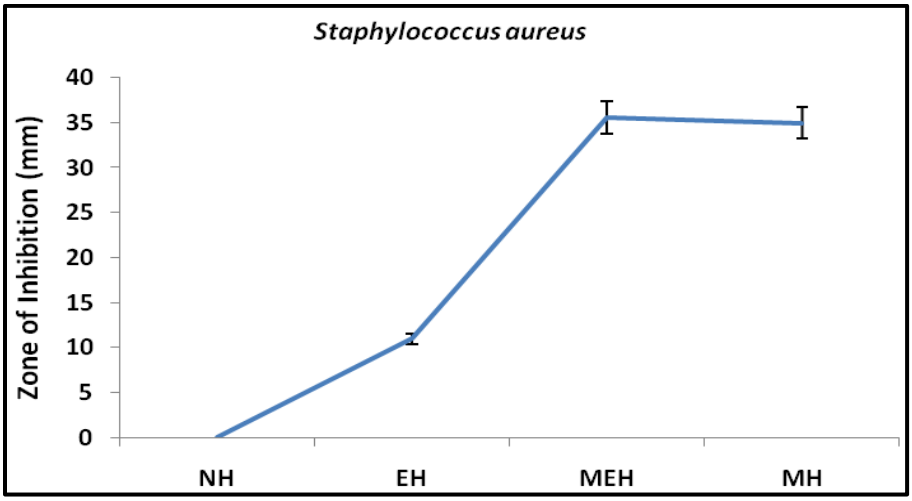

Fig. 4.13: Statistical graphical diagram of hydrogel against *Staphylococcus aureus*.

A sample of the zone of inhibition of *Escherichia coli* regarding this study is given in **Fig. 4.14** and its comparative sizes are given in the **Fig. 4.15**.

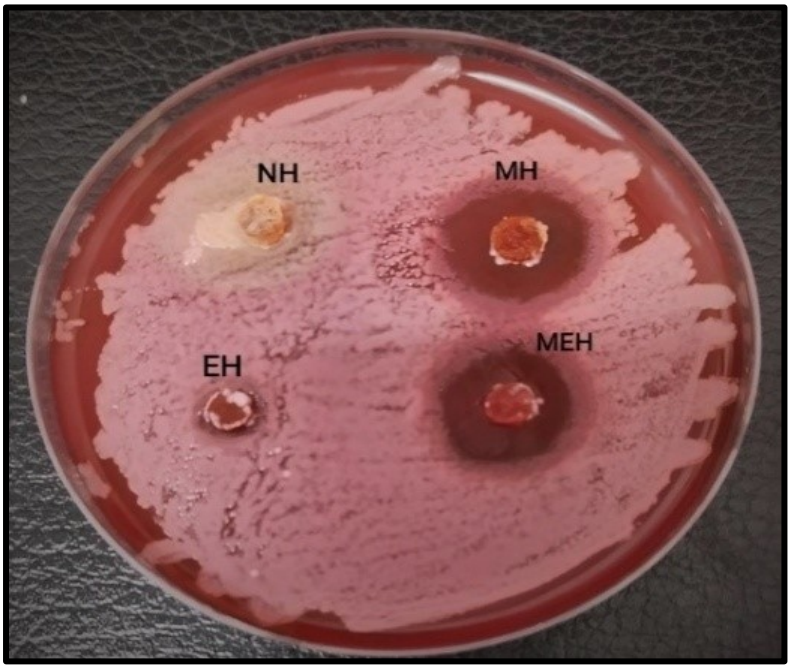

Fig. 4.14: Bacterial growth inhibition of hydrogel against *Escherichia coli*.

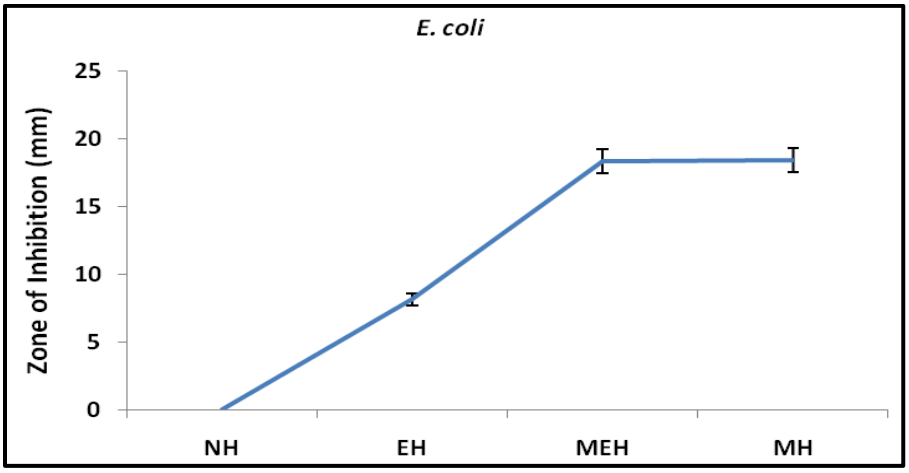

Fig. 4.15: Statistical graphical diagram of hydrogel against *Escherichia coli*.

Obvious zone of inhibition was observed for MEH and MH since mupirocin is strong antimicrobial agent and this result demonstrate that mupirocin activity was not changed due to incorporation in hydrogel (Breneman, D. L. 1990). A sample of the zone of inhibition of *Escherichia coli* regarding this study is given in **Fig. 4.16** and its comparative sizes are given in the **Fig. 4.17**.

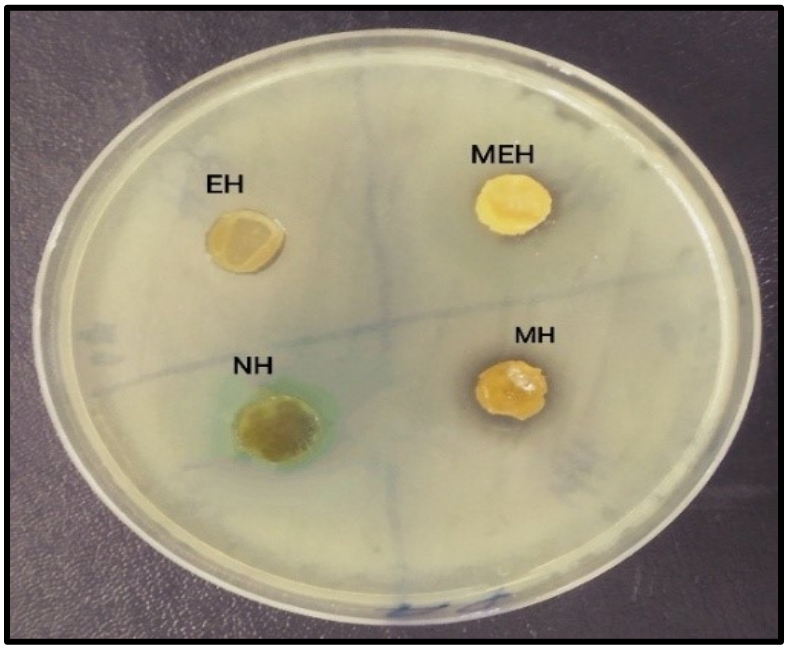

Fig.4.16: Bacterial growth inhibition of hydrogel against *Pseudomonas aeruginosa*.

The bacterial inhibition MH and MEH was higher against all experimented bacteria. Bacterial inhibition also observed again all experimented bacteria by EH and mild zone of inhibition was found against all bacteria.

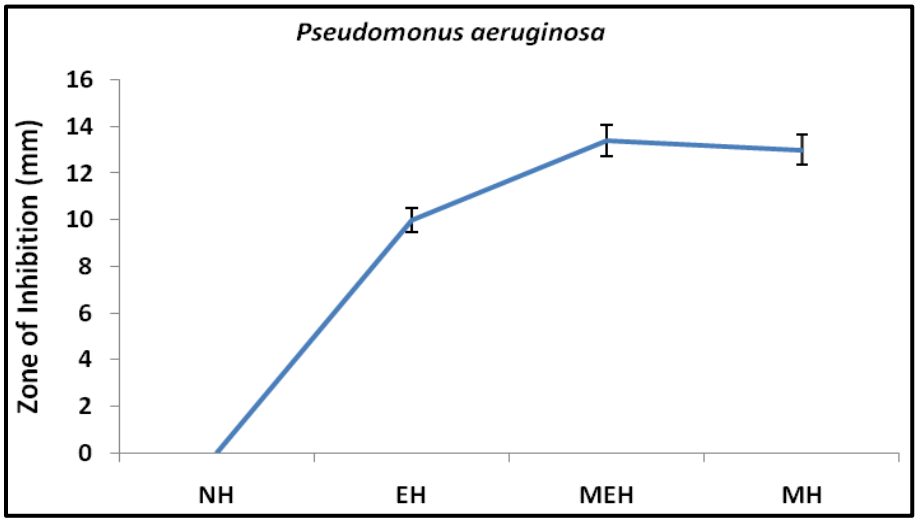

Fig.4.17: Statistical graphical diagram of hydrogel against *Pseudomonas aeruginosa*.

It has already reported that chicken egg white can inhibit bacteria because it possesses chemical components like ovalbumin, lysozyme etc. are responsible for antibacterial activity (Mine, Y. and Kovacs-nolan, J. 2004).Previous investigations have shown that lysozyme-derived polypeptides can damage the external membrane of *Escherichia coli* as well as inhibit DNA and RNA synthesis (Pellegrini, A. et al., 2000).

CHAPTER 5
CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusion

Bacterial infection is one of the principal challenges for wound healing, without controlling the bacterial infection after wound injury it is almost impossible to heal the wound. To address the issue, wound dressing hydrogels were developed. They not only support wound healing but also control bacterial infection. Naturally available and cost-effective egg white was used for bacterial inhibition in this study. It is noteworthy that egg white traditionally has been used for wound healing from ancient times. It is already proven by some experimental studies that it can inhibit bacteria and help in wound healing. On the other side, mupirocin is popular for topical use on the skin for containing the bacterial infection. To boost up antibacterial activity and wound healing egg white was incorporated with mupirocin in hydrogel for bacterial inhibition.

This is the first-ever use of natural egg white in combination with mupirocin antibiotic to produce hydrogels for application on bacterial inhibition. The prepared hydrogels were experimented to find out any bacterial inhibition activity against common bacteria which are generally responsible for creating wound infection. Physiochemical tests were conducted to confirm the presence of mupirocin and egg white in hydrogel and to ascertain the capability of hydrogel as a wound dressing. Swelling behavior, moisture retention capability, folding endurance, water vapor transmission rate, pH determination, gel fraction, spreadability measurement, and porosity experiments were conducted to check the wound dressing properties of prepared hydrogel.

To confirm the presence of the drug in the hydrogel, a drug compatibility study (FTIR analysis) and to confirm the porous structure of the hydrogel Scanning Electron Microscope (SEM) methods were applied. The outcomes of this current research clearly indicated that the mupirocin and egg white were successfully loaded into a hydrogel which showed improvement of the physical and biological properties considered essential for the wound healing process. The newly prepared hydrogel MEH and MH hydrogels have exhibited potential capability against experimented wound infection creating bacteria, which will accelerate wound healing by inhibiting bacterial infection.

5.2 Limitations

- Tensile strength test and X-ray Diffraction Analysis (XRD) analysis were not conducted because of the unavailability of machines during the time of the project work.
- SEM photos and analyses could have been better. Because of the time constraints it was not possible to repeat further more.

5.3 Recommendations

- The ability of inhibiting bacterial growth of newly prepared hydrogel could be compared with other dressing materials.
- The prepared hydrogel sample could be experimented with mice model for conducting in vivo test to check the wound healing capability of newly prepared hydrogel.

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