Nanocomposite based Smart Hydrogel for Wound Dressing

By

Muniba Zahra

(Registration No: 00000401846)

Department of Materials Engineering

School of Chemical and Materials Engineering

National University of Sciences & Technology (NUST)

Islamabad, Pakistan

(2024)

Nanocomposite based Smart Hydrogel for Wound Dressing

By

Muniba Zahra

(Registration No: 00000401846)

A thesis submitted to the National University of Sciences and Technology, Islamabad,

in partial fulfillment of the requirements for the degree of

Master of Science in

Nanoscience Engineering

Supervisor: Dr. Zakir Hussain

School of Chemical and Materials Engineering

National University of Sciences & Technology (NUST)

Islamabad, Pakistan

(2024)

THESIS ACCEPTANCE CERTIFICATE

Certified that final copy of MS Thesis entitled **"Nanocomposite based Smart Hydrogel for Wound Dressing"** written by Ms **Muniba Zahra** (Registration No 00000401846), of School of Chemical & Materials Engineering (SCME) has been vetted by undersigned, found complete in all respects as per NUST Statues/Regulations, is free of plagiarism, errors, and mistakes and is accepted as partial fulfillment for award of MS degree. It is further certified that necessary amendments as pointed out by GEC members of the scholar have also been incorporated in the said Thesis.

Signature: _____

Name of Supervisor: **Dr Zakir Hussain**

Date: 24/12/2024

Signature (HOD): _____

Date: 24-12-24

Signature (Dean/Principal): _____

Date: 24/12/24

Form TH-1

National University of Sciences & Technology (NUST)

MASTER'S THESIS WORK

Formulation of Guidance and Examination Committee (GEC)

e: Muniba Zahra NUST Reg No: 00000401846

ırtment: Department of Materials Engineering Specialization: Master of Science in Nanoscience & Engineering

it Hour Completed: 24.0 CGPA: 3.31

urse Work Completed

No:	Code:	Title:	Core/Elective:	CH:	Grade:
	MSE-854	Characterization Of Materials	Compulsory	3.0	B
	NSE-813	Essentials of Nanoscience and Engineering	Compulsory	3.0	A
	RM-898	Research Methodology	Additional	2.0	Q
	NSE-842	Nano Materials For Energy Applications	Elective	3.0	A
	MSE-856	Nano Material and Processing	Compulsory	3.0	B
	NSE-812	Environmental Nanotechnology	Elective	3.0	B+
	MSE-952	Materials For Biomedical Applications	Elective	3.0	B
	NSE-941	Nano Composite Materials	Elective	3.0	B
	ESE-837	Electrochemical Energy Storage and Conversion	Elective	3.0	B

1 - Dec - 2023 Student's Signature [redacted]

is Committee

ıe: Zakir Hussain (Supervisor) Signature Zakir Hussain

artment: Department of Materials Engineering

ıe: Muhammad Bilal Khan Niazi (Internal) Signature

ırtment: Department of Chemical Engineering

e: Usman Liaqat (Internal) Signature

ırtment: Department of Materials Engineering

11 - Dec - 2023 Signature of Head of Department:

APPROVAL

11 - Dec - 2023 Signature of Dean/Principal:

National University of Sciences &Technology (NUST)

FORM TH-4

MASTER'S THESIS WORK

We hereby recommend that the dissertation prepared under our supervision by

Regn No & Name: 00000401846 Muniba Zahra

Title: Nanocomposite based Smart Hydrogel for Wound Dressing.

Presented on: 21 Oct 2024 at: 1030 hrs in SCME Seminar Hall

Be accepted in partial fulfillment of the requirements for the award of Masters of Science degree in Nanoscience & Engineering.

Guidance & Examination Committee Members

Name: Dr M. Bilal Khan Niazi Signature:

Name: Dr Usman Liaqat Signature:

Supervisor's Name: Dr Zakir Hussain Signature:

Dated: 21/10/2024

Head of Department

24-10-24
Date

COUNTERSIGNED

Date 24/12/2024

24/10/24
Dean/Principal

School of Chemical &Materials Engineering (SCME)

AUTHOR'S DECLARATION

I Muniba Zahra hereby state that my MS thesis titled "Nanocomposite Based Smart Hydrogel for Wound Dressings" is my own work and has not been submitted previously by me for taking any degree from National University of Sciences and Technology, Islamabad or anywhere else in the country/ world.

At any time if my statement is found to be incorrect even after I graduate, the university has the right to withdraw my MS degree.

Name of Student: Muniba Zahra

Date: 12/24/2024

PLAGIARISM UNDERTAKING

I solemnly declare that research work presented in the thesis titled "Nanocomposite based Smart Hydrogel for Wound Dressing" is solely my research work with no significant contribution from any other person. Small contribution/ help wherever taken has been duly acknowledged and that complete thesis has been written by me.

I understand the zero-tolerance policy of the HEC and National University of Sciences and Technology (NUST), Islamabad towards plagiarism. Therefore, I as an author of the above titled thesis declare that no portion of my thesis has been plagiarized and any material used as reference is properly referred/cited.

I undertake that if I am found guilty of any formal plagiarism in the above titled thesis even after award of MS degree, the University reserves the rights to withdraw/revoke my MS degree and that HEC and NUST, Islamabad has the right to publish my name on the HEC/University website on which names of students are placed who submitted plagiarized thesis.

Student Signature: 

Name: Muniba Zahra

DEDICATION

I dedicate this thesis to my beloved parents and to my lovely siblings who have always been a strong pillar of support for me.

ACKNOWLEDGEMENT

I would like to express my deepest gratitude to Almighty Allah for His countless blessings. All praise be to Him. After that, I am profoundly grateful to my esteemed supervisor, Dr. Zakir Hussain, for choosing me as his student. His unwavering guidance, encouragement, and extensive knowledge were instrumental in laying the foundation of this project. It has been an honor to work under his distinguished supervision. I also present my heartful

My deepest thanks go to my parents and to my siblings. Their love and compassion have been my pillars of strength. Also, I am profoundly grateful to my friends and fellows, especially Dr. Dooa Arif, Dr. Sadaf Batool, Haleema Bibi, Fatima Maryam and Fareeha Batool who have always been there whenever I need any conceptual and technical support.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Page No.

LIST OF SYMBOLS, ABBREVIATIONS AND ACRONYMS

PVA	Polyvinyl Alcohol
HA	Hyaluronic Acid
AgNPs	Silver Nanoparticles
FTIR	Fourier Transform Spectroscopy
XRD	X-Ray Diffraction
SEM	Scanning Electron Microscopy
DI	Deionized Water
MOF-199	Metal Organic Framework-199
H1	PVA/HA Membrane
H2	PVA/HA/AgNPs Membrane
H3	PVA/HA/AgNPs/MOF-199(0.1mg) Membrane
H4	PVA/HA/AgNPs/MOF-199(0.2mg) Membrane
H5	PVA/HA/AgNPs/MOF-199(0.5mg) Membrane

ABSTRACT

There has been a surge in interest recently in the creation of innovative materials and therapeutic strategies to enhance the results of wound healing for chronic wounds as it is a painful condition that entails complex healing phenomena and would take years to recover. Hence, the use of nanoparticles to promote wound healing through cell proliferation and antimicrobial activity has proved to be an efficient way to increase the healing process. For this, a multipurpose smart dressing material with a variety of qualities that can encourage the best possible wound healing is required. This work used a PVA/HA biodegradable matrix for wound healing that used glycerin as a plasticizer and reinforced with AgNPs and MOF-199, to develop a smart nanocomposite hydrogel membrane. Different characterization techniques XRD, FTIR, SEM and mechanical testing were carried out to examine the membrane's shape and structural characteristics. Additionally, to assess the membrane's efficacy as a wound healing material, biomedical tests were carried out like moisture retention, gel fraction, swelling and antimicrobial. The membrane formed was hydrophilic, with excellent swelling properties and reasonable gel fraction and moisture retention values. It also showed remarkable mechanical strength and flexibility. These findings imply that the membrane created has promise for use as an efficient material for treating wounds and can prove be a good dressing for medium to high exudate absorption due to MOF-199 high surface area and porosity.

Keywords: Chronic Wound, Smart Wound Dressing, Hydrogel Membrane, MOF-199, Exudate Absorption, Polymer Materials, Polyvinyl Alcohol, Hyaluronic Acid, Wound Healing.

CHAPTER 1: INTRODUCTION

Skin acts as a primary natural barrier for a body that not only shields the internal organs from the harms of the external environment but also helps body cope with dehydration concerns [1]. However, the skin after facing some major injuries like burns, surgery or diseases could result in losing its protective barrier. In more severe conditions it could end up generating a wound that inhibits the skin's natural healing abilities due to microbial invasion. The harmful microbes like *S, aureus, P. aeruginosa and S. pyogenes* start to get colonized on a wound, becoming a reason for potential infections that could lead to a more severe chronic wound [2]. Pathogenic infection as a common complication of chronic wounds has become a global health concern because of its deleterious effect on the healing process [3]. These lesions, which include pressure sores, venous ulcers, and diabetic ulcers, place a significant burden on both patients and healthcare systems. This frequently led to serious side effects, such as infections, amputations, and protracted hospital stays, which raise morbidity and expense of care. Effective wound treatment is vital for clinical practice because in chronic wounds, healthy tissue can become infiltrated by bacteria, which compromise the immune system. This phenomenon holds particular importance in wound infections caused by Staphylococcus aureus, that would lead to severe tissue destruction [4]. In contrast, prolonged inflammation can cause tissue degeneration and impede the period of wound healing.

Wound healing as a primary process in effective wound treatment involves four steps i-e hemostasis, inflammation, cell proliferation, and tissue remodeling [5]. To enhance the treatment outcome during these stages effective control of bacterial infection [6]. Traditional antibiotics are more effective against bacteria but, using them frequently may result in developing a lasting drug resistance in organisms [7]. Numerous antimicrobial drugs have been created in response to these drawbacks, along with developments in nanotechnology and nanomaterials for the treatment of bacterial infections [8]. The nanomaterials have outstanding antibacterial properties; they can destroy bacteria by oxidative stress [9] by physical contact damage [10], photocatalytic treatment [11], antimicrobial photodynamic therapy [12] and photothermal therapy [13]. Some antibacterial nanomaterials like Ag [14],

Ag_3PO_4 [15], ZnO [16], CuO [17], TiO_2 [18] and MOS_2 [19] can be used directly to kill bacteria. However, these metal and metal oxides are unable to show durability and persistence against antibacterial action due to metal oxidation and agglomeration. That is why their extensive usage in clinical settings is hampered by their incompatibility with skin tissues [20].

As, metal and metal oxide antibacterial agents have been delivered using a range of scaffolds up to this point. The scaffolds comprise microgels [21], liposomes [22], yeast derived microparticles [23], polymeric nanoparticles [24], graphene [25], graphene oxide [26], single walled nanotubes [27], MXene [28] and metal organic frameworks (MOFs) [29]. Among them Metal-Organic Frameworks (MOFs) constitute a hybrid crystalline material with a periodic structure and a porous interior. MOFs are gaining popularity due to their large surface area, adaptable porosity, flexible architecture, and moveable and adjustable channels that make them so successful [29]. They can have extensive use in several biomedical domains, such as photodynamic treatment, drug delivery, bioimaging and wound healing [30]. Numerous metal-organic frameworks (MOFs) have been utilized as carriers for metal ions, including copper (Cu^{2+}), zinc (Zn^{2+}), and silver (Ag^{+}), which are potent antibacterial agents. Moreover, a delayed release of metal ions may be facilitated by the progressive breakdown of MOF structures brought on by the complicated physiological environment. Over time, this regulated release greatly helps in increasing the antibacterial efficacy[30].

Furthermore, some MOFs can produce reactive oxygen species (ROS), which cause oxidative stress in pathogens. Their antibacterial efficacy is facilitated by this oxidative stress [31]. Due to the regular pores and large specific surface area MOFs offer a high loading capacity. They have attracted a lot of interest such as delivery systems for antibacterial components like antibiotics, metal nanoparticles (NPs), and natural antibacterial compounds. MOFs increase the efficiency of these chemicals by reducing cytotoxicity and facilitating their prolonged release [32]. Still, most studies have focused on using MOFs in conjunction with natural antibacterial compounds or antibiotics. Usually, these mixtures use chemical antibacterial methods to fight germs, but that causes medication resistance [33]. To get over this restriction, MOFs are commonly coupled with other metal

nanoparticles (Ag, Cu, ZnO, TiO_2, and Fe_3O_4) that have remarkable antibacterial qualities [34]. These MOF-based composites can exhibit several bacteriostatic processes, making them unique antibacterial systems [35].

Hydrogels have been developed using a variety of biocompatible polymers, including hyaluronic acid, gelatin, chitosan, and sodium alginate, for use in wound healing applications [36]. Due to hemostatic, mucoadhesive, and antibacterial properties, hyaluronic acid has been extensively studied for its potential applications as a remarkable polymer for the manufacture of hydrogels and for accelerating wound healing. Hyaluronic Acid (HA) is a naturally occurring biopolymer with a range of molecular weights. It is made up of units of D-glucuronic acid and N-acetyl-D-glucosamine joined by alternating β-1,4 and β-1,3 glycosidic linkages [37,38]. Several functional groups found in hyaluronic Acid (HA), including acetamide, carboxyl, and hydroxyl groups, can be used to chemically modify the material's surface. HA is widely found in various human organs, including the eye, skin, and cartilage [39,40,41] because of its special physicochemical characteristics, which include viscoelasticity [37], oxidative degradation, biocompatibility [42] and biodegradability [43]. HA is a desirable biopolymer with a broad range of uses in cosmetics, medicines, textiles, and pharmaceuticals [44,45,46]. Hyaluronic Acid based composites have attracted a lot of attention because of their multiple uses in medicine, particularly with inorganic metal nanoparticles (such Ag, Au, and ZnO-NPs) [47,48,49,50,51]. For advanced material applications, there has been an increasing interest in creating nanocomposite materials made of metallic nanoparticles combined with polymers [52,53,54,55].

A lot of work has gone into producing silver nanoparticles (AgNPs) with excellent distribution, regulated size, and form [38,56,57]. Because silver nanoparticles have numerous applications in bioengineering, electronics, photonics, and catalysis, their production for use in nanocomposites is of particular interest [58,59,60,61,62,63]. AgNPs are well-known as potent antibacterial and antifungal agents in the world of bioengineering [64]. Ag NPs can be delivered to bacterial cell walls, where they destroy bacterial cells and cause the leakage of intracellular contents. This is achieved through a combination of physical interactions, the generation of reactive oxygen species (ROS), and ion release. These mechanisms help to overcome bacterial resistance to Ag NPs based antimicrobial

therapies [65]. A range of MOFs have been created to date for loading silver nanoparticles (Ag NPs). These comprise HKUST-1 [66], MIL-125(Ti)-NH_2 [67], peroxidase-like NH_2-MIL-88B(Fe) [43], cyclodextrin-MOF [44]. The regulated release of Ag+ and metal ions from the MOF matrices can be facilitated by integrating different MOFs with Ag NPs, hence augmenting the antibacterial action.

In the context of wound healing research, hydrogels are particularly noteworthy because of their remarkable capacity to both efficiently absorb tissue exudates and promote oxygen permeability. These materials can chemically adhere to the skin's surface and promote blood clotting by interfering with the actions of naturally occurring tissue proteins, also creating a favorable moist environment that promotes cell growth and division [68]. Hydrogels are polymeric crosslinked networks, which can absorb water without dissolving, and are widely employed in many different biomedical applications [69]. Hydrogels stand out in these applications because of their high surface-area-to-volume ratio, which promotes maximum tissue engagement and quick reaction. Achieving the desirable features of hydrogel thin films requires the creation of a mesoporous structure in films. Large open spaces within the crosslinked network are provided by hydrogels in their swollen condition, and these spaces can serve as nano- or micro-reactors for the creation of AgNPs by giving them enough room to nucleate and proliferate. Silver ions can establish advantageous connections with the functional groups in the polymeric chains inside the hydrogel network, resulting in a homogeneous distribution across the network. After reduction, controlled size, uniformly dispersed AgNPs can be obtained and then immobilized inside the gel network [70]. Polyvinyl alcohol (PVA) is a synthetic polymer obtained by hydrolyzing vinyl acetate. It is a highly appreciated medical substance due to its extraordinary elasticity and room-temperature chemical stability and is recognized as a biocompatible, biodegradable, and non-toxic polymer [71].

By combining the advantageous qualities of both synthetic (PVA) and natural polymer (HA), the formed hybrid nanofibrous hydrogels display a synergistic effect that can serve as an excellent biomaterial system [72]. As PVA and HA hydrogels contain 90% water, they resemble the human body, making them ideal for drug administration and transport [73]. The hydrogels constructed of PVA and HA can be used as biomaterials; their

potential applications can be understood by examining their gelation time, gel strength, and rheological properties [74,75]. Glycerin is used as a crosslinker because of its bifunctionality that reacts with the hydroxyl groups in PVA and HA and can create three-dimensional network structures by forming crosslinks between polymer chains.

In conclusion, wound healing is a complicated process that involves many variables and calls for a multimodal strategy. This following research aims to address the previously described problem by developing a novel PVA/HA based hydrogel enhanced with MOF-199 and Ag nanoparticles. By utilizing these constituents, a multifunctional hydrogel compound was produced that could control the release of contained nanoparticles, provide a favorable microenvironment for wound healing, providing mechanical strength to the damaged area.

CHAPTER 2: LITERATURE REVIEW

2.1 Skin

The skin is the largest organ in the human body and covers an area of around 1.856 square meters. Our skin not only serves as a purpose of regulating our body's temperature but also provides shielding from microbial invasion [76]. Human skin has remarkable healing properties that act as a powerful barrier, preventing excessive moisture loss and limiting the entry of environmental contaminants. Furthermore, it retains control over other physiological processes, including temperature regulation, variations in blood supply to the extremities, and fluid balance through sweating. Because of its layered composition, the skin is incredibly flexible and strong. Still, every time a wound develops, the barricade's function is compromised, increasing the danger of infection.

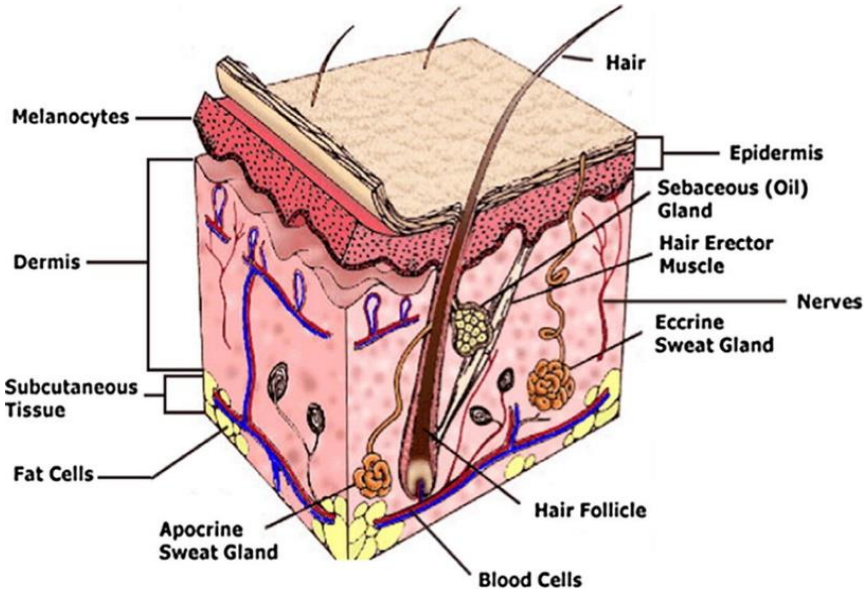

Figure 1: Structure of human skin [76].

The hypodermis, epidermis, and dermis are the three main layers that make up the integumentary system of skin as shown in (fig.1). The functions and structural makeup of the skin give each layer unique characteristics. The skin's outermost layer, the epidermis, has a strong and resilient 12 features. It serves the vital purpose of protecting the underneath

components. This organism's cellular design provides an incredibly effective defense mechanism against microbial invasion. One superior quality that sets the epidermis apart from other body cells is its ability to reproduce itself. When a body is injured, the damaged cells are repaired and make room for new cells to proliferate. The dermis is the underlying layer of tissue that lies next to the epidermis. Dermis, which is made up of sweat glands, lymphatic vessels, and hair follicles, is essential for controlling body temperature and generating sebum, which keeps skin hydrated. The material gives the integumentary system robustness and tensile strength. The hypodermis, the underlying layer beneath the dermis, is responsible for attaching the skin to the surrounding muscles and bones [77]. The distinction between the hypodermis and dermis is still imperceptible.

2.2 Wound and its Classification

A wound is a defect or rupture in the skin on the outermost layer of the body caused by systemic deficiencies or traumatic experiences. The process of classifying wounds entails identifying the precise area of the dermis damaged as well as the degree of damage sustained by the skin layers. The categories include full thickness wounds that injure subcutaneous layers and underlying tissues, partial thickness wounds that penetrate deeper than the epidermis, and superficial wounds that only impact the skin's topmost layer. Wounds are further categorized as follows:

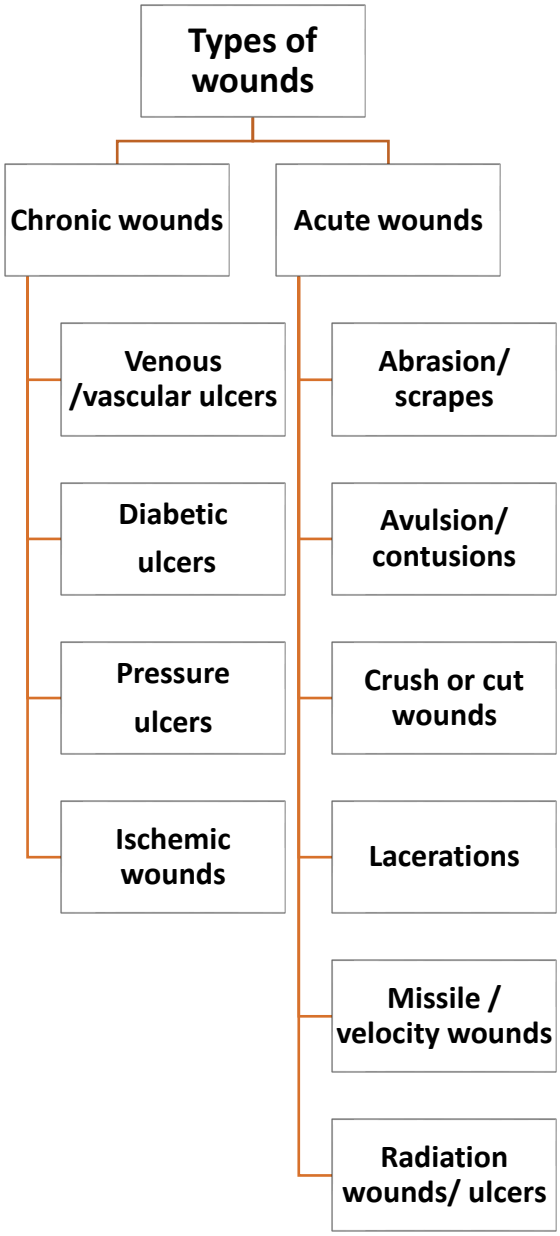

Figure 2: Types of Wounds.

Acute wounds have a predictable healing trajectory that follows the standard protocol for wound healing, with resolution usually taking place in 4–12 weeks (about 3 months). Abrasion, incision, cuts from sharp objects, and tissue ripping from strong blows are some of the mechanisms that can result in these types of injuries. **Chronic wounds** begin as acute wounds but become chronic because the natural healing process is not followed, which results in a protracted recovery period that can last for months or even years [78]. The

manifestation thus appears as thermal burns, persistent ulcers, or incised wounds. Chronic wounds may require a lengthy healing period, lasting several months or even years, and frequently leave permanent skin markings. Due to the prolonged healing process, dressing must be changed frequently, which puts financial strain on patients and extends their hospital stays.

2.2.1. Wound Healing

The human skin's remarkable capacity for regeneration is essential for both protecting the epidermis from outside contaminants and ensuring that it is properly transpiring. The process of wound healing is a complex event in the human body that requires special attention [79]. The process of healing wounds consists of several stages that occur simultaneously, such as hemostasis, inflammation, tissue proliferation, and tissue remodeling. Tissue restructuring following injury is a dynamic process marked by complex interactions between cells, extracellular matrix (ECM) and growth factors [80]. The migration of keratinocytes and the proliferation of fibroblasts are key processes involved in epithelialization and full wound healing [81]. After a cutaneous lesion, platelet aggregation is triggered by tissue factors and collagen. This in turn, causes the release of growth factors and chemokines, which facilitate the formation of clots.

The following stages in wound healing are as follows: The first stage of wound healing, known as **hemostasis,** signifies the beginning of the process being studied. The phenomenon described above refers to the primary and natural response that the human body displays in an accident or trauma. To avoid hemorrhaging, the blood flow is slowed down which allows clot formation. Blood in the next phase converts to a gelatinous fluid from liquid state. Neutrophils are the first cells to actively respond at the wound site thus playing a crucial role in creating a conducive environment for wound healing. Its primary duty also involves the elimination of superfluous cellular material and bacterial infections from wound sites. During the second stage of **inflammation**, macrophages gather and aid in the phagocytosis of injured tissues. This strategy leads to the inflammatory phase's successful completion. It will take seventy-two hours to finish each of these steps. The dilation of blood arteries facilitates the delivery of vital nutrients, leukocytes, and antibodies to the site of

injury. This helps to reduce the chance of infection. Patients experience physiological symptoms including oedema, erythema, or nociception during this phase.

Proliferation, the next stage, is characterized by a considerable number of cells and connective tissues accumulating. Granulation tissue development thus began to take shape. These newly formed tissues are composed of a composite mixture of collagen and extracellular matrix (ECM), which leads to the development of a complex vascular network that replaces broken blood vessels. This phase involves many strains of growth factors, cytokines, and extracellular matrix. This period lasts anywhere from a few days to potentially several weeks as shown in fig. 3. The last stage i-e., **Remodeling** calls for a precise balance between newly formed and pre-existing cells. The recently created tissues mature during this stage. Skin tissues go through a rejuvenation process to increase their tensile strength. Ultimately, functional fibroblasts take the place of the non-functional ones.

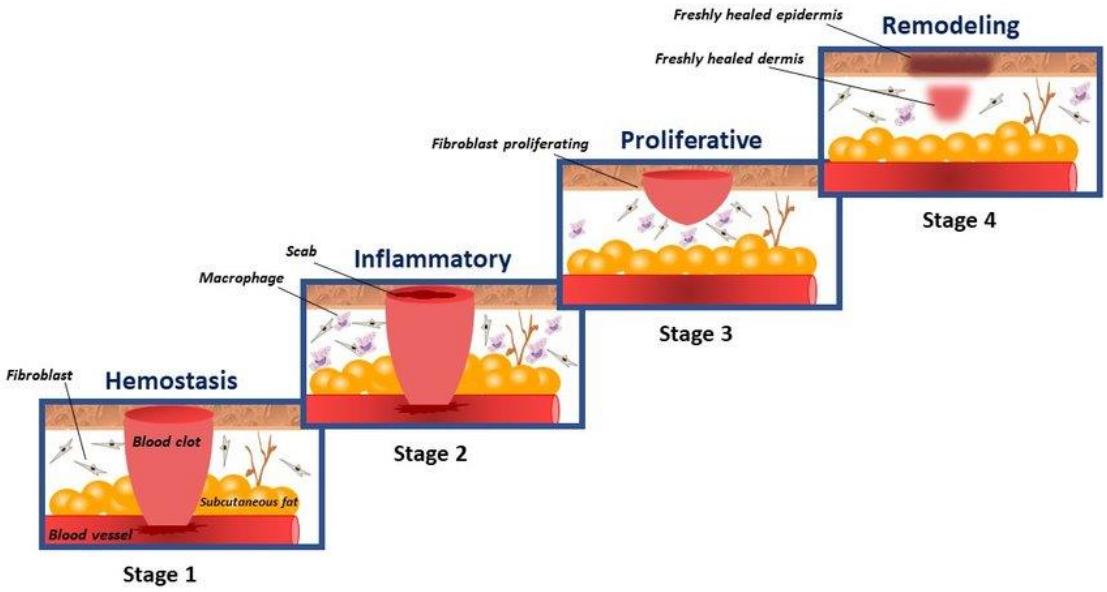

Figure 3: Different stage of wound healing [82].

2.2.2. Wound Dressing

Cotton gauze or rayon mix dressings were frequently used to promote wound healing and were regarded as dry dressings until the 1970s [83]. Earlier people thought that when wounds were left open and dried, they would heal quickly and methodically. It was clear that desiccated dressings are unable to provide the essential environment needed for the care of long-term wound problems through Winter's research [79]. The goal of using wound dressings is to manage wounds. Depending on how wettable they are, wound dressings can be divided into the following categories (fig. 4).

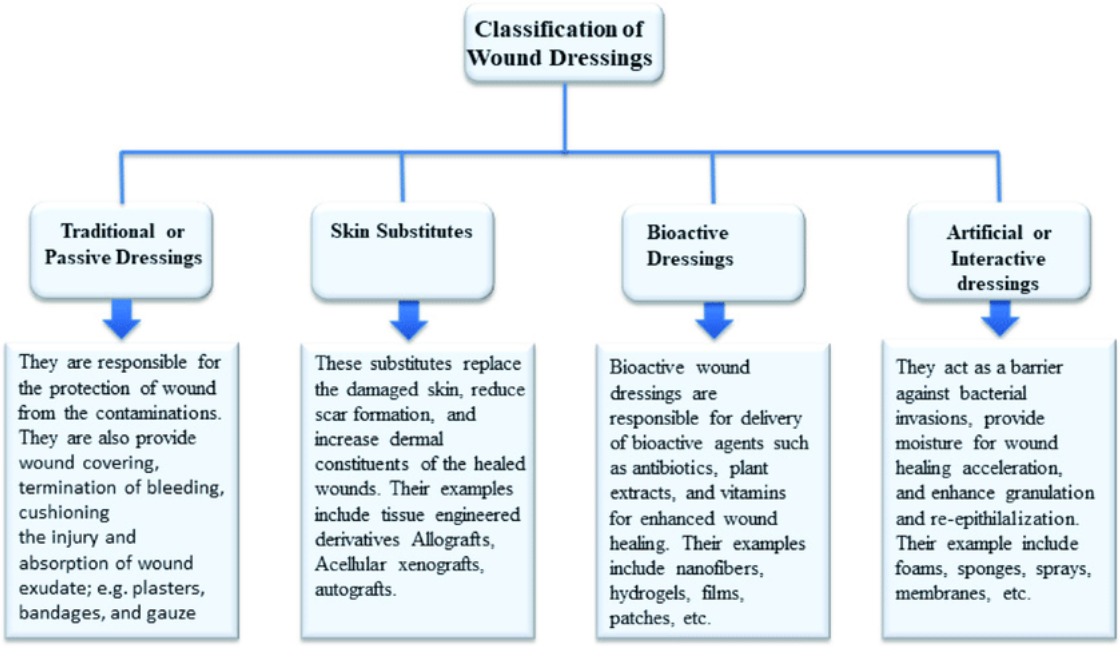

Figure 4: Classification of wound dressings [84].

2.2.3 Characteristics of Ideal Wound Dressing

The perfect wound dressing should have several essential features to help promote the best possible wound healing, offering protection, by improving patients' comfort. Among these qualities are:

1. Moisture Retention

It keeps the wound wet to encourage tissue regeneration and speed up the healing process. It keeps the wound from being overly wet or dry (effectively regulates exudate).

2. Permeability

Permit the gaseous exchange (carbon dioxide and oxygen) to promote wound healing and cell respiration. It should be impermeable to impurities and microorganisms, avoiding illness.

3. Biocompatibility

Non-irritating and non-toxic to the tissue around the incision. It should not elicit an immunological response or allergic responses.

4. Antimicrobial Properties

Protects against infection by preventing the growth of microorganisms. Antimicrobial compounds (such as silver and copper) should preferably be included to treat or prevent infections without endangering healthy tissue.

5. Absorbency

Absorbs wound exudate without causing the surrounding skin to become macerated. It should balance extra fluid in heavily secreting wounds while maintaining a moist atmosphere.

6. Adequate adhesion & Easy Removal

Minimize discomfort and harm upon removal by adhering sufficiently to stay in place but not to the wound bed. It should be simple to remove without damaging the healing tissue or leaving behind residues.

7. Flexibility and Conformability

Must be pliable and adapt to the contours of the wound, particularly in challenging areas, like joints. It must adjust to the movements of the body without hurting the wound or disturbing it.

8. Mechanical Protection

Offers a tangible shield to shield the wound from outside influences including pressure, friction, and stress.

9. Thermal Insulation

Should keep the wound region at the ideal temperature to encourage cellular activity and the healing process. Should stop the wound from cooling, which may cause the healing process to go more slowly.

10. Not-Cytotoxicity

Should not discharge any hazardous materials that can damage immature cells or hinder the healing process. The dressing components should ideally encourage tissue healing and cell proliferation.

11. Cost Efficient & Readily Available

Accessible and priced, particularly for the management of chronic or long-term wounds. It should be simple to use and remove, requiring neither specific equipment nor expertise.

12. Extended Wear Life

Made to last for a long time to minimize the need for frequent dressing change. It should lessen the possibility of wound disruption and patient pain with repeated changes.

13. Encourage Healing

Should promote angiogenesis, tissue regeneration, and cell development using bioactive substances such as peptides, growth factors, or other medicinal compounds.

2.3 Hydrogel Wound Dressing

Wichterle and Lim made the first attempt to create synthetic hydrogel in 1960 [85]. An example of a hydrogel is a three-dimensional lattice made of hydrophilic polymers that are cross-linked chemically or physically to produce a strong framework. As they are hydrophilic, these materials can expand or contract in response to changes in chemical or physical circumstances. Water soluble materials have a far larger volume expansion potential than weight. They show resistance to dissolution in water, saline solutions, and other biological fluids, depending on the degree of crosslinking, that can be enhanced using synthetic or natural polymers with them [86].

1. Polymer chains are joined by chemical reactions

2. Ionic Radiations

3. Physical interactions, such as creation of crystallites, entanglements, and electrostatics

Hydrogels are a useful, biocompatible, and long-lasting drug delivery platform that can hold both larger molecules, like peptides and proteins, and smaller ones, like non-steroidal anti-inflammatory drugs.

2.3.1 Smart Hydrogels in Wound Dressings

As there are many kinds of chronic wounds, they all have unique traits that are impacted by modifications to the wound microenvironment. The nature and state of the wound affects various parameters, including blood glucose levels, temperature, pH, and pressure. Hydrogels are a promising material for building multifunctional platforms since they are made of hydrophilic polymers. These hydrogels have a 3D network structure, high

oxygen and water permeability, and biocompatibility, among other beneficial characteristics. Hydrogel dressings with pH-sensitive, temperature-sensitive, glucose-sensitive, and pressure-sensitive qualities can be created by using hydrogels as substrates [87]. The smart types of hydrogels wound dressings include those that are pH-sensitive, thermo-sensitive, blood-glucose-sensitive, pressure-sensitive, and nanocomposite. Based on the monitoring data, these hydrogel dressings with wound-monitoring features may also make therapy easier.

The characteristics like surface functionality, biodegradability, biorecognition, and environmental friendliness have made hydrogels extremely important in the field of biomedical. These are developed with a goal to release therapeutic chemicals in a stimulus-dependent manner and are sensitive to their surroundings. A variety of elements are included in the physical characterization of wound sites such as pressure, temperature, electric or magnetic forces, and so forth. Conversely, the chemical characteristics include a range of factors, including the molecular species involved, pH level, ionic strength, and solvent type [88]. The internal environment of the human body is characterized by high temperatures and low pH levels, making these elements extremely important [89]. Hence, it is possible to maximize the medication release rate by utilizing all these characteristics. Ionizable groups, which can donate or absorb protons in reaction to changes in the external environment, are frequently included in polymers that display pH or temperature responsiveness. In scientific study, poly(acrylamide), poly (methacrylic acid), and poly (acrylic acid) have often been used as polymers that hold a potential for stimulus sensitivity [90].

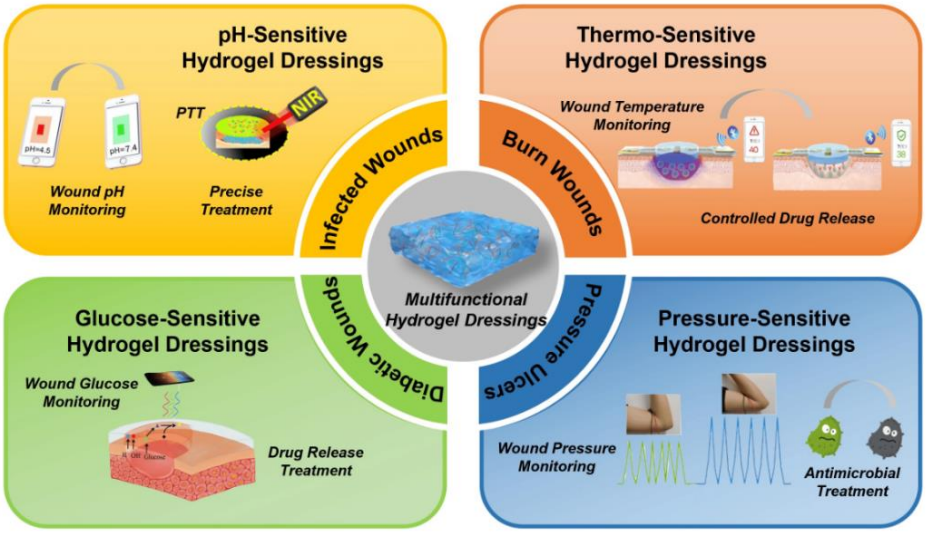

Figure 5: Smart hydrogel wound dressings for different applications [87].

2.3.2 Classification of Hydrogels

As of right now, there is no accepted system for categorizing hydrogels. Hydrogels, however, can be divided into the following groups according to differences in specific characteristics:

Based on degradation there are two types of hydrogels:

1. Physical or reversible gels

2. Permanent or chemical gels

2.3.2.1 Physical gels:

Ionic or hydrogen bonding are examples of secondary forces that build the network in these kinds of hydrogels. They can dissolve in the presence of changes in temperature, ionic strength, pH, or other environmental factors. They are frequently reversible.

2.3.2.2 Chemical gels:

Here, covalent bonds are formed between the molecular chains by cross-linking polymer chains, which can occur in a solution or in a dry state. The charges and exemptions for functional categories vary based on their characteristics.

They are prepared by:

a) Polymerization in three dimensions

b) Direct polymer-water soluble cross-linking

2.3.3 Hydrophilicity in Hydrogels

The incorporation of water into hydrogel structures plays a crucial role in promoting the transfer of nutrients and cellular products. The beginning of the absorption process causes the arriving molecules to connect with the groups that are most polar and hydrophilic. In academic discourse, the mechanism outlined above is usually referred to as **"primary attached water"**. The hydrogels show signs of swelling when the hydration process inside polar networks is finished. Hydrophobic networks are exposed, so they are formed. This process is known as **"secondary attached water"** and happens following a volume increase.

Both primary and secondary attached water forms make up the overall bond water. Osmotic pressure is responsible for the hydrogel's capacity to absorb further water once its polar and non-polar components have fully hydrated [33]. The revocation force phenomenon, which prevents more swelling, creates an innate elasticity in the network. With this method, the hydrogel reaches its maximal swelling point. Unbound water molecules occupy the leftover spaces in the network when the polar, non-polar, and ionic groups are fully saturated. As water is absorbed, the polymer dissolves and dissociates; the degree to which this process occurs depends mostly on the properties and composition of the polymers in question.

2.3.4 Synthesis Mechanism

One of two well-established techniques that can be used to create polymeric hydrogels are:

- Polymerization of hydrophilic monomers
- Modification of preexisting polymers by cross-linking

The monomer polymerization process and the subsequent hydrogel's synthesis are shown in (fig.6 fig.7) show how the joining of water-soluble polymers results in the

development of hydrogel. In both cases, covalent bonding plays a critical role in enabling molecular chain interconnection through polymer chain cross-linking.

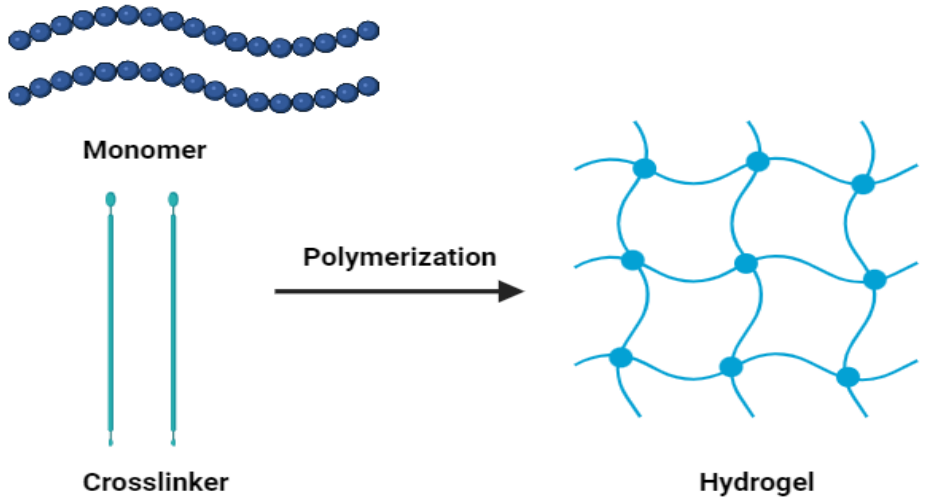

Fig.6: Synthesis of hydrogels by polymerization.

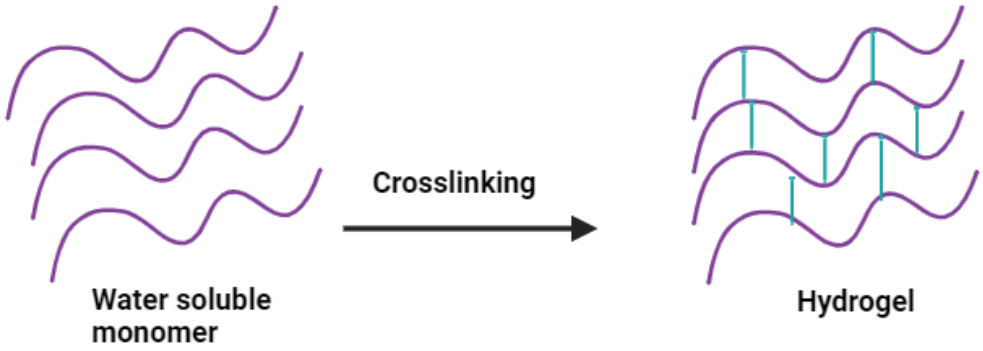

Fig.7: Synthesis of hydrogels by crosslinking.

2.3.5 Properties of Hydrogel

The incorporation of hydrophilic pendant groups into natural or synthetic polymers offers various advantages for creating hydrogels for medical use. These groups can boost the hydrogels' ability to absorb water, which will improve their interactions with mucous membranes and epithelial tissues. When hydrogels meet biological fluids, they often exhibit

properties related to viscoelastic behavior, softness, lubricity, and a decreased interfacial angle when they are fully hydrated. These characteristics have a crucial role in reducing the likelihood of an adverse immunological response. The combined existence of these components helps to support hydrogels' biocompatibility. In hydrogels, the degree of degradation is usually erratic and contingent upon the crosslinker employed [91].

2.3.5.1 Swelling Behavior

Hydrogels have a noticeable swelling feature that is important to their overall survival. Hydrogel swelling is a phenomenon that happens in three distinct stages in a sequential process. Water molecules diffuse into the network because of the first step, known as primary bound water ingress, which happens into the hydrogel matrix. Later, the polymer chains relax and absorb additional water, which is referred to as secondary bound water. In the end, when additional water, also referred to as free water, is infused into the hydrogel framework, it expands. According to the Flory-Reihner theory, there is a strong correlation between the swelling phenomena and the elastic properties of polymer chains, particularly about their ability to interact amicably with molecules of water [92].

2.3.5.2 Response to External Stimuli

Changes in their environment, which are mostly classified as physical stimuli, cause hydrogels to exhibit a wide spectrum of behaviors. Living things are susceptible to three distinct kinds of stimuli: chemical stimuli (such as pH and salt concentration variations), biological stimuli (involving enzymes), and physical stimuli (such as heat, light, and pressure). Except for temperature, which can originate from both exterior and internal sources, physical stimuli typically occur in the external environment. In contrast, chemical and biological stimuli are naturally occurring phenomena [93].

2.3.5.3 Mechanical Properties

Because of their high mechanical resilience, hydrogels have attracted a lot of interest in the biological and pharmacological fields. Assessing the mechanical resilience of hydrogels is crucial for ensuring their efficacious functioning in various physiological contexts. These fields cover a wide range of topics, including tissue engineering, wound dressing, cartilage replacement, biomedical technology, tendon and ligament healing, and

drug delivery systems. When considering hydrogel's progressive release of medicinal chemicals over a predetermined duration, maintaining its physical integrity is crucial [91].

2.3.5.4 Degree of Crosslinking

Hydrogels' mechanical properties are optimized through the careful control of crosslinking intensity and the addition of specific crosslinkers, co-monomers, and polymers. Even while these connections have many facets, having too many of them might lead to a decreased ability to be flexible and adaptive, which in turn increases the likelihood of fragility. Elasticity is an attribute of the highest importance as it offers enhanced flexibility to the networked system and permits the smooth movement of complementary medicinal components. Therefore, maintaining a fine balance between structural integrity and flexibility requires hydrogels to reach the maximum degree of crosslinking possible [94].

2.3.5.5 Biocompatibility

Hydrogels must demonstrate both non-toxicity and biocompatibility. Hydrogels must demonstrate both non-toxicity and biocompatibility. Essential elements of biocompatibility include biological safety and biological functionality. The hydrogels might then become fouled if they do not match these requirements. The creation of hydrogels using hazardous compounds often poses challenges to the achievement of in vivo biocompatibility. Since polysaccharides are widely recognized as non-toxic and biologically friendly polymers, their use in food contexts has been approved. Tokura et al. did a study. As stated in the reported literature, numerous biodegradable polysaccharides were the subject of in vivo cytotoxicity investigations. There were no symptoms of acute injury found in the rats' blood systems [95]. In our current study, Chan et al. examined the possible negative effects of chitosan, chitosan-24 PEG, and chitosan-PEG/DNA complexes coupled with folic acid. The results showed that 90% of the cells were viable on average [96]. A specific study was carried out to evaluate the effects of chitosan, N, O-carboxymethyl chitosan, and O-carboxymethyl chitosan on the growth of MCF-7 breast cancer cells in a lab setting. The experiment's findings demonstrated that the chemicals under study were significantly less hazardous, leading to a 98% cell survival rate [97].

2.3.5.6 Biodegradability

One important biomedical research project is the use of biodegradable hydrogels. "Biodegradability" refers to the process by which an organism converts hydrogels into non-toxic metabolites through enzymatic breakdown. Hydrogels' capacity to decompose naturally depends on the components used in the systems and the techniques used to prepare them. The degradation processes involve the hydrolysis and solubilization of biological constituents in hydrogels, leading to the creation of final materials or products. The human body can eliminate hydrogels by means of procedures known as bio-absorption and bio-erosion [91].

2.4 Polymers for Hydrogels

Natural polymers have attracted a lot of attention lately because of their abundance, affordability, biodegradability, and biocompatibility [98]. Nonetheless, the enduring problem with natural polymers is their vulnerability to mechanical fragility. Polyvinyl alcohol (PVA) is hindered in its capacity due to low hydrophilicity and flexibility to produce a persistent hydrogel on its own [99]. As a result, combining PVA with natural and synthetic polymers has been considered as a potential solution to these problems. PVA hybrid hydrogels are becoming increasingly used as polymeric membranes for wound treatment in the medical field. Effective synthesis has been achieved for the hydrogels made of PVA/Dextran [100], PVA/Sodium Alginate [101] PVA/Chitosan [102], PVA/Glucan [103], PVA/Gelatin [104], PVA/Hyaluronic Acid[105], and PVA/Keratin [106].

2.4.1 Polyvinyl alcohol (PVA)

F. Klatte discovered polyvinyl alcohol (PVA) in 1915. W.O. Hermann and W. Haehnel initially developed the method of stoichiometrically saponifying a polyvinyl acetic acid derivative with sodium hydroxide to generate polyvinyl alcohol (PVA) in 1924. The literature states that polyvinyl alcohol (PVA) exhibits a recurrent fundamental unit vinyl acetate (H3C-CO2-CH=CH2) that is notable for not requiring a monomeric structure [107]. This event can be attributed to the free radical polymerization of vinyl acetate, which is followed by the acetate moieties' inclusion of alcoholic functional groups [108].

2.4.2 Role of PVA in Hydrogel formation

Polyvinyl alcohol (PVA) as an artificial biopolymer has remarkable features, including the ability to retain oxygen and oil in water and remarkable visual attributes. Furthermore, PVA demonstrates several positive attributes in a learning environment. The characteristics include remarkable tensile strength, excellent thermal stability, resistance to organic solvents and oils, and non-toxicity as demonstrated by reference [108]. One noteworthy property of polyvinyl acetate (PVA) is its ability to prevent solvents, oils, and greases from penetrating. This material dissolves in water to produce a thick, obviously clear solution. The PVA films had a noticeably lower inclination to absorb water and showed an exceptionally elevated level of cleanliness. The items under observation demonstrate a deficiency in adaptability and durability. To address these issues, the production of hydrogels usually involves the mixing of polyvinyl alcohol (PVA) with other natural polymers such chitosan, cellulose, starch, PLA, gelatin, and Hyaluronic acid [109, 110].

2.4.3 Role of PVA in Blends formation

PVA has a low rate of biodegradability and a high material cost. Therefore, it is frequently required to blend in additional polymers, such as starch, chitosan, cellulose, and lignocellulose [111]. The hydrogel composite was created using free radical polymerization. Monomer acrylamide is combined with AgNPs on a PVA substrate and sodium alginate (Na-Alg). Methylene Bis Acrylamide (MBA) served as the cross-linking agent in this synthesis. A green method was used to create silver nanoparticles, with sodium borohydride serving as the reducing agent. This study indicates that when Na-Alg content rises, so does the size of silver nanoparticles which results in increasing the hydrogel's density but shows a decrease in AgNPs loading at higher crosslinker amounts [77]. Similarly, in another research a freeze-thawing process was used to combine PVA with natural fillers, such as starch, gelatin, and chitosan, through a bath immersion coagulation method. The objective of this strategy was to improve cell attachment as PVA's natural hydrophilicity restricts its ability to do so. When the hydrogel's physical characteristics were compared to the protein solution and cell proliferation, the gelatin/PVA hydrogel performed better in both scenarios [112]. The networks of PVA and PMMA poly (N, N-dimethyl acrylamide) hydrogels were created using free radical polymerization. After being added to the hydrogel, silver

nanoparticles (Ag NPs) helped the Ag+ ions form weak interactions with nitrogen and oxygen atoms to generate complex molecules [113]. Similarly, PVA composite containing chitosan and glycerol was made for use as a wound dressing by freeze-thaw process. After 11 days (about 1 and a half weeks), the wounds treated with this composite had a considerable healing response, as seen by the formation of a well-formed epidermal layer. This suggests that the composite is non-toxic, especially when it comes to its resistance to toxicity in L929 mouse fibroblasts [114].

A plasticizer consisting of glycerol and urea was employed in thermoplastic PVA/starch mixtures. Compared to utilizing a single plasticizer, this combination created stronger and more stable connections with the water in the hydrogels. The PVA/starch blend's continuous phase shape was validated by SEM examination and the mechanical characteristics and rheological performance were improved using the mixture of urea and glycerol (20wt% and 10wt%, respectively) [115]. By using the freeze-thaw method, the composite PVA/PVP and hydroxyl apatite was created, and a comparison of the PVA/PVP/HA and plain PVA/PVP was made. PVA/PVP/HA composite hydrogel exhibits a more prominent denser structure and a high rate of dehydration. The crystalline structure of HA, as shown by the XRD pattern, results in a high diffusion activation energy and improved molecular attachment when it is incorporated into the PVA/PVA hydrogel. Since HA filled up the gaps in PVA/PVP, the hydrogel's water content decreased. When compared to ordinary hydrogel, PVA/PVP/HA exhibits greater stability and strength when stress is applied[116].

2.5 Nanoparticles

Nanoparticles (NPs) are highly valued for their innovative technology, which makes them perfect for usage in a wide range of high-end products and applications. They are thought to be a promising way to handle difficult problems since they provide capabilities that would be challenging to obtain in any other way. NPs are used in many different applications, including the treatment of cancer, the filtration of water, the absorption of solar energy, energy storage, and the improvement of industrial processes. The notion of nanotechnology was first introduced by Nobel laureate and well-known scientist Richard P. Feynman, making him a pioneer in this subject.

NPs have existed since prehistoric times; naturally occurring examples include carbon-based materials produced by combustion and volcanic activity[117]. Moreover, it has been noted that there are specific microbes actively producing these compounds [94]. NPs are made up of materials such as metal, metal oxide, or carbon-based compounds. Their size distribution is usually found in the region of about 100 nm. Due to their size-dependent characteristics, nanoparticles, especially metallic ones, are important in biomedical applications [118]. The shape, size, content, and crystallinity of NP are some of the aspects that affect its inherent features. The factors described above are dependent on the conditions and procedures used during the synthesis process [119]. Four separate categories can be used to classify NP (fig. 8), and these are based on the chemical characteristics, morphology, and size.

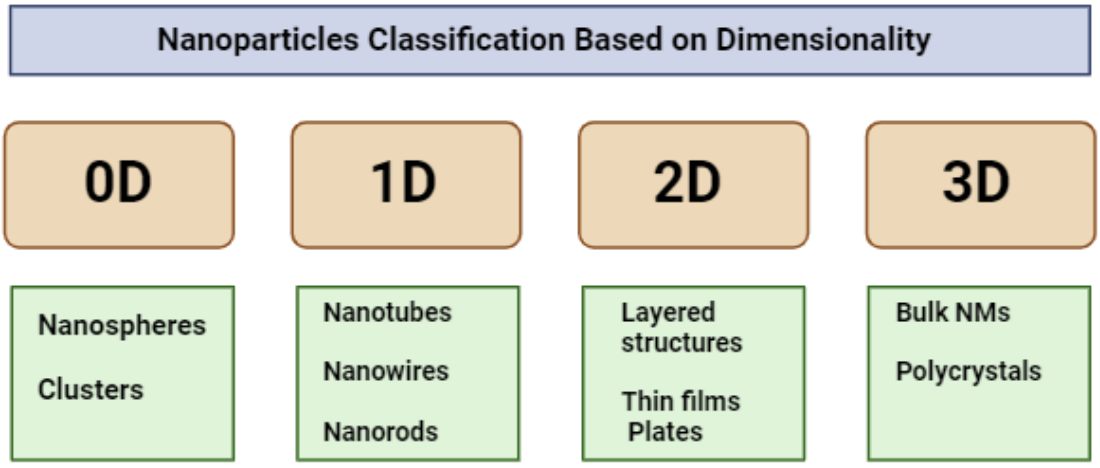

Figure 8: Classification of Nanoparticles based on dimensionality.

2.5.1 Metallic Nanoparticles

In academic research, metallic nanoparticles including ZnO, CuO, FeO, Au, and Ag are of interest. Although NPs have been used in many other applications throughout the business, their introduction into the biomedical sector stands out as one of the most significant and common. The antibacterial characteristics of these nanoparticles against both Gram-positive and Gram-negative bacteria account for their significance in biomedical application. Numerous fields, including agriculture, information technology, optics, catalysis, and energy, have found use for metallic nanoparticles [97]. There is a specific size parameter of NPs that determines their chemical and magnetic properties [98].

2.5.2 Nanoparticles as Antibacterial Agents

Antibacterial nanoparticles (NPs) are extremely small particles, usually smaller than 100 nm, that can be created with a variety of materials such as polymers, metal oxides, and metals. It is widely acknowledged that one of the most common types of antibacterial nanoparticles is silver nanoparticles (AgNPs) [106–108]. The antibacterial qualities of silver, and especially silver nanoparticles, have long been recognized. These particles have demonstrated efficacy against a variety of bacteria, including antibiotic-resistant ones. The antibacterial efficacy of various other forms of nanoparticles, particularly copper, gold, and zinc oxide nanoparticles, has been studied and assessed. Antimicrobial nanoparticles have several uses in various fields, including medicine [111], water filtration [112], and food product packaging [113]. Medical gadgets have been coated with bioactive chemicals to help stop bacterial infections. To accelerate healing and guard against bacterial infections, they are also utilized in wound dressings. Within the field of water treatment, various substances have been employed to achieve the objective of purifying water by eliminating bacteria and other microbes. In the field of food packaging, nanoparticles have been used to effectively extend food products' shelf life by preventing bacterial growth.

2.5.3 Silver nanoparticles and their antimicrobial mechanism

AgNPs' antibacterial qualities are primarily influenced by the medium's size, pH, and ionic strength as well as the kind of capping agent used. Nevertheless, the precise mechanism underlying the AgNPs' toxicity or antibacterial actions remains unclear and needs further explanation. AgNPs may release silver ions (Ag^+) on a regular basis, which could be one of the mechanisms behind their bactericidal activity. The silver (Ag) must be in its ionized state to retain its antibacterial or toxicity properties, and the positively charged Ag^+ is needed for the Ag to display these properties. Compared to phosphate groups, the Ag^+ ions have been observed to form complexes with nucleic acids and to interact with nucleosides of nucleic acids. Ag^+ ions are thus ultimately derived from any kind of silver or composites containing silver that exhibit antibacterial properties. Ag^+ ions bind to the cytoplasm and cell wall and dramatically increase permeability due to their affinity for sulfur proteins and electrostatic attractions. This causes the bacterial casings to break. Reactive oxygen species (ROS) are produced, and the release of adenosine triphosphate (ATP) is disrupted as soon as the

respiratory enzymes are inhibited by the uptake of free Ag+ ions by the cells. ROS may have a key role in exacerbating cellular membrane breakdown and altering deoxyribonucleic acid (DNA). DNA is mostly composed of phosphorus and sulfur, and when these elements interact with Ag+ ions, it can result in a variety of DNA-related problems, such as cell division and DNA replication. Furthermore, Ag+ ions have the potential to effectively impede protein synthesis by denaturing components of the cytoplasmic ribosome. In addition to its capacity to discharge Ag+ ions, AgNPs have the potential to eliminate bacteria or other microorganisms. The accumulated AgNPs cause denaturation of the cell membranes, and because of their nanoscale size, they can enter bacterial cell walls and change the configuration of the cell membranes. Denaturation of the cell membrane may cause the organelles to burst, which may also result in cell lysis. AgNPs may also be involved in the transduction of microbial signals. Phosphorylation of protein substrates affects the transduction of microbial signals; AgNPs may dephosphorylate the tyrosine residues over the peptide substrates. Moreover, a major reduction in cell division and death could result from a disturbance of microbial signal transduction [120].

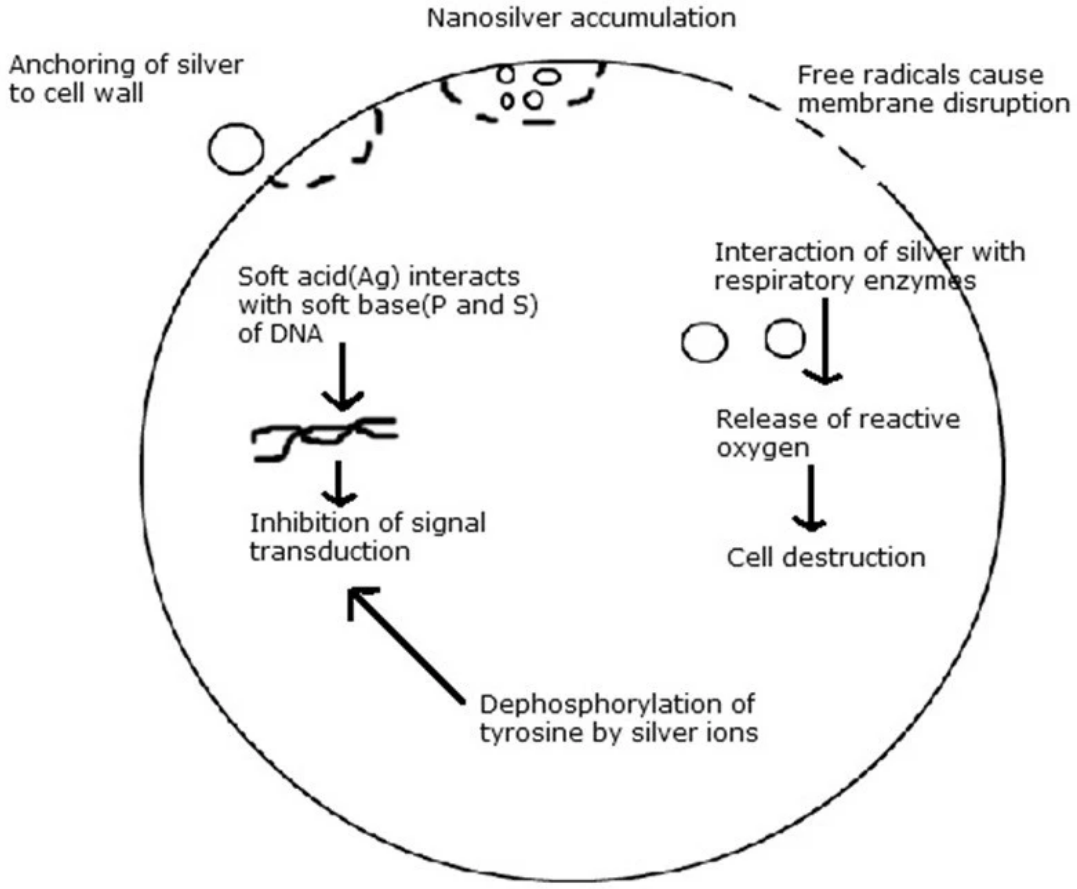

Figure 9: Antimicrobial action of AgNPs.

2.5.4 Use of Silver nanoparticles in Wound Dressings

Even though antibiotic-resistant bacteria are currently one of the biggest threats to public health, nanoscience can provide novel treatment strategies for the post-antibiotic age. The main medical issue in the past few years has been bacterial infection, particularly in chronic wounds, for which conventional treatment is insufficient owing to the rise in bacterial resistance. Therefore, antimicrobial therapy is frequently required for effective wound healing. Strong substitutes for antibiotics are being extensively researched due to the high frequency of infection and rising danger of antibacterial resistance. In those situations, silver and silver nanoparticles have the power to kill microbes and promote skin regeneration at the same time as it uses can be seen in (fig. 10). Compared to conventional topical treatments, Ag-NPs' distinct features imply that they can both successfully prevent wound infections and accelerate the healing process of damaged tissues. Naturally, as was already

indicated, bacteria can also acquire resistance mechanisms, which lower the efficacy of silver ion therapy [24]. Fortunately, silver has antibacterial activity through distinct mechanisms in comparison to conventional antibiotics. Because of this, the authors predict that soon, combined therapy of AgNPs and conventional antibiotics will be used to treat the most severely infected wounds. These treatments work, as evidenced by the trials that investigated dressings that combined silver with neomycin or colistin. In pre-clinical and clinical investigations, several natural or synthetic biomaterials incorporating silver nanoparticles have shown encouraging outcomes. At the same time the next generation of wound healing nanoscience has been designed using the developed nano systems and current capabilities in advanced manufacturing, along with understanding of molecular pathology, phenotype-genotype features, and chronic wounds.

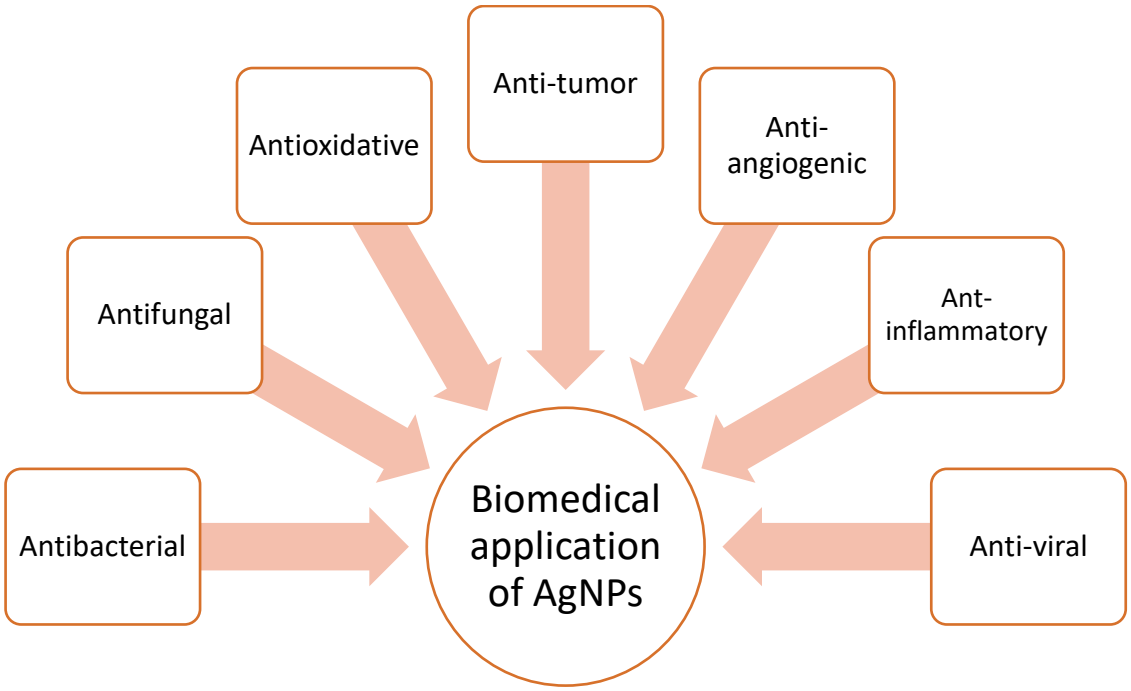

Figure 10: Biomedical Application of AgNPs used in wound dressings.

2.5.5 Combined effect of Silver Nanoparticles and Hydrogel Matrix

AgNPs with hydrogel matrix combination offer several benefits for wound healing and antimicrobial activity. Many applications of tailored matrices and AgNPs have been documented, along with their physical, chemical, and biological characteristics. However, there are still a lot of untapped chances to learn more about AgNPs activity in human cells

and the differences between local and systemic toxicity. One kind of device that was sold before to the Medical Device Amendments of 1976 was a hydrogel wound dressing paired with a medication (such as AgNPs).89 In general, the complexity and invasiveness of dressings determine their classification. Class II dressings are subject to the 510(k) pathway and need a clearance letter before the device is commercialized, whereas Class I dressings are regarded as low risk and do not require regulatory approval.89,90 If the indication for use, technological features, safety, and efficacy of Class II dressings are substantially similar to those of the designated predicate device, then they are certified for sale.89,90 The US Food and Drug Administration provides a precise and well-defined approval process that fosters innovation and new developments in technology.

Chitosan-PEG Hydrogels Impregnated In diabetic patients with chronic wounds, the efficacy of hydrogel materials for AgNPs administration has been evaluated through the examination of impregnated chitosan–PEG hydrogels. While the system's antibacterial activity was investigated, the primary goal was to ensure that AgNPs material was released steadily during the wound-healing process. A chitosan-based hydrogel was suggested elsewhere as a potentially effective material for wound healing due to its biodegradability, cell-binding ability, and antibacterial activity. The first study's PEG-prepared hydrogels allowed for the slow, continuous release of AgNPs, which was essential for making use of the particles' antibacterial properties [121]. Also, in another study, when compared to a bare chitosan hydrogel, it was discovered that the hydrogel with silver integrated showed better bactericidal action against both bacterial strains. The hydrogel with the highest concentration of silver (3 g/100 ml (about 3.38 oz) AgNO3) had the highest rate of inhibition for both bacteria [122].

2.6 Hyaluronic Acid

Natural anionic polysaccharide hyaluronic acid is made up of N-acetyl-D-glucosamine and D-glucuronic acid connected by β-1,3 or β-1,4 glycosidic linkages (Fig. 11). It is one of the exemplary biomaterials capable of designing microenvironment-responsive polymer nanoparticles in an easier way. In an aqueous solution, hydroxyl and carboxyl groups can create intra- and intermolecular hydrogen bonds that give the hyaluronic acid molecule an extended coil structure and give the molecular chain stiffness

in the form of a helical configuration. It is also compatible with biological systems and is appropriate for applications needing gel-like qualities due to its high viscoelasticity, biocompatibility, and biodegradability. Furthermore, hyaluronic acid's capacity to bind to cell surface receptors permits tailored distribution and promotes intercellular communication. Ionic crosslinkers, such as cationic compounds like chitosan, are used to produce hyaluronic acid-based nanoparticles. As hyaluronic acid drug conjugates, a few methods have been established for the synthesis of hyaluronic acid-based nanocarriers. Hyaluronic acid has been thoroughly investigated in several fields since the advent of nanotechnology, including the delivery of anti-cancer treatments, imaging agents, medications, photosensitizers, and gene plasmids [123].

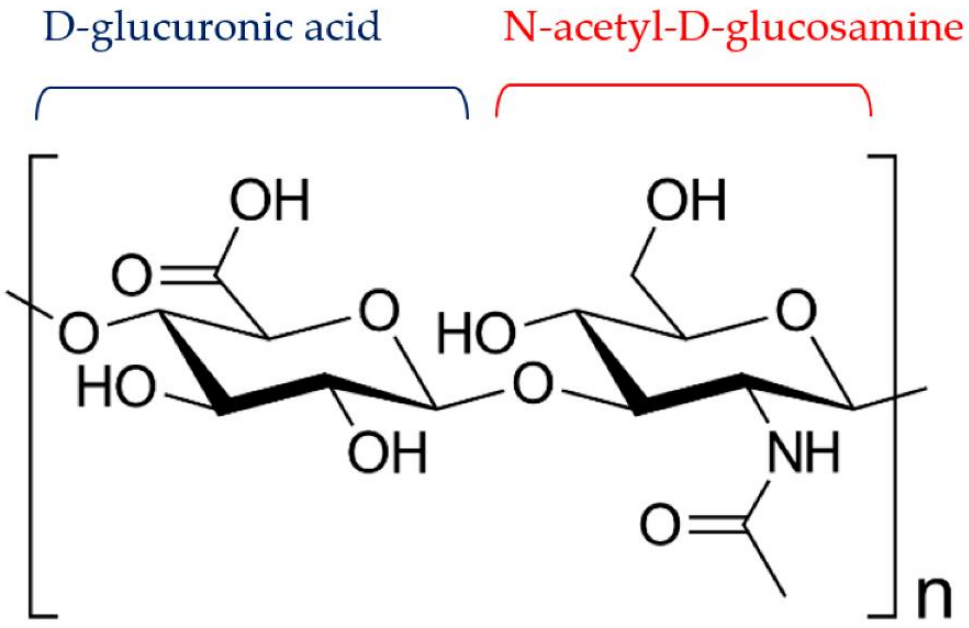

Figure 11: Structure of Hyaluronic Acid [123].

2.6.1 Hyaluronic Acid in Wound Healing

The binding of HA to various receptors is what gives it its biological function. In addition to contributing to the osmotic equilibrium, high-molecular-weight HA oversees maintain the extracellular matrix structure and hydrate the tissue. It also possesses anti-inflammatory qualities. In addition to its angiogenic properties, low-molecular-weight HA may also have pro-inflammatory properties. As a result, it can affect a wide range of

physiological or pathological processes, including angiogenesis, inflammation, and wound healing.

An essential part of the healing process of wounds is played by HA. There has been a steady rise in interest in HA products due to their diverse biological activities and physiological roles. HA can keep an environment wet, which encourages healing and increases the development of keratinocytes, fibroblasts, and growth factors. HA promotes cell motility by absorbing exudate and is extremely hydrophilic. Regarding wound healing, it has positive effects that reduce inflammation, regulate tissue remodeling, and increase angiogenesis when combined with HA [124]. As a major constituent of the extracellular matrix (ECM) of connective tissue in all vertebrates, HA is essential for wound healing because it promotes the production of fibrin clots and the release of proinflammatory cytokines and interleukins. Because of its hemophilicity, biocompatibility, and easily adjustable chemical properties, HA-based wound dressings have been produced to date in a range of shapes and sizes (scaffolds, films, fibers, non-woven fabrics, etc.). Moreover, HA has a significant bacteriostatic effect by aiding in the decrease of bacterial adherence and biofilm formation, especially greater molecular weight HA. This is a beneficial feature for wound healing because bacterial infections can obstruct the healing process [125].

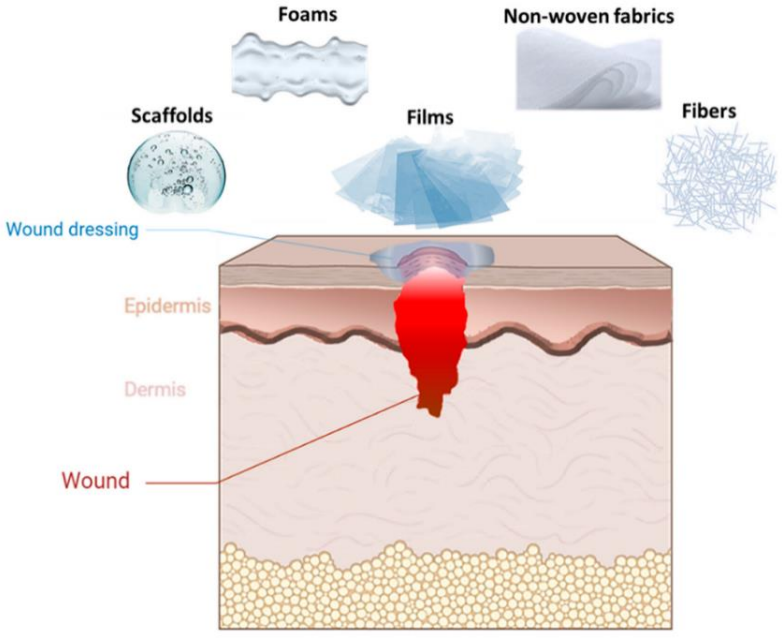

Figure 12: *Application of Hyaluronic Acid in Wound Healing* [125].

2.6.2 PVA and Hyaluronic Acid as a potential Biomaterial Systems

Excellent mechanical qualities and potential uses in biological and medical fields characterize functionally modified polyvinyl alcohol (PVA). Hyaluronic acid (HA), a polysaccharide polymer and a fundamental component of human tissue is used to create biomaterials because of its remarkable biocompatibility and biodegradation properties. The HA-based hydrogels may find use in tissue engineering, 3-D printing, and wound healing. It is utilized as a scaffold in numerous injectable medication delivery systems due to its quick biodegradation. Contemporary materials combining natural and synthetic polymers show promise for a range of biomedical uses. PVA and HA hydrogels together may be good options because of their superior mechanical and biocompatibility properties. These materials could find application in many biomaterial systems due to their variable functionality.

PVA and HA hydrogels that have been developed for a variety of uses in the form of semi-permeable and inter-permeable networks can be applied to a range of biological applications. At this point, understanding the mechanism underlying the crosslinking interaction between PVA and HA is essential. When glutaraldehyde and PVA crosslink, the glutaraldehyde first assumes the form of hemiacetal when an acid is present. Hemiketal's now experience an elimination reaction, losing the oxygen atom that was formerly a part of the carbonyl group of the parent aldehyde. The steps involved in this reaction would be as follows: 1) protonation of the hemiacetal's hydroxyl group 2) Water loss by excretion 3) PVA is added to the oxonium ion, rupturing the π link. 4) Acetal is produced by losing a proton. Divinyl sulfone crosslinks are said to develop in the case of HA during the crosslinking reaction with divinyl sulfone in an alkaline environment. It is claimed that bridge formation is caused by the –OH group in primary alcohol. Deprotonation of the hydroxyl group in the alkaline medium is the initial stage of the process. Next, each deprotonated OH interacts with the vinyl sulfone group of DVS's electron-deficient double bond [126].

2.7 RESEARCH GAP

Many hydrogels face challenges related to biocompatibility and the potential for foreign body reactions. Also, most of the hydrogels commercially available these days lack somehow in their absorption abilities. Research into novel hydrogel compositions and structures that minimize inflammation and promote tissue integration along with the release of growth factors, antimicrobial agents, or pain relief drugs in a controlled manner to help promote wound healing and prevent infections is a prime focus. This can be achieved by designing smart nanocomposite-based hydrogels by incorporating such materials that aid in increasing the porosity of hydrogels so that the goal of medium to maximum exudate absorption can be achieved easily. With the rising concern of antibiotic-resistant bacteria, creating hydrogels with inherent antimicrobial properties that can prevent or treat infections in chronic wounds is vital. Along with that research into hydrogel stability and long-term performance with tunable mechanical properties to match various wound types along with cost efficiency and patient comfort, ease of application, and wearability is an important aspect of wound dressings and is an ongoing challenge. Through literature it is discovered that the potential of MOF-199, metal organic framework as a highly porous structure is discovered and it is studied that how the incorporation of even the slightest amount of it can affect the overall morphology of hydrogels and how it can help in increasing the exudate absorption of membranes. As the biomedical application of MOF-199 is not much studied, the following study is to cover this research gap. Further, hyaluronic acid as a most beneficial component of our skin structure is also not much reported in literature, so the following study is also to cover the research gap regarding the use of HA as a biodegradable biopolymer so that the valuable characterization results can be published on such materials that are not under much study is also covered in this research.

2.8 OBJECTIVES

The main objective of this research is as follows:

- To develop a smart hydrogel that contributes to treating different types of wounds by maximizing the absorption capacity of hydrogel membranes.
- Synthesis and characterization of MOF-199 to meet the exudate absorption gap due to its highly porous structure.
- Synthesis and characterization of AgNPs to add antibacterial property to the hydrogel.
- Synthesis of nanocomposite (MOF-199 & AgNPs) based hydrogel membrane and its characterization.
- Antimicrobial and physical characterization of hydrogel membranes to know its medical efficacy.

CHAPTER 3: MATERIALS AND METHODS

The following materials are used for the complete synthesis of hydrogel films:

- Polyvinyl Alcohol (MW: 145000g/mol) was purchased from Sigma Aldrich
- Hyaluronic Acid (purity: 97%) was obtained from scientific hub
- Glycerin (molecular weight: MW: 92.09g/mol)
- Silver Nitrate ($AgNO_3$) from Sigma Aldrich
- Sodium Acetate from Sigma Aldrich
- Copper Nitrate from Sigma Aldrich
- DMF from Sigma Aldrich
- Ethanol from Sigma Aldrich
- Deionized water was used throughout the experiment

3.1 Synthesis of Silver Nanoparticles (AgNPs):

Silver Nanoparticles (AgNPs) synthesized using a chemical reduction method in which Hydrazine hydrate ($N_2H_4.H_2O$) was employed as a reducing agent. An aqueous solution of 4.3mM of $AgNO_3$ and 0.05M (0.2721g) of sodium acetate were prepared separately with 40 ml of deionized water and stirred simultaneously. Upon addition of 0.02ml of $N_2H_4.H_2O$ (98%) to the above mixture with vigorous stirring, the solution rapidly turned black, indicating the reduction of silver ions to silver metal. After 2-3 hours of stirring, the solution became transparent with visible, fine silver particles. These particles were isolated by filtration, washed three times with deionized water. Finally dried overnight at 60 degrees [127].

3.2 Synthesis of MOF-199:

MOF-199 was made by a hydrothermal method. First, 22.5 ml of absolute ethanol and 22.5ml of DMF were added to 1.50g BTC. Then, 3.11g $Cu(NO_3)_2$ is added with 22.5ml of DI water. Both solutions stir for 5 minutes until they are homogeneously mixed.

Then, add Cu $(NO_3)_2$ mixture to BTC followed by 15 minutes of stirring. Then, the solution is placed in an autoclave overnight at 60 °C. Finally, the formed blue crystals are filtered, washed and vacuum dried for 12 hrs. at 60°C [128].

3.3 Synthesis of Hydrogel Membranes:

3.3.1 PVA/HA Blank Film

To create polymeric films, 2.5 % w/w HA and 15 % w/w PVA (MW: 145000g/mol) solutions were mixed in various ratios. A 4 ml PVA/HA blank film is developed by solution casting technique, for this both polymeric solutions were prepared separately in DI water under magnetic stirring (100 rpm) at room temperature. 150 mg PVA is taken and is stirred at 500 rpm and heated at 90◦C to ensure homogeneity. After cooling the PVA solution, 105 mg HA solution was added into it while stirring (100 rpm) for 5 min. Finally, 28mg of glycerin is added to the above mixture with gentle stirring. The mixture was made to stand for 24h followed by spreading it on the glass plate and dried in oven at 70◦C for 1h [129].

3.3.2 PVA/HA/AgNPs Membrane

A membrane made of nanocomposite hydrogel was created using the solution casting method. 15% w/w PVA solution was prepared on heating at 90◦C in DI water at room temperature. After cooling the PVA solution 50ul AgNPs were added while stirring (100 rpm) for 10 min. Then dissolve 2.5 % w/w HA solution in the above solution followed by gentle stirring. At the end glycerin is added as a plasticizer to give final shape to the hydrogel film. Rest the mixture for 24 hours. Afterwards it is cast on the petri dish and dried overnight at 70◦C for 1h.

3.3.3 PVA/HA/AgNPs/MOF-199 Membranes

Solution casting was employed in the creation of hydrogels. Initially, 15% w/w PVA solution is prepared in DI water at 90◦C and stirred at 500rpm until homogenous mixture is obtained. After cooling, AgNPs were added and stirred for 5 min followed by adding different concentrations of MOF-199 (0.05%, 0.08%, 0.1%) into the solution mixture accompanied by gentle stirring (100 rpm) for 5 mins each. The HA solution is dissolved in the above mixture while gently stirred for 5 min. To create a crosslinker

solution, 28mg of Glycerin was dissolved in the above mixture. After placing the mixture for 24 hours, it is casted in the petri dish and then dried at 70◦C for 1h. The composition of the films is detailed in Table 1.

TABLE 1: COMPOSITION OF HYDROGEL FILMS

Hydrogel Compositions	**HA**	**PVA**	**DI Water**	**Glycerol**	**AgNPs**	**MOF-199**
H1	105mg	150mg	4ml	28mg	50 µL	-
H2	105mg	150mg	4ml	28mg	50 µL	-
H3	105mg	150mg	4ml	28mg	50 µL	0.05%
H4	105mg	150mg	4ml	28mg	50 µL	0.08%
H5	105mg	150mg	4ml	28mg	50 µL	0.10%

3.4 Characterization Techniques

3.4.1 X-ray Diffraction (XRD)

The XRD analysis was performed using the STORE-Germany instrument to analyze the membrane's nanocomposite properties and crystallinity. An acceleration voltage of 40 kV and a current of 40 mA were used to obtain the X-ray diffraction (XRD) patterns. The step size was set at 0.04 s, while the scan speed was set to 0.5 s. The measurement was performed using 1.54060-wavelength Cu-K radiation.

Objectives of XRD

- Identification of the materials' crystal structure and lattice characteristics.
- Clarifies the crystallographic architecture of a particular material.
- Recognition of various polymeric structures in fingerprints
- Distinguishing crystalline and amorphous forms
- Determination of the specimen's percentage crystallinity

3.4.2 Fourier Transform Infrared Spectroscopy (FTIR)

Using the attenuated total reflectance (ATR) technique (ATR-FTIR, BRUKER), Fourier transform infrared spectroscopy (FTIR) was used to examine the functional groups found in the nanocomposite membranes. The FTIR spectra were acquired at a resolution of 4 cm-1 and a scanning frequency of 32 in the 500–4000 cm^{-1} range. The following method was also used to study the molecular makeup and structures of the nanocellulose and nanoparticles in the samples.

Objectives of FTIR

- Characterize unidentified materials
- Identify molecules that are polymeric, organic, or occasionally inorganic.
- Determine any contamination (on the materials or in them).
- Determine oxidation, decomposition, or uncured compounds in failure analysis.

3.4.3 Scanning Electron Microscopy (SEM)

An energy-dispersive X-ray (EDX) spectrometer and a scanning electron microscope (JSM-64900) were used to examine the surface morphology of hydrogel films. Before examination, a thin layer of palladium/platinum was added to the test sample to improve conductivity. Throughout the investigation, a 20 kV acceleration voltage was used, and an additional electron locator was employed to view the surface structure.

Objectives of SEM

- A scanning electron microscopes (SEM) goals are as follows:
- **High-Resolution Imaging:** To produce richly detailed, highly magnified, and high-resolution images of a specimen's surface.
- **Surface Topography Analysis:** To investigate the micro- and nanoscale surface characteristics and texture of materials.
- **Elemental content Analysis:** Using energy-dispersive X-ray spectroscopy (EDS) coupled with the SEM, ascertain the elemental content of a sample.
- **Morphological Studies:** To examine how particles or structures are arranged, sized, and shaped inside a specimen.

- **Material Characterization:** To research the characteristics and actions of various materials, such as metals, ceramics, polymers, and living things.
- **Failure Analysis:** Using fracture surfaces and flaws as a guide, this technique looks at the reasons behind material failure.

3.5 Physical Characterization of a Hydrogel Wound Dressing

3.5.1 Mechanically Testing

The hydrogel membranes' tensile characteristics were evaluated by the utilization of Shimadzu Corporation's Trapezium-X Universal Testing Machine (AG 20RRKNXD Plus). Using the membranes, rectangular specimens of 20 mm in length and 7 mm in width were created for testing. The experiments were conducted at a crosshead speed of 10 mm/min and a gauge length of 40 mm, as recommended by previous studies [114]. Every membrane was tested three times, and the average value was noted, to guarantee the precision and dependability of the outcomes.

Objectives of UTM

- **Tensile test:** Determines how strong a material is when pulled apart.
- **Compressive Test:** Determines how strong a material is under compression.
- **Flexural Test:** Determines how resistant a material is to distortion under load.
- **Shear Test:** The material's resistance to forces that could cause layers to slide against one another is determined by the shear test.

3.5.2 Swelling Test

Hydrogel membranes, both pure and nanocomposites, were tested for their ability to absorb water using distilled water, 0.9% NaCl and 0.9% $MgCl_2$ [130]. Since these salts are the main components of bodily fluids, the swelling tendency of the nanocomposite's membrane was measured using the NaCl and $MgCl_2$ solutions. While the pH values of 0.9% NaCl and 0.9% $MgCl_2$ were 6.47 and 6.70, respectively, the pH of the distilled water was 7.18. The hydrogel membrane of nanocomposites with a uniform thickness of 0.16 mm was divided into equal pieces of 1x1 cm^2 and weighed. For twenty-four hours, the previously mentioned salt solution, water, and blood were submerged in each piece.

Followed by equilibrium (24h), the hydrogel membrane pieces were taken out of the corresponding fluids and carefully blotted with cellulose paper to remove any excess water that was on their surface before being weighed once more. Shortly after the synthesis, the newly manufactured (dried) membranes were used for the test. As a result, drying the membrane before the test was conducted was not necessary. The following formula was used to estimate the water uptake ability:

$$\textbf{Degree of Swelling (\%)} = (W_e - W_o)/W_o \times 100$$

Where W_o is the initial weight of the dry hydrogel membrane, and We is the weight of the expanded hydrogel membrane after 24 hours [130]. To find the mean value, three duplicates of each sample were taken.

3.5.3 Water Solubility or Gelation Test

The PVA/Hyaluronic Acid hydrogel's level of crosslinking was determined using the gel fractionation test. Because they both have hydroxyl groups on their surfaces, PVA and hyaluronic acid are soluble in water and have a significant propensity to absorb water. Equally thick hydrogel membranes from both the pristine and nanocomposites were divided into equal portions measuring 1 × 1 cm before being weighed. When they reach a constant weight, these pieces of the same thickness were put in a vacuum oven set at 40 °C and 36 mbar of pressure. All membrane samples reached similar weights after 12 hours. After being submerged in distilled water for four days, the hydrogel membranes were once more placed in a vacuum oven until they reached a consistent weight. The membrane samples were able to reach their constant weight after 9h. After being removed from the oven, they were weighed. The following formula was used to calculate the membrane's gel fractionation:

$$\textbf{Gel fraction (\%) is equal to } (W_t/W_i) \times 100.$$

Where Wi is the initial weight of the membranes after reaching equilibrium weight and Wt is the sample's final weight[104]. To find the mean value, three replicates of each sample were taken.

3.5.4 Moisture Retention Capability Test

Both PVA and Hyaluronic Acid have a significant propensity to absorb water. With a few minor adjustments, the moisture retention test was carried out in accordance with the previously published procedure [131]. Equally thick hydrogel membranes, both pristine and nanocomposites, were divided into equal sections measuring 1 × 1cm and equal weighted. After that, these samples were baked for six hours at 40 °C. After six hours, the membranes were removed from the oven and weighed. The following formula was used to determine the synthetic hydrogel membranes' moisture retention capacity:

Capacity to retain moisture (%) = (Wt/Wi) × 100

Where Wt is the hydrogel membranes' ultimate weight after six hours and Wi is their starting weight. Every measurement was made three times.

3.5.5 Antibacterial Test

The disc diffusion method was followed by conducting the antibacterial test. Two strains of bacteria, gram-positive Staphylococcus aureus and gram-negative Pseudomonas Aeruginosa, were cultured for 24 hours at 37 °C in nutritional broth. After making agar broth, agar plates were incubated for twenty-four hours. The agar plates were taken out of the incubator after 24 hours, and microbial strains were evenly distributed over the agar. After inserting the hydrogel membranes with discs and 50 μL Ag NPs into the agar, the antibacterial activity data was taken 24 hours later. Moreover, dried membranes were used in the antibacterial test, which involved cutting the membrane into round discs to combat S. aureus and E. coli. After 24 hours, the antibacterial activity of the membrane portions was evaluated on an agar plate.

CHAPTER 4: RESULTS AND DISCUSSION

4.1 XRD Analysis

X-ray diffraction (XRD) analysis was performed to study the structural formation of the prepared materials with diffracted intensities recorded at the range of 0°-90°. The XRD patterns of AgNps, MOF-199 and all hydrogels are shown in (fig. 1). XRD analysis of AgNPs showed strong Bragg reflections at 30.8°, 40.5°, 60.5° and 70.8°indexed to the crystal planes (111), (200), (220), (311)[127]. The XRD analysis of MOF-199 showed peaks at (9.2°), (11.1°), (13.1°), (14.39°), (16.5°), (17.02°), (23.05°), (25.8°), (29.17°), (35.15°) indexed to the crystal planes at (220), (222), (420), (400), (331), (422), (511), (551), (751), (951) [128]. The diffraction peaks for PVA appear at 19.5° (101) that is not sharp representing its semicrystalline nature [132]. Similarly, the XRD peak for HA is at 20° (200) that is more like a hump is assigning the amorphous nature of hyaluronic acid [133]. Analyzing the peaks for all the hydrogels, similar peaks are present in a region around 20° that is an indication of amorphous nature of polymers present in the hydrogels. As both PVA and HA polymers are present in greater quantity as compared to the AgNPs and MOF-199, so their peaks are more significant in the XRD pattern and overall represent the semi-crystalline nature of hydrogels.

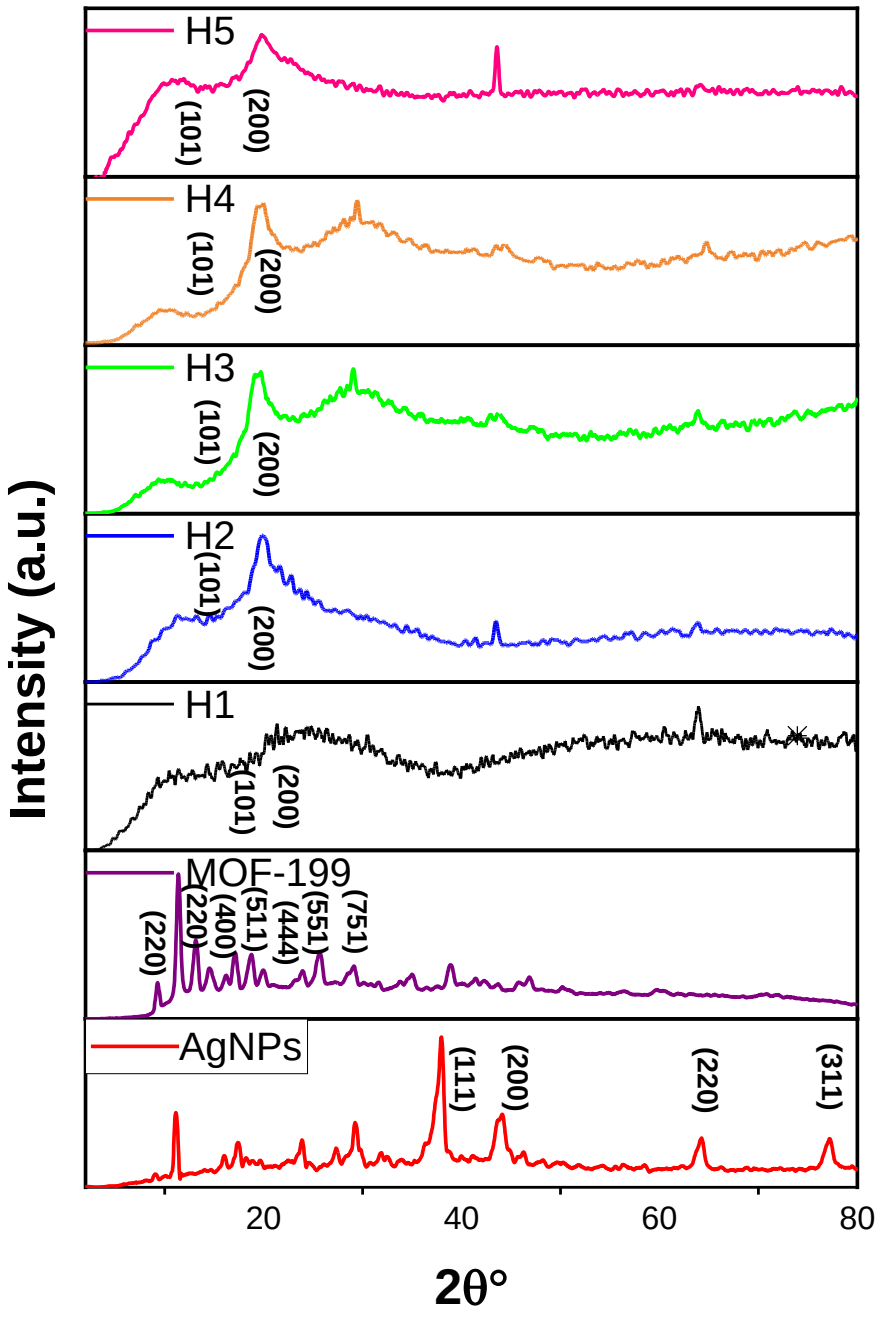

Figure 13: XRD Spectrums of a) AgNPs b) MOF-199 c) H1 d) H2 e) H3 f) H4 g) H5.

4.2 FTIR Analysis

FTIR spectroscopic analysis of all the hydrogels was carried out to identify their structural characteristics to confirm the available functional groups present in it (fig. 14). PVA is used as a matrix material to synthesize hydrogel membranes, HA is used as a biopolymer for tissue regeneration and MOF-199 as a reinforcement material. The spectrum showed specific similar peaks around 3435, 3411, 2418, 3419 cm^{-1} which is an indication of OH stretching present in the structure of PVA [134], HA [135] and in MOF-199 [128]. Second similar peaks that are present in all the spectrums are 2925, 2929, 2921, 2922 cm^{-1} that is an indication of asymmetric CH_2 stretching from an alkyl group of PVA, acetamido group of HA and phenyl group of MOF-199. Another similar peak that is available in all the spectrums is 1644, 1624, 1625, 1629, 1630 cm^{-1} that is due to the C=O stretching of acetamido group of HA and carboxylate group of MOF-199. The spectrum also shows alike peaks at 1419, 1441, 1451, 1428, 1446 cm^{-1} that is due to bending of CH_2 groups present in PVA, HA and in MOF-199. The peaks at 1059, 1056, 1101 cm^{-1} are due to stretching of C=O and bending of OH bonds present in PVA, HA and MOF-199. Other peaks at 854, 618 and 760 cm^{-1} are due to C-C stretching.

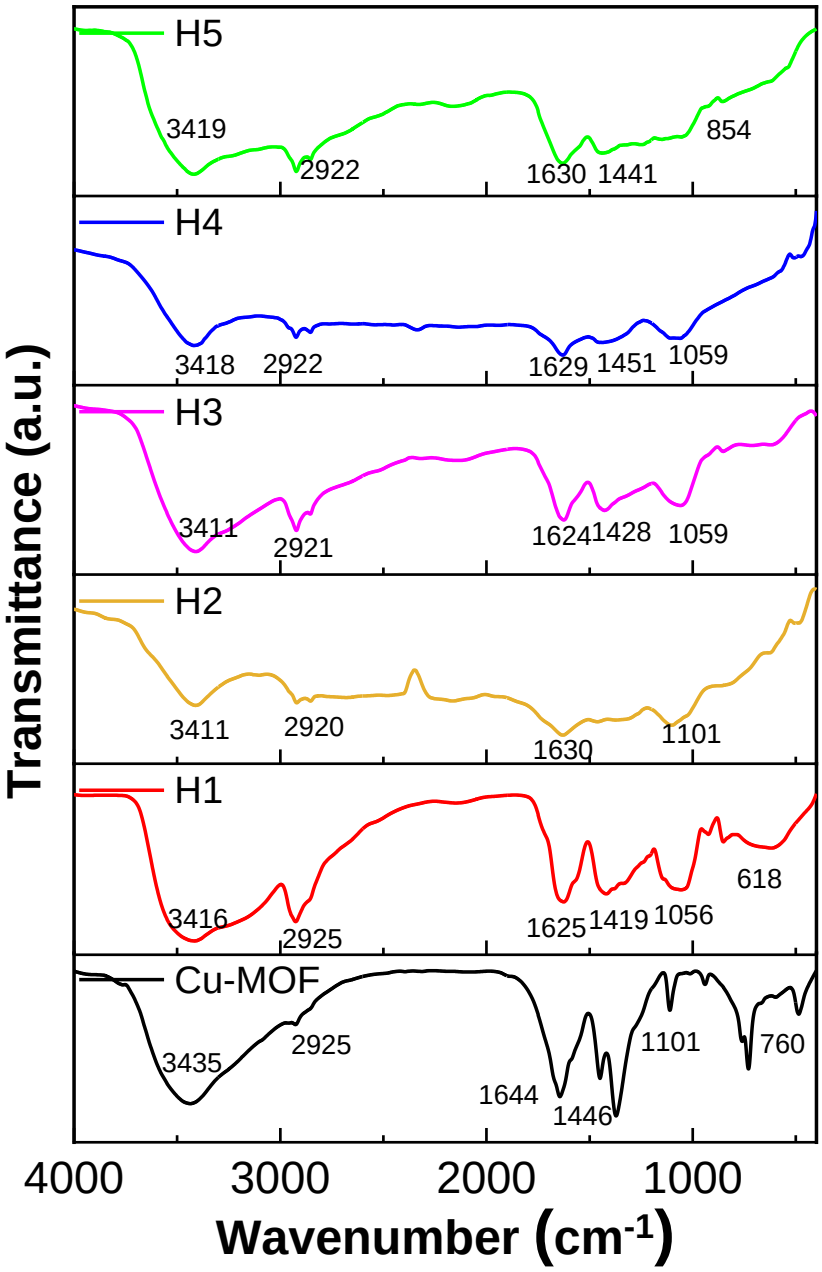

Figure 14: FTIR Spectrums of a) MOF-199 b) H1 c) H2 d) H3 e) H4 f) H5.

4.3 SEM Analysis

SEM analysis is performed to examine the surface morphology and cross-section of formulated membranes. SEM findings of all the materials formed are displayed in fig.15. The results in fig.15(a) show uniformly dispersed AgNPs with an average size of 26.3 nm. Fig. 15(b) represents the nanoscale image of MOF-199 that is a highly porous structure due to the metal ions and organic linker frameworks, and its porosity can be confirmed by a SEM image in fig.15(c). Both AgNPs and MOF-199 are loaded to form the biocompatible hydrogel membranes.

The results in fig.15(e) demonstrated that the combination of PVA/Glycerin hydrogels would lead to a good absorbent thin film that transforms wound exudates into the hydrogel matrices and supports wound healing by providing a clean, moist environment to the wound site [136]. The addition of hyaluronic acid to the PVA matrix would lead to a stable polymeric network as PVA's hydroxyl groups give it a semi-crystalline structure that contributes to the physical crosslinking and hydrogen bonding. On the other hand, a naturally occurring polysaccharide i-e hyaluronic acid forms a loose, water-absorbing matrix that enhances the structure's hydrophilicity and biocompatibility of the overall membrane giving it a malleable matrix. As a result, a stable polymer network is formed, crucial to maintain the hydrogel's integrity.

As seen in the fig.15(f) of H2 membrane it can be possible that AgNPs can provide extra porosity to the membrane by acting as nucleation sites during the gelation or physical crosslinking process [137]. Also, it can be possible that AgNPs can disrupt the polymer chains' packing, creating pores in the structure. Three different concentrations of MOF-199 are varied in the H3, H4 and H5 membranes to check the concentration effect on the porosity of the hydrogel membrane. As, MOFs are highly porous structures that can have a range of pore shapes in addition to high porosity [32]. Thus, it can be seen in fig.15(g, h, i) MOF-199 has participated in increasing the overall porosity of the hydrogel membrane. As, H3 (0.05% MOF-199) fig.15(f) shows more porous structure as compared to H2 fig. 15(e) with only AgNPs incorporated inside the PVA/HA membrane. Similarly, on increasing the concentration of MOF-199 in H4 (0.08% MOF-199), the number of pores seems to be increased as shown in fig.15(g). It is further confirmed by cross

sectional SEM analysis of H4 that shows clear porous structure inside the membrane fig.15(i) but on further increasing the concentration of MOF-199 in H5 (0.1% MOF-199) fig.15(h) porosity does not seem to be increased but caused the hydrogels to become dense and that pores are absent. It may depict the optimum limit that is no further loading of MOF-199 is favored on the hydrogel membrane at this concentration.

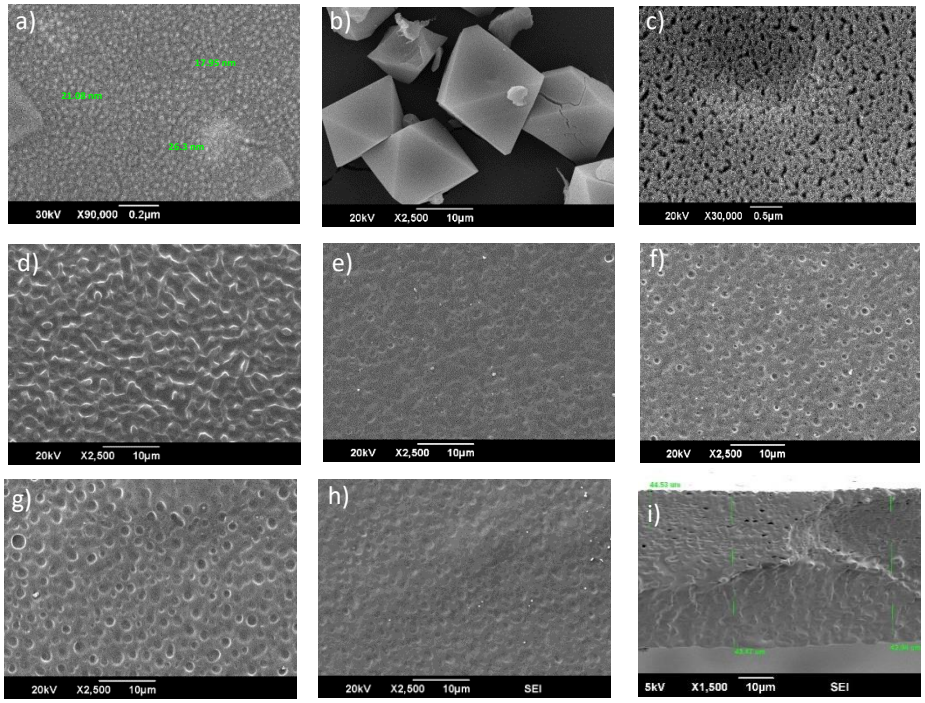

Figure 15: (a) SEM images of AgNPs (b) MOF-199 (c) Porous structure of MOF-199 (d) H1 hydrogel (e) H2 hydrogel (f) H3 hydrogel g) H4 hydrogel (h) H5 hydrogel i) Cross sectional analysis of H4 hydrogel.

4.4 Physical Analysis of Hydrogels

4.4.1 Mechanically Testing

In case of H1 (pure PVA/HA) hydrogel exhibits a moderate stress-strain behavior (black curve), peaking at a strain of about 1.25 and a maximum stress just over 5 N/mm² as shown in fig.16. Prior to failure (break point at around 1.5 strain), the behavior is typical for a hydrogel with moderate elasticity and respectable mechanical strength. It is because it lacks extra points to a less porous which means it forms a more homogeneous structure with moderate mechanical strength and brittle structure.

While in H2 (PVA/HA/AgNPs) hydrogel the maximum stress has increased to around 6.5 N/mm² (red curve) due to a small alteration in the mechanical characteristics caused by the introduction of silver nanoparticles. AgNPs nanoparticles add reinforcement effect to the hydrogels by strengthening microscopic networks. Adding AgNPs not only improves the mechanical characteristics but also increases porosity thus enhancing load distribution in hydrogels as demonstrated by the higher maximum stress in comparison to H1. The failure point, however, happens at a comparable strain level (around 1.6), suggesting that even as the stress capacity rises, the stretchability remains constant.

In case of H3 (PVA/HA/AgNPs/0.1mgMOF-199) the greater maximum stress (~7.8 N/mm^2) indicates a considerable increase in mechanical strength with the addition of 0.1 mg of MOF-199 (blue curve). The hydrogel has grown stronger and more elastic as seen by the strain at break increasing as well (~3.5). This implies that a more robust structure and higher porosity have been produced by adding 0.1 mg MOF-199. It is well known that MOF-199 particles create stiff frameworks that enhance both elasticity and strength.

In H4 (PVA/HA/AgNPs/0.2mgMOF-199) hydrogel the mechanical strength reaches a peak of around 9 N/mm² at 0.2 mg MOF-199. It is because of the ideal porosity that MOF-199 creates, the strain at break occurs at about 4.0, indicating that the hydrogel becomes much stretchier and more durable. This exhibits the optimal strength-to-elasticity ratio, indicating that this concentration improves the network's ability to extend without breaking too soon.

Remarkably, in H5 (PVA/HA/AgNPs/0.4mgMOF-199) hydrogel the strain at break is greatly reduced (~1.2) and the maximum stress drops (around 5.5 N/mm²) when the concentration of MOF-199 is increased to 0.4 mg (purple curve) as, H5 is showing almost the same trend as in H1 This decline in mechanical performance most likely results from overuse of MOF-199, which also decreases porosity and weakens the hydrogel's overall structural integrity, making it more brittle. The system may become less flexible if MOF-199 is overloaded, which could result in an early mechanical failure.

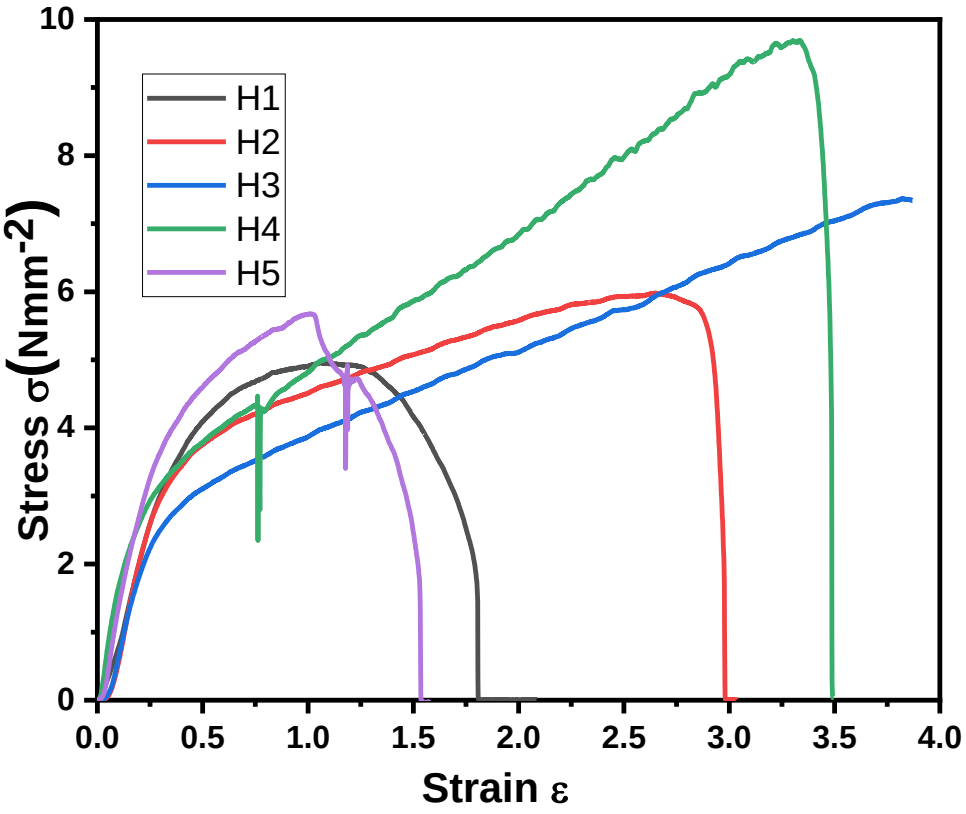

Figure 16: Mechanical Behavior of Hydrogels.

4.4.2 Swelling Test

An ideal wound dressing should promote skin cell proliferation along with the ability to both absorb exudates from the wound surface and create a moist environment around the wound site. Hyaluronic Acid and PVA both are hydrophilic in nature and promote water absorption due to the presence of (-OH) groups in their structures. The hydrogel nanocomposites' ability to form hydrogen bonds is further enhanced by the addition of glycerol. As a result, the hydrogel membrane from the nanocomposites absorbed more water than the pure membrane and the trend is especially pronounced in the case of hydrogel membranes containing AgNPs and MOF-199 as compared to the H1 (pure PVA/HA) membrane. In H2 AgNPs present at the intercalation sites may be the cause of more absorptions of fluids (H_2O, NaCl and $MgCl_2$) [138], by interacting with PVA and HA crosslinking sites AgNPs raise the total -OH levels. The H3 membrane's propensity to swell increased as the concentration of MOF-199(0.1mg) is added to it. This is because MOF-199 is a highly porous structure that adds to the overall absorption

capacity of hydrogel membrane. On further increasing the concentration of MOF-199(0.2mg) in H4 membrane results clearly showed an excellent absorption capacity for three fluids i-e H_2O, NaCl and $MgCl_2$. The trend in fig. 15 showed an unexpected decrease in case of H5 that is on further increasing the concentration of MOF-199(0.4mg) in the hydrogel, nanoparticles instead of adding the reinforcement effect inside the membrane thus made the membrane more brittle i-e non-porous structure can be seen from the SEM images that is further confirmed by the mechanical testing trend in fig. 17.

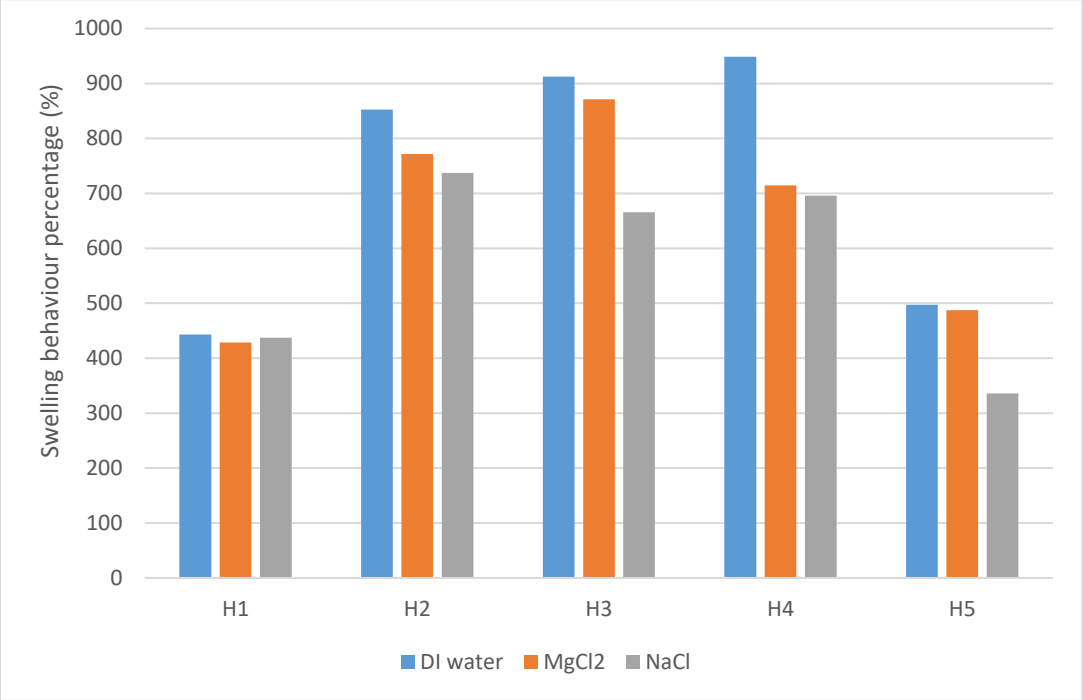

Figure 17: Swelling Behavior of Hydrogels in DI Water, NaCl and $MgCl_2$.

TABLE 2: SWELLING RATIOS (%) OF HYDROGELS IN DI, NaCl and $MgCl_2$

FLUIDS	H1	H2	H3	H4	H5
DI Water	442.85	852.85	912.85	948.57	497.142
MgCl2	428.57	771.42	871.42	714.28	487.142
NaCl	437.14	737.14	665.71	712.857	335.714

4.4.3 Water Solubility or Gelation Test

A membrane used as a wound dressing should not dissolve in water. PVA and HA combine to produce a well-defined, cross-linked network. H1 is likely to have a distinct gel point because of the cross-linking of PVA and hyaluronic acid so it is anticipated that the gelation time will be reasonable and free from significant variations. In H2 in which AgNPs are added, which causes the polymer matrix to become less homogeneous and acts as nucleation sites, increasing the porosity of the hydrogel. A larger surface area is accessible for cross-linking because of the increased porosity, which also improves the swelling capacity and permits more solvent uptake. Since the increased porosity enhances the network's capacity to generate cross-links, the percentage of gelation increases in H2 as compared to H1. H3 includes the integration of a tiny amount (0.1 mg) of MOF-199, which further increases the hydrogel's porosity because of the highly porous structure of MOF-199. A more open structure brought about by the increased porosity makes more cross-linking sites within the hydrogel network accessible. Because the open structure allows for more contact between polymer chains and hence increased cross-linking effectiveness, the gelation percentage increases further in H3. Similarly, as in H4 that has a larger concentration of MOF-199 (0.2 mg), the hydrogel's porosity is much increased. More MOF-199 in the network means that there are more voids and cavities, which promotes more contact between the polymer chains and accelerates the cross-linking process. The highest porosity at this point optimizes the network construction, allowing a high degree of gelation, which is why the gelation % peaks in H4. Inverse happens in case

of H5 where on further increasing the concentration of concentration of MOF-199 by o.4mg makes the structure more brittle, hence the decrease in porosity would lead to a more brittle structure as discussed above (SEM & Mechanical testing) results. Thus, the percentage decreased due to less cross-linking sites within the polymer matrix that would lead to less voids between the nanoparticles and polymer chains as shown in trend in fig. 18.

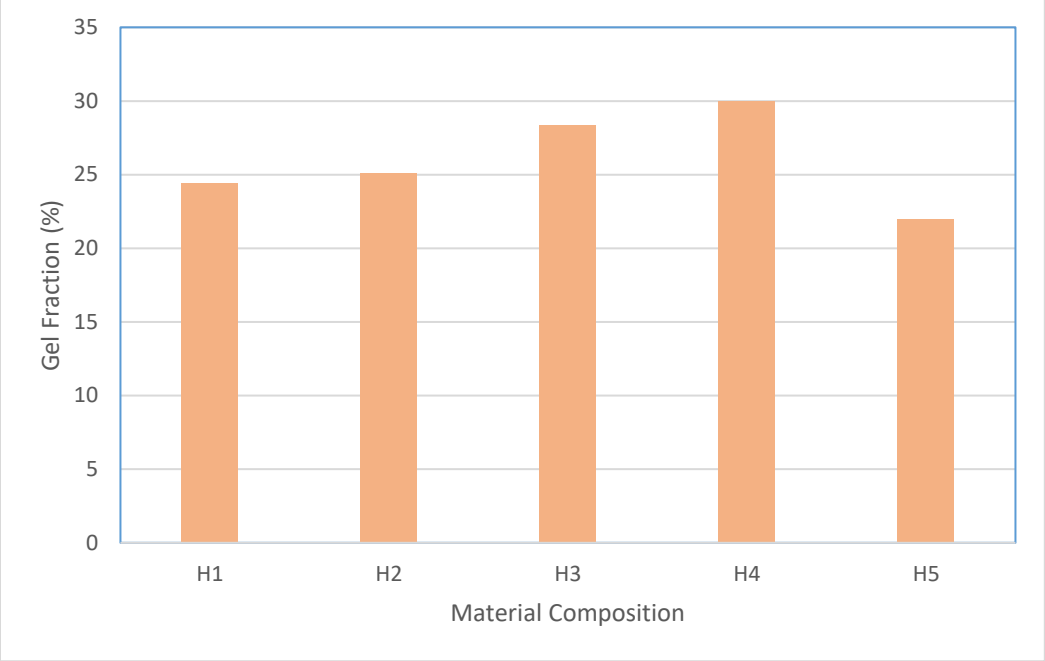

Figure 18: Swelling Behavior of Hydrogels in DI Water, NaCl and $MgCl_2$.

4.4.4 Moisture Retention Capability

It was shown in the trend in (fig.19) as AgNPs and MOF-199 concentrations rose, the membrane's capacity to retain moisture increased as well from H1 to H4 as shown in the trend in (fig.19). Crosslinking produced three-dimensional structures that aided in the membrane's ability to lose water [139, 140] that is a great benefit of nanocomposite formation that adds reinforcement effect to the membrane. The inclusion of NPs may have assisted in preventing the creation of these structures, leading to more smooth membranes with less water loss, except in H5 in which the ability to retain water is reduced this is

because as the porosity is decreased as discussed in mechanical test discussion, the increase in MOF-199 concentration to 0.1mg is making the structure more brittle.

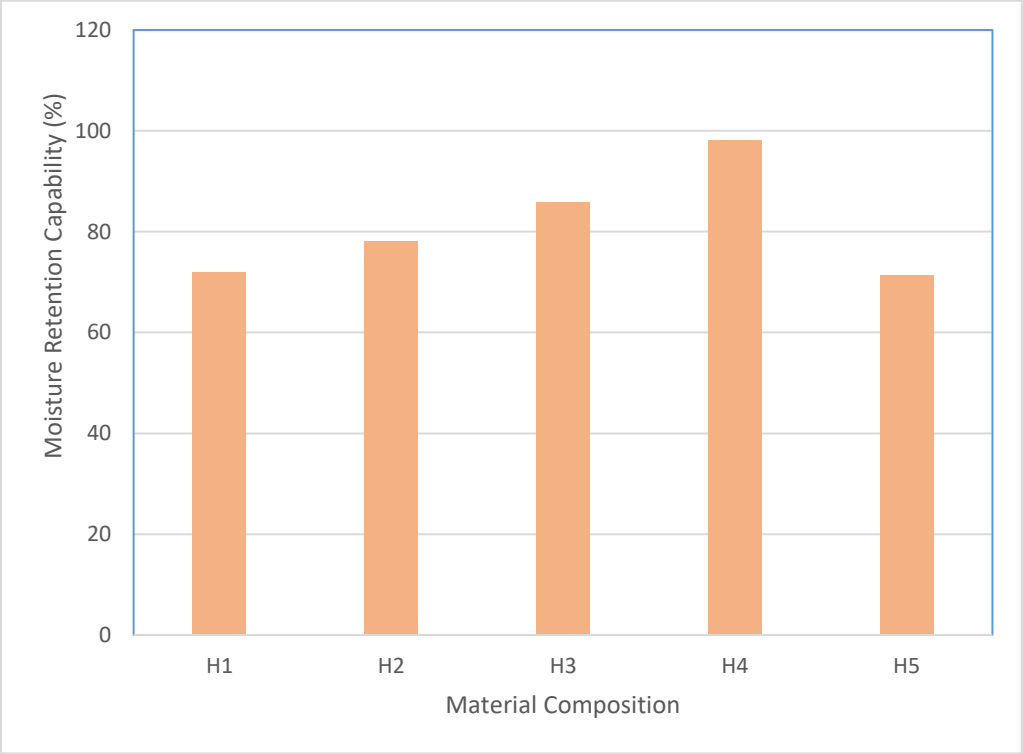

Figure 19: Moisture retention capability of Hydrogels.

4.4.5 Antibacterial Test

The antibacterial experiment is performed against S. aureus and Pseudomonas Aeruginosa as a gram-positive and gram-negative bacterium. Silver nanoparticles (AgNPs), which are well known for their strong antibacterial capabilities, were added to the hydrogels. In this experiment, the zone of inhibition generated by Augmentin (350 mg), an antibiotic, was compared to that created by AgNPs. AgNPs were consistently added to the hydrogels from samples H2 to H5 in an amount of 0.5 µL as shown in trend in (fig. 20). Due to the steady AgNPs content in these samples, the resulting zone of inhibition stayed quite consistent. From H2 to H3 the trend decreased. This may be due to the releasing factor that may be because as the concentration of MOF-199 is increasing that is making the compact nanocomposite hydrogel structure that may be reducing the releasing rate. On the other hand, hydrogel H5 showed a discernible decrease in the zone of inhibition, which was caused by the enhanced brittleness in hydrogel structure's which

may have prevented the AgNPs from being released.

AgNPs' antibacterial action is ascribed to their oxidative characteristics, specifically the oxidative stress that is caused by reactive oxygen species (ROS). AgNPs have been shown to produce hydroxyl and superoxide radicals, which aid in their antibacterial properties. AgNPs can also target proteins and bacterial membranes, exhibiting peroxidase-like activity and causing bacterial cell death. Despite being a part of the hydrogels, MOF-199 does not appear to have a major impact on antibacterial activity. This is probably because of its low concentration. Additionally, some antibacterial activity was shown by the PVA/hyaluronic acid hydrogel itself, confirming that it is bactericidal[105] as it is reported in the paper that the inhibitory zones enhanced, when HA was added to PVA hydrogel as was shown with Staphylococcus aureus.

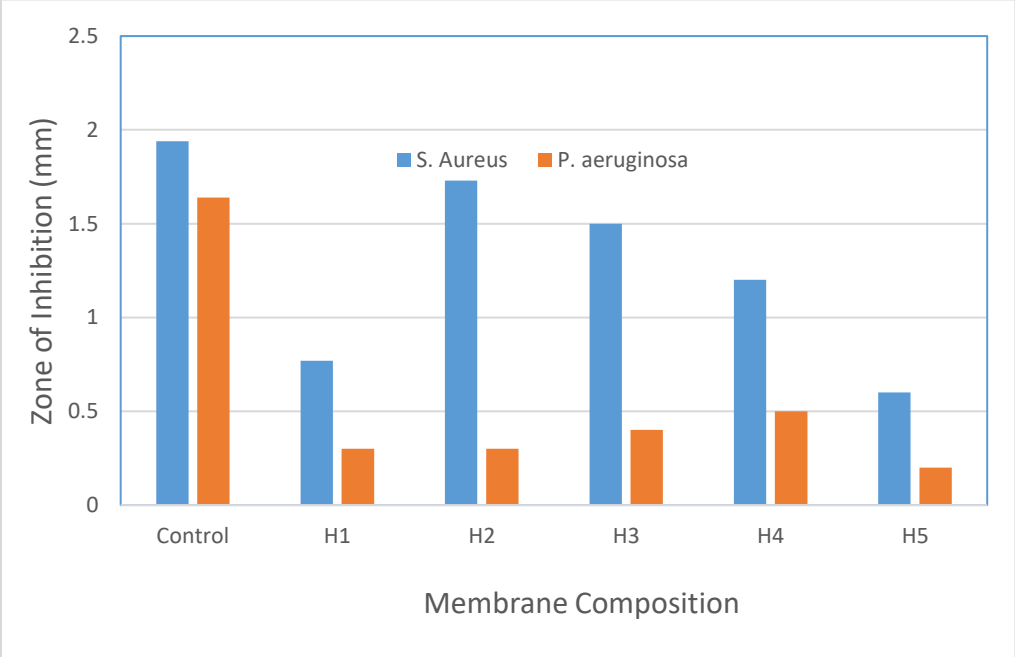

Figure20: Antibacterial Activity of hydrogels against A. Aureus and P. Aeruginosa.

CHAPTER 5: CONCLUSION

The following work conducted a comprehensive analysis of the development and assessment of PVA/HA hydrogel membranes loaded with AgNPs and MOF-199 that is crosslinked with glycerin, for potential use in wound healing. Hyaluronic Acid is used as a biopolymer for fast tissue regeneration abilities and PVA as a synthetic polymer was used to provide a matrix for membrane formation and material loading. The membranes showed favourable characteristics such hydrophilicity, excellent mechanical strength, flexibility, and high swelling behaviour with improved moisture retention ability when loaded with MOF-199. Significant inhibition was shown for both gram-positive and gram-negative bacteria prooving that the membranes have good antibacterial properties due to AgNPs. Majorly the effect of concentration of MOF-199 is varied and studied in the hydrogels to check how porosity can effect the absorption capacity of hydrogel membranes. SEM results have confirmed the porous structure formation inside the membrane due to the reinforcement effect of MOF-199 and AgNPs with hydrogel matrix.

The change in concentration effect was further analyzed by the mechanical test confirms that the flexibility and mechanical strength enhanced on increaisng the concentration of MOF-199 upto 0.08% but on further increasing the concentration the structure becomes brittle due to overloading of materials. The maximium fluid absorption is confirmed by the swelling test in which the H4(0.2mg) concentration has showed the maximum absorption of 940% in DI water. This means that all the components of the hydrogel that is OH groups of PVA, HA, Glycerin and MOF-199 along with AgNPs are aiding in enhancing the overall absorption capacity of hydrogel that is much beneficial for medium to high exudate absorption. All the results are further confirmed by the contact angle measurement test that shows as the concentration of MOF-199 is increased the hydrophilicity of membrane also increases due to the formation of more porous structure in the membranes and H4 is showing the best absorption, hydrophilicity and mechanical flexibility. Similarly, hydrogel H4 with 0.08% MOF-199 proved to be a best loaded concentration for MOF-199 showing the best swelling, mechanical and moisture retention ability.

FUTURE RECOMMENDATIONS

Future research should be done on the potential market for wound dressing.

1) The production of nanoparticles may be explored through green synthesis that can be more economical and eco-friendly descreasing the toxicity level of hydrogels for human skin.

2) The biomedical effects of MOF-199 can be further studied by varying its concentrations along with the different cytoxicity tests.

3) Different smart hydrogels can be made exploring the different polymer types by loading the MOF-199 and studying the release rate of Copper from membranes is further to be explored.

4) Different pH, temperature sensitive hydrogels can be developed with smart system that can check the wound conditions and can tell when the drug can be released according to wound conditions.

4) It is possible to perform biological characterization, which includes protein absorption, tissue growth, cell proliferation and animal model for the MOF-199 based hydrogels.

5) Different concentrations of MOF-199 can be placed into the matrix for drug delivery, and their release profiles can be examined.

REFERENCES

1. Huang, X., et al., *Influence of radiation crosslinked carboxymethyl-chitosan/gelatin hydrogel on cutaneous wound healing.* Materials science and engineering: C, 2013. **33**(8): p. 4816-4824.

2. He, J., et al., *Conductive adhesive self-healing nanocomposite hydrogel wound dressing for photothermal therapy of infected full-thickness skin wounds.* Chemical Engineering Journal, 2020. **394**: p. 124888.

3. Dargaville, T.R., et al., *Sensors and imaging for wound healing: a review.* Biosensors and Bioelectronics, 2013. **41**: p. 30-42.

4. Zhang, L.-j., et al., *Dermal adipocytes protect against invasive Staphylococcus aureus skin infection.* Science, 2015. **347**(6217): p. 67-71.

5. Baltzis, D., I. Eleftheriadou, and A. Veves, *Pathogenesis and treatment of impaired wound healing in diabetes mellitus: new insights.* Advances in therapy, 2014. **31**: p. 817-836.

6. Zhou, J., et al., *Bacteria-responsive intelligent wound dressing: Simultaneous In situ detection and inhibition of bacterial infection for accelerated wound healing.* Biomaterials, 2018. **161**: p. 11-23.

7. Levy, S.B. and B. Marshall, *Antibacterial resistance worldwide: causes, challenges and responses.* Nature medicine, 2004. **10**(Suppl 12): p. S122-S129.

8. Yousefi, M., et al., *Anti-bacterial activity of graphene oxide as a new weapon nanomaterial to combat multidrug-resistance bacteria.* Materials Science and Engineering: C, 2017. **74**: p. 568-581.

9. Yang, H., et al., *Comparative study of cytotoxicity, oxidative stress and genotoxicity induced by four typical nanomaterials: the role of particle size, shape and composition.* Journal of applied Toxicology, 2009. **29**(1): p. 69-78.

10. Xin, Q., et al., *Antibacterial carbon-based nanomaterials.* Advanced Materials, 2019. **31**(45): p. 1804838.

11. Wang, W., et al., *Photocatalytic nanomaterials for solar-driven bacterial inactivation: recent progress and challenges.* Environmental Science: Nano, 2017. **4**(4): p. 782-799.

12. Qi, M., et al., *Novel nanomaterial-based antibacterial photodynamic therapies to combat oral bacterial biofilms and infectious diseases.* International journal of nanomedicine, 2019: p. 6937-6956.

13. Ray, P.C., et al., *Nanomaterials for targeted detection and photothermal killing of bacteria.* Chemical Society Reviews, 2012. **41**(8): p. 3193-3209.

14. Ramalingam, B., T. Parandhaman, and S.K. Das, *Antibacterial effects of biosynthesized silver nanoparticles on surface ultrastructure and nanomechanical properties of gram-negative bacteria viz. Escherichia coli and Pseudomonas aeruginosa.* ACS applied materials & interfaces, 2016. **8**(7): p. 4963-4976.

15. Xie, X., et al., *Tuning the bandgap of photo-sensitive polydopamine/Ag3PO4/graphene oxide coating for rapid, noninvasive disinfection of implants.* ACS central science, 2018. **4**(6): p. 724-738.

16. Baek, S., S.H. Joo, and M. Toborek, *Treatment of antibiotic-resistant bacteria by encapsulation of ZnO nanoparticles in an alginate biopolymer: Insights into treatment mechanisms.* Journal of Hazardous materials, 2019. **373**: p. 122-130.

17. Maruthapandi, M., et al., *Antimicrobial activities of Zn-doped CuO microparticles decorated on polydopamine against sensitive and antibiotic-resistant bacteria.* ACS Applied Polymer Materials, 2020. **2**(12): p. 5878-5888.

18. Xu, J., et al., *Upconversion nanoparticle-assisted payload delivery from TiO2 under near-infrared light irradiation for bacterial inactivation.* ACS nano, 2019. **14**(1): p. 337-346.

19. Karunakaran, S., et al., *Simultaneous exfoliation and functionalization of 2H-MoS2 by thiolated surfactants: applications in enhanced antibacterial activity.* Journal of the American Chemical Society, 2018. **140**(39): p. 12634-12644.

20. Makvandi, P., et al., *Metal-based nanomaterials in biomedical applications: antimicrobial activity and cytotoxicity aspects.* Advanced Functional Materials, 2020. **30**(22): p. 1910021.

21. Lei, K., et al., *The innovative fabrication and applications of carvacrol nanoemulsions, carboxymethyl chitosan microgels and their composite films.* Colloids and Surfaces B: Biointerfaces, 2019. **175**: p. 688-696.

22. Hallaj-Nezhadi, S. and M. Hassan, *Nanoliposome-based antibacterial drug delivery.* Drug delivery, 2015. **22**(5): p. 581-589.

23. Huang, K., F. Dou, and N. Nitin, *Biobased sanitizer delivery system for improved sanitation of bacterial and fungal biofilms.* ACS applied materials & interfaces, 2019. **11**(19): p. 17204-17214.

24. Sikder, P. and S.B. Bhaduri, *Antibacterial Hydroxyapatite: An Effective Approach to Cure Infections in Orthopedics.* Racing for the Surface: Pathogenesis of Implant Infection and Advanced Antimicrobial Strategies, 2020: p. 583-612.

25. Ji, H., H. Sun, and X. Qu, *Antibacterial applications of graphene-based nanomaterials: recent achievements and challenges.* Advanced drug delivery reviews, 2016. **105**: p. 176-189.

26. Akhavan, O. and E. Ghaderi, *Toxicity of graphene and graphene oxide nanowalls against bacteria.* ACS nano, 2010. **4**(10): p. 5731-5736.

27. Zhu, Y., et al., *Silver nanoparticles-decorated and mesoporous silica coated single-walled carbon nanotubes with an enhanced antibacterial activity for killing drug-resistant bacteria.* Nano Research, 2020. **13**: p. 389-400.

28. Zhu, X., et al., *A near-infrared light-mediated antimicrobial based on Ag/Ti 3 C 2 T x for effective synergetic antibacterial applications.* Nanoscale, 2020. **12**(37): p. 19129-19141.

29. Ali, A., et al., *Tailoring metal-organic frameworks-based nanozymes for bacterial theranostics.* Biomaterials, 2021. **275**: p. 120951.

30. Wyszogrodzka, G., et al., *Metal-organic frameworks: mechanisms of antibacterial action and potential applications.* Drug Discovery Today, 2016. **21**(6): p. 1009-1018.

31. Yang, B., Y. Chen, and J. Shi, *Reactive oxygen species (ROS)-based nanomedicine.* Chemical reviews, 2019. **119**(8): p. 4881-4985.

32. Shen, M., et al., *Antibacterial applications of metal–organic frameworks and their composites.* Comprehensive Reviews in Food Science and Food Safety, 2020. **19**(4): p. 1397-1419.

33. Munita, J.M. and C.A. Arias, *Mechanisms of antibiotic resistance.* Virulence mechanisms of bacterial pathogens, 2016: p. 481-511.

34. Alavi, M. and M. Rai, *Recent advances in antibacterial applications of metal nanoparticles (MNPs) and metal nanocomposites (MNCs) against multidrug-resistant (MDR) bacteria.* Expert review of anti-infective therapy, 2019. **17**(6): p. 419-428.

35. Liu, J., et al., *Antibacterial mechanisms and applications of metal-organic frameworks and their derived nanomaterials.* Trends in Food Science & Technology, 2021. **109**: p. 413-434.

36. Tian, B. and J. Liu, *Smart stimuli-responsive chitosan hydrogel for drug delivery: A review.* International Journal of Biological Macromolecules, 2023. **235**: p. 123902.

37. Radhakrishnan, J., et al., *Injectable and 3D bioprinted polysaccharide hydrogels: from cartilage to osteochondral tissue engineering.* Biomacromolecules, 2017. **18**(1): p. 1-26.

38. Abdel-Mohsen, A., et al., *Green synthesis of hyaluronan fibers with silver nanoparticles.* Carbohydrate polymers, 2012. **89**(2): p. 411-422.

39. Broguiere, N., et al., *Factor XIII cross-linked hyaluronan hydrogels for cartilage tissue engineering.* ACS Biomaterials Science & Engineering, 2016. **2**(12): p. 2176-2184.

40. Seror, J., et al., *Articular cartilage proteoglycans as boundary lubricants: structure and frictional interaction of surface-attached hyaluronan and hyaluronan–aggrecan complexes.* Biomacromolecules, 2011. **12**(10): p. 3432-3443.

41. Rojas, F.P., et al., *Molecular adhesion between cartilage extracellular matrix macromolecules.* Biomacromolecules, 2014. **15**(3): p. 772-780.

42. Valachová, K., et al., *Influence of tiopronin, captopril and levamisole therapeutics on the oxidative degradation of hyaluronan.* Carbohydrate Polymers, 2015. **134**: p. 516-523.

43. Valachová, K., et al., *Hydrogen peroxide generation by the Weissberger biogenic oxidative system during hyaluronan degradation.* Carbohydrate polymers, 2016. **148**: p. 189-193.

44. Ji, Y., et al., *Electrospun three-dimensional hyaluronic acid nanofibrous scaffolds.* Biomaterials, 2006. **27**(20): p. 3782-3792.

45. Zhu, X., et al., *Biomimetic bacterial cellulose-enhanced double-network hydrogel with excellent mechanical properties applied for the osteochondral defect repair.* ACS Biomaterials Science & Engineering, 2018. **4**(10): p. 3534-3544.

46. Tamer, T.M., et al., *MitoQ loaded chitosan-hyaluronan composite membranes for wound healing.* Materials, 2018. **11**(4): p. 569.

47. Han, H.S., et al., *Gold-nanoclustered hyaluronan nano-assemblies for photothermally maneuvered photodynamic tumor ablation.* ACS nano, 2016. **10**(12): p. 10858-10868.

48. Bano, F., et al., *Interaction of hyaluronan with cationic nanoparticles.* Langmuir, 2015. **31**(30): p. 8411-8420.

49. Dreaden, E.C., et al., *Bimodal tumor-targeting from microenvironment responsive hyaluronan layer-by-layer (LbL) nanoparticles.* ACS nano, 2014. **8**(8): p. 8374-8382.

50. Boddohi, S., et al., *Polysaccharide-based polyelectrolyte complex nanoparticles from chitosan, heparin, and hyaluronan.* Biomacromolecules, 2009. **10**(6): p. 1402-1409.

51. Cai, Z., et al., *Hyaluronan-inorganic nanohybrid materials for biomedical applications.* Biomacromolecules, 2017. **18**(6): p. 1677-1696.

52. Gaharwar, A.K., N.A. Peppas, and A. Khademhosseini, *Nanocomposite hydrogels for biomedical applications.* Biotechnology and bioengineering, 2014. **111**(3): p. 441-453.

53. Kao, Z.-K., et al., *Low temperature synthesis of conductive silver tracks with polymer addition.* Journal of the Taiwan Institute of chemical engineers, 2012. **43**(6): p. 965-970.

54. El-Mahdy, G.A., A.M. Atta, and H.A. Al-Lohedan, *Synthesis and characterizations of Fe3O4 nanogel composite for enhancement of the corrosion resistance of steel in HCl solutions.* Journal of the Taiwan Institute of Chemical Engineers, 2014. **45**(4): p. 1947-1953.

55. Tseng, C.-C., et al., *Synthesis of vinyl acetate/Pd nanocomposites as activator ink for ink-jet printing technology and electroless copper plating.* Journal of the Taiwan Institute of Chemical Engineers, 2011. **42**(6): p. 989-995.

56. Chen, C.-H., et al., *Dual functional core–sheath electrospun hyaluronic acid/polycaprolactone nanofibrous membranes embedded with silver nanoparticles for prevention of peritendinous adhesion.* Acta biomaterialia, 2015. **26**: p. 225-235.

57. Abdel-Mohsen, A., et al., *A novel in situ silver/hyaluronan bio-nanocomposite fabrics for wound and chronic ulcer dressing: in vitro and in vivo evaluations.* International journal of pharmaceutics, 2017. **520**(1-2): p. 241-253.

58. Wu, J., et al., *In situ synthesis of silver-nanoparticles/bacterial cellulose composites for slow-released antimicrobial wound dressing.* Carbohydrate polymers, 2014. **102**: p. 762-771.

59. Cao, X., et al., *Preparation of silver nanoparticles with antimicrobial activities and the researches of their biocompatibilities.* Journal of Materials Science: Materials in Medicine, 2010. **21**: p. 2861-2868.

60. Bai, W., et al., *Novel silver nanoparticle–manganese oxyhydroxide–graphene oxide nanocomposite prepared by modified silver mirror reaction and its application for electrochemical sensing.* ACS Applied Materials & Interfaces, 2014. **6**(8): p. 5439-5449.

61. Liu, J.H., D.S. Wu, and K.Y. Tseng, *Fabrication and characterization of GRIN plastic rods containing silver nanoparticles with novel surfmers.* Macromolecular Chemistry and Physics, 2004. **205**(16): p. 2205-2213.

62. You, J., et al., *Fabrication of high-density silver nanoparticles on the surface of alginate microspheres for application in catalytic reaction.* Journal of Materials Chemistry A, 2014. **2**(22): p. 8491-8499.

63. Otari, S., et al., *A novel microbial synthesis of catalytically active Ag–alginate biohydrogel and its antimicrobial activity.* Dalton transactions, 2013. **42**(27): p. 9966-9975.

64. Prabhu, S. and E.K. Poulose, *Silver nanoparticles: mechanism of antimicrobial action, synthesis, medical applications, and toxicity effects.* International nano letters, 2012. **2**: p. 1-10.

65. Mi, G., et al., *Reducing bacterial infections and biofilm formation using nanoparticles and nanostructured antibacterial surfaces.* Advanced Healthcare Materials, 2018. **7**(13): p. 1800103.

66. Li, Y., et al., *Significant enhancement in hydrolytic degradation of sulfur mustard promoted by silver nanoparticles in the Ag NPs@ HKUST-1 composites.* Journal of hazardous materials, 2018. **358**: p. 113-121.

67. Arenas-Vivo, A., et al., *An Ag-loaded photoactive nano-metal organic framework as a promising biofilm treatment.* Acta Biomaterialia, 2019. **97**: p. 490-500.

68. Wang, J., et al., *UV cross-linked injectable non-swelling dihydrocaffeic acid grafted chitosan hydrogel for promoting wound healing.* Carbohydrate Polymers, 2023. **314**: p. 120926.

69. Nguyen, N.-T. and J.-H. Liu, *A green method for in situ synthesis of poly (vinyl alcohol)/chitosan hydrogel thin films with entrapped silver nanoparticles.* Journal of the Taiwan Institute of Chemical Engineers, 2014. **45**(5): p. 2827-2833.

70. Mohan, Y.M., et al., *Hydrogel networks as nanoreactors: A novel approach to silver nanoparticles for antibacterial applications.* Polymer, 2007. **48**(1): p. 158-164.

71. Karthick, S.A., K. Manjari, and M.G. Devi, *Biocompatible and bioactive PVA/Sericin/Chitosan nanofibrous wound dressing matrix.* Applied Surface Science Advances, 2023. **13**: p. 100362.

72. Khorasani, M.T., et al., *Enhanced antimicrobial and full-thickness wound healing efficiency of hydrogels loaded with heparinized ZnO nanoparticles: in vitro and in vivo evaluation.* International Journal of Biological Macromolecules, 2021. **166**: p. 200-212.

73. Kodavaty, J. and A.P. Deshpande, *Self-assembly and drying assisted microstructural domain formation in poly (vinyl alcohol) and hyaluronic acid gels.* Polymer Bulletin, 2017. **74**: p. 3605-3617.

74. Kodavaty, J. and A.P. Deshpande, *Mechanical and Swelling Properties of Poly (vinyl alcohol) and Hyaluronic Acid Gels used in Biomaterial Systems--a Comparative Study.* Defence Science Journal, 2014. **64**(3).

75. Kodavaty, J. and A.P. Deshpande, *Regimes of microstructural evolution as observed from rheology and surface morphology of crosslinked poly (vinyl alcohol) and hyaluronic acid blends during gelation.* Journal of Applied Polymer Science, 2014. **131**(22).

76. Kamoun, E.A., E.-R.S. Kenawy, and X. Chen, *A review on polymeric hydrogel membranes for wound dressing applications: PVA-based hydrogel dressings.* Journal of advanced research, 2017. **8**(3): p. 217-233.

77. Potts, R., *Skin barrier: principles of percutaneous absorption.* Archives of dermatology, 1997. **133**(7): p. 924-924.

78. Boateng, J.S., et al., *Wound healing dressings and drug delivery systems: a review.* Journal of pharmaceutical sciences, 2008. **97**(8): p. 2892-2923.

79. Gurtner, G.C., et al., *Wound repair and regeneration.* Nature, 2008. **453**(7193): p. 314-321.

80. Winter, G.D., *Formation of the scab and the rate of epithelization of superficial wounds in the skin of the young domestic pig.* Nature, 1962. **193**(4812): p. 293-294.

81. Ndlovu, S.P., et al., *Gelatin-based hybrid scaffolds: promising wound dressings.* Polymers, 2021. **13**(17): p. 2959.

82. De Luca, I., et al., *Nanotechnology development for formulating essential oils in wound dressing materials to promote the wound-healing process: a review.* Applied sciences, 2021. **11**(4): p. 1713.

83. Edwards, J.V., et al., *Modified cotton gauze dressings that selectively absorb neutrophil elastase activity in solution.* Wound Repair and Regeneration, 2001. **9**(1): p. 50-58.

84. Alven, S., et al., *Polymer-based scaffolds loaded with Aloe vera extract for the treatment of wounds. Pharmaceutics 13: 961*. 2021.

85. LIM, D., *Hydrophilic gels for biological use.* Nature, 1960. **185**: p. 4706.

86. Mathur, A.M., S.K. Moorjani, and A.B. Scranton, *Methods for synthesis of hydrogel networks: A review.* Journal of Macromolecular Science, Part C: Polymer Reviews, 1996. **36**(2): p. 405-430.

87. Jin, S., et al., *Progress of hydrogel dressings with wound monitoring and treatment functions.* Gels, 2023. **9**(9): p. 694.

88. Alexander, C., *Temperature-and pH-responsive smart polymers for gene delivery.* Expert Opinion on Drug Delivery, 2006. **3**(5): p. 573-581.

89. Bignotti, F., et al., *Synthesis, characterisation and solution behaviour of thermo-and pH-responsive polymers bearing L-leucine residues in the side chains.* Polymer, 2000. **41**(23): p. 8247-8256.

90. Liu, F. and M.W. Urban, *Recent advances and challenges in designing stimuli-responsive polymers.* Progress in polymer science, 2010. **35**(1-2): p. 3-23.

91. Bashir, S., et al., *Fundamental concepts of hydrogels: Synthesis, properties, and their applications.* Polymers, 2020. **12**(11): p. 2702.

92. Buenger, D., F. Topuz, and J. Groll, *Hydrogels in sensing applications.* Progress in Polymer Science, 2012. **37**(12): p. 1678-1719.

93. Guo, W., et al., *pH-stimulated DNA hydrogels exhibiting shape-memory properties.* Advanced Materials (Deerfield Beach, Fla.), 2014. **27**(1): p. 73-78.

94. Das, N., *Preparation methods and properties of hydrogel: A review.* Int. J. Pharm. Pharm. Sci, 2013. **5**(3): p. 112-117.

95. Yang, Z., et al., *Acute toxicity of high dosage carboxymethyl chitosan and its effect on the blood parameters in rats.* Journal of Materials Science: Materials in Medicine, 2012. **23**: p. 457-462.

96. Chan, P., et al., *Synthesis and characterization of chitosan-g-poly (ethylene glycol)-folate as a non-viral carrier for tumor-targeted gene delivery.* Biomaterials, 2007. **28**(3): p. 540-549.

97. Anitha, A., et al., *Synthesis, characterization, cytotoxicity and antibacterial studies of chitosan, O-carboxymethyl and N, O-carboxymethyl chitosan nanoparticles.* Carbohydrate polymers, 2009. **78**(4): p. 672-677.

98. Mohamed, N.A. and M.M. Fahmy, *Synthesis and antimicrobial activity of some novel cross-linked chitosan hydrogels.* International journal of molecular sciences, 2012. **13**(9): p. 11194-11209.

99. Zhang, D., et al., *Carboxyl-modified poly (vinyl alcohol)-crosslinked chitosan hydrogel films for potential wound dressing.* Carbohydrate polymers, 2015. **125**: p. 189-199.

100. Hwang, M.-R., et al., *Gentamicin-loaded wound dressing with polyvinyl alcohol/dextran hydrogel: gel characterization and in vivo healing evaluation.* Aaps Pharmscitech, 2010. **11**: p. 1092-1103.

101. Kamoun, E.A., et al., *Poly (vinyl alcohol)-alginate physically crosslinked hydrogel membranes for wound dressing applications: characterization and bio-evaluation.* Arabian Journal of Chemistry, 2015. **8**(1): p. 38-47.

102. Yang, J.M., W.Y. Su, and M.C. Yang, *Evaluation of chitosan/PVA blended hydrogel membranes.* journal of Membrane Science, 2004. **236**(1-2): p. 39-51.

103. Huang, M.-H. and M.-C. Yang, *Evaluation of glucan/poly (vinyl alcohol) blend wound dressing using rat models.* International journal of pharmaceutics, 2008. **346**(1-2): p. 38-46.

104. Hago, E.-E. and X. Li, *Interpenetrating polymer network hydrogels based on gelatin and PVA by biocompatible approaches: synthesis and characterization.* Advances in Materials Science and Engineering, 2013. **2013**(1): p. 328763.

105. Fahmy, A., et al., *Poly (vinyl alcohol)-hyaluronic acid membranes for wound dressing applications: synthesis and in vitro bio-evaluations.* Journal of the Brazilian Chemical Society, 2015. **26**: p. 1466-1474.

106. Padol, A.R., et al., *Safety evaluation of silk protein film (a novel wound healing agent) in terms of acute dermal toxicity, acute dermal irritation and skin sensitization.* Toxicology international, 2011. **18**(1): p. 17.

107. Bhardwaj, U., et al., *PLGA/PVA hydrogel composites for long-term inflammation control following sc implantation.* International journal of pharmaceutics, 2010. **384**(1-2): p. 78-86.

108. Abdullah, Z.W., et al., *PVA, PVA blends, and their nanocomposites for biodegradable packaging application.* Polymer-Plastics Technology and Engineering, 2017. **56**(12): p. 1307-1344.

109. Jayasekara, R., et al., *Biodegradability of a selected range of polymers and polymer blends and standard methods for assessment of biodegradation.* Journal of Polymers and the Environment, 2005. **13**: p. 231-251.

110. Fukushima, K. and G. Camino, *Polymer nanocomposites biodegradation.* Functional and physical properties of polymer nanocomposites, 2016: p. 57-91.

111. Tănase, E.E., et al., *Preparation and characterization of biopolymer blends based on polyvinyl alcohol and starch.* Romanian Biotechnological Letters, 2015. **20**(2): p. 10307.

112. Liu, Y., et al., *Physically crosslinked composite hydrogels of PVA with natural macromolecules: structure, mechanical properties, and endothelial cell compatibility.* Journal of Biomedical Materials Research Part B: Applied Biomaterials: An Official Journal of The Society for Biomaterials, The Japanese Society for Biomaterials, and The Australian Society for Biomaterials and the Korean Society for Biomaterials, 2009. **90**(2): p. 492-502.

113. Luo, Y.-L., et al., *Assembly, characterization and swelling kinetics of Ag nanoparticles in PDMAA-g-PVA hydrogel networks.* Materials chemistry and Physics, 2009. **118**(2-3): p. 329-336.

114. Yang, X., et al., *Cytotoxicity and wound healing properties of PVA/ws-chitosan/glycerol hydrogels made by irradiation followed by freeze–thawing.* Radiation Physics and Chemistry, 2010. **79**(5): p. 606-611.

115. Zhou, X.Y., et al., *Effect of a complex plasticizer on the structure and properties of the thermoplastic PVA/starch blends.* Polymer-Plastics Technology and Engineering, 2009. **48**(5): p. 489-495.

116. Ma, Y., T. Bai, and F. Wang, *The physical and chemical properties of the polyvinylalcohol/polyvinylpyrrolidone/hydroxyapatite composite hydrogel.* Materials Science and Engineering: C, 2016. **59**: p. 948-957.

117. Heiligtag, F.J. and M. Niederberger, *The fascinating world of nanoparticle research.* Materials today, 2013. **16**(7-8): p. 262-271.

118. Li, N., P. Zhao, and D. Astruc, *Anisotropic gold nanoparticles: synthesis, properties, applications, and toxicity.* Angewandte Chemie International Edition, 2014. **53**(7): p. 1756-1789.

119. Taylor, M.G., et al., *Catalyst design based on morphology-and environment-dependent adsorption on metal nanoparticles.* ACS Catalysis, 2015. **5**(11): p. 6296-6301.

120. Ahmad, S.A., et al., *Bactericidal activity of silver nanoparticles: A mechanistic review.* Materials Science for Energy Technologies, 2020. **3**: p. 756-769.

121. Pangli, H., et al., *Incorporation of silver nanoparticles in hydrogel matrices for controlling wound infection.* Journal of Burn Care & Research, 2021. **42**(4): p. 785-793.

122. Xie, Y., et al., *Novel chitosan hydrogels reinforced by silver nanoparticles with ultrahigh mechanical and high antibacterial properties for accelerating wound healing.* International journal of biological macromolecules, 2018. **119**: p. 402-412.

123. Bushra, R., et al., *Polysaccharide-based nanoassemblies: From synthesis methodologies and industrial applications to future prospects.* Advances in Colloid and Interface Science, 2023. **318**: p. 102953.

124. Antoszewska, M., E.M. Sokolewicz, and W. Barańska-Rybak, *Wide Use of Hyaluronic Acid in the Process of Wound Healing—A Rapid Review.* Scientia Pharmaceutica, 2024. **92**(2): p. 23.

125. Della Sala, F., et al., *Hyaluronic acid-based wound dressing with antimicrobial properties for wound healing application.* Applied Sciences, 2022. **12**(6): p. 3091.

126. Kodavaty, J., *Poly (vinyl alcohol) and hyaluronic acid hydrogels as potential biomaterial systems-A comprehensive review.* Journal of Drug Delivery Science and Technology, 2022. **71**: p. 103298.

127. Agasti, N. and N. Kaushik, *One pot synthesis of crystalline silver nanoparticles.* Am. J. Nanomater, 2014. **2**(1): p. 4-7.

128. Nisar, A., M.A. Khan, and Z. Hussain, *Synthesis and characterization of PANI/MOF-199/Ag nanocomposite and its potential application as non-enzymatic electrochemical sensing of dopamine.* Journal of the Korean Ceramic Society, 2022. **59**(3): p. 359-369.

129. Ghezzi, M., et al., *Hyaluronic acid–PVA films for the simultaneous delivery of dexamethasone and levofloxacin to ocular tissues.* International Journal of Pharmaceutics, 2023. **638**: p. 122911.

130. Hassan, A., et al., *Development of anti-bacterial PVA/starch based hydrogel membrane for wound dressing.* Journal of Polymers and the Environment, 2018. **26**: p. 235-243.

131. Zahran, M., H.B. Ahmed, and M. El-Rafie, *Surface modification of cotton fabrics for antibacterial application by coating with AgNPs–alginate composite.* Carbohydrate polymers, 2014. **108**: p. 145-152.

132. Tang, C.-M., Y.-H. Tian, and S.-H. Hsu, *Poly (vinyl alcohol) nanocomposites reinforced with bamboo charcoal nanoparticles: mineralization behavior and characterization.* Materials, 2015. **8**(8): p. 4895-4911.

133. Cassimjee, H., et al., *Genipin-crosslinked, proteosaccharide scaffolds for potential neural tissue engineering applications. Pharmaceutics 2022; 14: 441*.

134. Jipa, I.M., et al., *Potassium sorbate release from poly (vinyl alcohol)-bacterial cellulose films.* Chemical Papers, 2012. **66**: p. 138-143.

135. Garg, A., et al., *Hyaluronic acid embedded cellulose acetate phthlate core/shell nanoparticulate carrier of 5-fluorouracil.* International journal of biological macromolecules, 2016. **87**: p. 449-459.

136. Gwon, H.-J., et al., *Characterization of PVA/glycerin hydrogels made by γ-irradiation for advanced wound dressings.* Korean Journal of chemical engineering, 2009. **26**: p. 1686-1688.

137. Zhang, F., et al., *Development of a complex hydrogel of hyaluronan and PVA embedded with silver nanoparticles and its facile studies on Escherichia coli.* Journal of Biomaterials Science, Polymer Edition, 2013. **24**(12): p. 1410-1425.

138. Bhowmick, S. and V. Koul, *Assessment of PVA/silver nanocomposite hydrogel patch as antimicrobial dressing scaffold: Synthesis, characterization and biological evaluation.* Materials Science and Engineering: C, 2016. **59**: p. 109-119.

139. Zahran, M., H.B. Ahmed, and M. El-Rafie, *Alginate mediate for synthesis controllable sized AgNPs.* Carbohydrate polymers, 2014. **111**: p. 10-17.

140. Ding, L., et al., *Spongy bilayer dressing composed of chitosan–Ag nanoparticles and chitosan–Bletilla striata polysaccharide for wound healing applications.* Carbohydrate Polymers, 2017. **157**: p. 1538-1547.