TOPICAL HYDROGEL SCAFFOLD BASED THERAPEUTIC DRUG DELIVERY SYSTEM FOR CONTROLLED RELEASE TREATMENT OF CHRONIC WOUNDS

A dissertation submitted to the Faculty of Health Sciences, University of the Witwatersrand, in fulfilment of the requirements for the degree of Master of Science in Medicine in the field of Pharmaceutical Affairs.

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DECLARATION

I, Sewela Stanford Molapo declare that this Dissertation is my own work. It is being submitted for the degree of Master of Science in Medicine in the field of Pharmaceutical Affairs in the Faculty of Health Sciences at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.

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Signature

This …02...day of …August 2024

DEDICATION

This dissertation is dedicated to everyone who has inspired and helped me to get to this level.

ACKNOWLEDGMENT

On reaching the finishing point of this challenging task I would like to extend my heartfelt gratitude to all those who have made it possible for the achievement of this goal.

To the Wits School of Therapeutic Sciences – WDDP, Supervisors, Dr Hillary Mndlovu and Prof Yayha E Choonara, thank you for the effort, time, and commitment endowed in guiding me and ensuring the successful completion of this research.

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ABSTRACT

The process of wound healing is a dynamic, effectively orchestrated, and complex process, in which several factors become inhibited and/or activated in steps of sequences. The quality of millions of population's health continues to suffer a major impact caused by compromised healing of skin wounds, more especially chronic wounds. There are treatment options available for treatment of various kinds of wounds in the form of wound dressings, however, most of the treatments have limitations. Majority do not possess full properties of best possible wound healing dressings system to meet every property in the cascade of healing. Current research is showing interest in dressings that are therapeutically advanced to actively participate in the process of wound healing to achieve complete and fast healing of all types of wounds (acute and chronic wounds). Due to the compromised quality of lives and extended financial burdens, it is every researcher's desire to achieve expeditious and complete wound healing using advanced strategies. A researcher's understanding of wound healing process, conditions associated with patients (socioeconomics, health and environmental) and properties associated with types of wound healing materials (physical and chemical dressing properties), achieves successful formulation for treatment of wounds. In the current study, the review section therefore outlines characteristics of skin and wounds, the three key elements involved in the process of wound healing (inflammation, proliferation, and remodelling), current treatment options including advanced management and wound treatments, and physical characteristic of advanced wound dressings. The main purpose of this study was to synthesize a phase morphing tannic acid and carbopol scaffold for targeted chronic wound application. The synthesized scaffolds were subjected to characterization by physicochemical analysis to evaluate their properties employing Scanning Electron Microscopy (SEM), Fourier Transform Infrared Spectroscopy (FTIR), Differential Scanning Colorimetry (DSC), X-Ray Diffraction (XRD), Texture analyser, Zeta potential, as well as Ultraviolet/Visual (UV/Vis) spectroscopy (nanophotometer). The maximum wavelength (λ_{max}) of Tannic Acid was 265 nm. The *in vitro* drug release indicates an initial short burst effect for up to 24% of release within 48 hrs, then followed by a slow release for up to 7th day. These ensure initial fast and a later slow and sustained drug release at the site of application. The initials burst release ensure immediate enough bioavailability to trigger therapeutic effects. SEM analysis confirmed spherical conformation of the scaffolds. The mean zeta potential of -15 mV over 7 days which is indicative of great stability of the scaffold. The combined analysis and results from the formulation of TA-carbopol scaffold showed evidence that these scaffolds dosage form can be utilized as potential invaluable formulation for transdermal controlled drug delivery of TA with improved efficacy and patient conformity.

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DSC	Differential Scanning Calorimetry
ECM	Extracellular Matrix
FTIR	Fourier Transformation Infrared Spectroscopy
LDDS	Local Drug Delivery System
PBS	Phosphate Buffer Solution
PDGF	Platelet Derived Growth Factors
ROS	Reactive Oxygen Species
SD	Standard Deviation
SEM	Scanning Electron Microscopy
UV	Ultraviolet
WHO	World Health Organization
WHS	Wound Healing Society
XRD	X-Ray Diffraction

CHAPTER 1 BACKGROUND AND INTRODUCTION

1.1. Introduction and background on wounds and treatments

The main primary protector of the human body that is capable of safeguarding the body from infections caused by microorganisms and external environmental damages is known as the skin (Jingjing et al., 2021). The very same skin sometimes become susceptible to these damages, thus resulting in wounds. Generally, a wound is known as a defect or break of the skin tissue, caused by thermal damage, mechanical damage, or as a cause of the existence of underlying physiological or medical state. These damages compromise the integrity of the skin and its capability to safeguard the human body (Mayet et al., 2014). There are types of wounds, and their categorizations are decided in accordance with the affected area of the skin, their healing periods and rate, and as well as the affected number of layers of the skin. Wounds have two types of classes/categories. They can be classified as acute and chronic wounds. Wounds become acute when the healing process takes less than 8 weeks and chronic when the healing process takes longer than 8-12 weeks (Aulton et al., 2013).

The healing process of wounds it is a dynamic and integrates complex of biological and molecular phases. During ordinary conditions, the wound healing process takes place through four stages that overlap. The overlapping stages include remodeling, inflammation, proliferation, and migration. The phase at which the skin surface is reforming and regaining its tensile strength is considered as a wound healing phase. The stem cells are used for creation of complex structure due to their ability to differentiate types of tissues through asymmetrical replication (Aulton et al., 2013; Mayet et al., 2014). The process of wound healing and the rate at which it occurs can be affected by various conditions. Both local and systemic conditions such as lack of blood flow to the wound area and diabetic condition delays and disrupts the healing process. Microbial infections from environmental conditions also disrupts the healing process of several types of wounds. Surgical, bite, pressure ulcers, burns, and diabetic wounds are examples of wounds that can be affected by bacterial infections, thus, delaying and disrupting the healing process (Daniel et al., 2015).

The perceptive understanding of cutaneous burns and wound healing requires thorough comprehension and knowledge with regard to the function of the skin, its anatomy and physiology (Mihm et al., 1976). Abnormalities may occur during the healing process, which could result in longer healing times and/or turn the wound from acute into a chronic wound, a not healing wound. This is despite the fact that, healing process is a natural and predicated process. Poor treatment as well as bad local, environmental, and systemic conditions turn a wound from acute to chronic at any stage of the healing process. Also, factors such as the

health status of the patient, and bad nutritional status disrupts the healing process and inhibit the ability of the body to fight infections (Aulton et al., 2013).

Wounds from the largest organ of the body (skin) are also contributors of main causes of deaths worldwide. Surgical wounds, diabetic and pressure ulcers and burn wounds are some of wound types that can lead to death (Daniel et al., 2015). Both types of wounds, but more especially chronic wounds cause pain and discomfort to patients and are of a great disadvantage to the quality of patients' lives. Also, financially millions of people tend to incur a huge cost in finances trying to treat painful wounds. Furthermore, at most, one out of five diabetic foot ulcers result in amputation (Bohl et al, 2002). Therefore, treating wounds requires correct wound treatment approach and special attention invested into these treatments. Because wound dressings are important for speedy and successful healing of wound, dressing are the commonly used to treat wounds. Dressing serves as a barrier between external environment and the wound itself, thus, it resists further infections and injuries to the wound (Baljit et al., 2013; Jingjing et al., 2021).

Traditional wound treatment dressing products have been in use for centuries and these dressing types are still in use to date. Each having a specific role as either primary or secondary dressing. These traditional dressings include, cotton wool, gauze, lint, and synthetic or natural bandages. All possess varying degrees of absorbency of moisture and are having specific functions to keep the wound dry and clean. They enable permeability of excess wound exudate to evaporate, as described on below (Table 1.1). However, as most common types of wound dressings are formulated to provide a moist environment for wound healing, they at the same time provide a permeable layer which enable moisture evaporation, resulting in a dry desiccated wound. Innovations to new dressing products has led to the discoveries and developments of innovated dressings (Aulton et al., 2013).

There has been transitions from simple wound management aids to advanced specialised devices with specific characteristics, such advanced wound dressings are infused with various components in optimised proportion, which may include polymers and hydrocolloids (Mayet et al., 2016). The development of new intelligent dressings is underway, that promise to play an active role in modifying and promoting healing of both acute and chronic wounds. Advanced dressings that have the ability to transport active substances to the wound site by the design of dressings composed of materials having endogenous activity or by the delivery of active substances in wound healing are regarded as bioactive products. These products include collagen, proteoglycans, chitosan, and alginates (Paul et al., 2004). In recent past years, research interest on the design of innovative hydrogels for wound healing applications has

significantly increased, as confirmed by the huge amount of literature published on this topic. In this regard, the Pubmed database reports an exponential increase in the number of publications concerning the use of hydrogels in wound healing over the last five decades, with around 550 works published in 2020. Specifically, the literature reports the design of both naturally derived and synthetic polymer-based hydrogels for wound healing applications (Rossella et al., 2022).

Current treatment options for wound dressings fall under various classes, classified according to their roles, the type of wound applied on or the physicochemical properties of the dressing (see Table 1.1). Other types of dressings are combined with active pharmaceutical agents such as antibacterial agents or wound debridement agents. Dressing induced with human Platelet Derived Growth Factors (PDGF) has been discovered to treat chronic diabetic ulcers (Aulton et al., 2013). The field of nanotechnology relates to structures like nanofibres, nanoparticles, nanorods and nanotubes with unique chemical, mechanical and optical properties to promote novel opportunities for delivering wound dressings with efficient drug delivery (Broudriot et al., 2006; Patzke et al., 2002. New dimensions can be envisioned for areas such as wound healing and tissue engineering drug delivery with regards to enhancing the therapeutic aspect whilst reducing side effects and risks when introducing novel concepts such as nanotubes, nanofibres, nanorods and branched nanoobjects (Miyoshi et al., 2006). Techniques such as the application of nanotechnology will ensure the fabrication of a biodegradable scaffold for directing a series of tissue regeneration processes in a more active manner promoting faster wound healing (Mayet et al., 2016).

Table 1-1 The key properties of common types of wound dressings.

Dressing type	Dressing characteristics	Uses of dressing	References
Impregnated gauze (sodium chloride or soft paraffin)	This type of dressing sometimes sticks to the wound. Therefore, it gets damaged and causes pain. It requires frequent changing. The dressings have many levels of absorption.	It is used as a medical device to apply ointments and creams to a wound.	Aulton et al., 2013
Films	The dressing is not absorbent. It is porous to oxygen, moisture, and vapour, while allowing evaporation of some exudate. Not permeable microorganism.	It is used on wounds that have little exudate and at a later stage of wound healing. Not for use on fragile and/or thin and infected areas of the skin.	Mayet at el., 2014

Foams	Permeable to exchange of gaseous and absorbent. Not permeable to microorganisms and water.	Used on exuding wounds that are low or moderate.	Mayet at el., 2014
Alginates	When on contact with exudate wound, it forms hydrophobic gel to encourage a moist healing process. It is easy to remove and clean from the wound without causing pain.	Used on exuding wounds that are low or moderate. Appropriate for use on infected wounds and not appropriate for use on dry wounds.	Aulton et al., 2013
Gels/Hydrogels	The gel controls hydration around the wound to maintain the moisture of the wound. It is absorbent to moisture	Used for cleansing of necrotic wounds through rehydrating of dead tissues.	Aulton et al., 2013
Hydrocolloids	It is absorbent to moist. Sticks to dry and wet wounds. Secondary dressing is not required.	Suitable for use on non-infected wounds that are light to moderately exuding.	Mayet at el., 2014

Studies have investigated the potential of antioxidant activity of Tannic Acid (TA) and mucoadhesive effect of Carbopol hydrogel materials. Tannins are plant phenol derivatives of various molecular weights naturally synthesized by plants as metabolic products (Haslam et al., 1996 ; Akiyama et al., 2001). Tannic acid (TA) is the simplest and principal hydrolyzable tannin that has astringent, antioxidant, antimicrobial, antiviral and anti-inflammatory properties (Fernandez et al., 2002 ; Buzzini et al., 2008). Carbopol's application for topical dressing delivery was discovered to have the ability to improve swelling and release properties of the hydrogel. It is biocompatible in nature and mucoadhesive poly(acrylic acid) polymer (Baljit et al., 2013). Also, studies have indicated that an ideal hydrogel for wound dressing will consist of the following properties; good mechanical properties, low toxicity, antibacterial, antioxidant, and be able to facilitate cell growth at area of the wound. Furthermore, a good quality, safe and efficacious hydrogel dressing consists of properties such as, (i) the ability to stop and maintain bleeding due to its blood compatibility and biocompatibility properties; (ii) ability to prevent microbial (bacterial) infections onto the wound through their adhesion and good mechanical properties; (iii) encourage cell proliferation and maintain a moist environment at the wound area; (iv) be non-ecotoxic (Jingjing et al., 2021).

1.2. Study rational and motivation.

Hydrogel scaffold wound dressings have unique properties that makes them ideal dressings which meets the essential requirements of a good wound dressing. Due to hydrogel wound dressing's unique properties that makes them an ideal dressing that meet essential requirements, exceptional attention has been invested into hydrogels more, as compared to

other available wound dressings (Baljit et al., 2013). Since the inception of the idea to use the gels as the basic dressing materials for treatment for wounds by Rosiak and co-workers, more research attention has been focused on developing advanced hydrogels with improved physical and chemical properties for effective wound healing (Rosiak at el., 1989). Hydrogels have water content of about 80%–90%, promoting donation of fluids to help control the moisture environment at the wound area (Mayet et al., 2014).

This study aims to design a hydrogel wound dressing using a solution of (5%, w/v) Tannic Acid (TA) and (5%, w/v) Carbopol® solution (Baljit et al. 2013). Tannins are plant phenol derivatives of various molecular weights naturally synthesized by plants as metabolic products (Haslam et al., 1996 ; Akiyama et al., 2001). Tannic acid (TA) is the simplest and principal hydrolyzable tannin that has astringent, antioxidant, antimicrobial, antiviral and anti-inflammatory properties. (Fernandez et al., 2002 ; Buzzini et al., 2008). It is believed that tannins can promote wound healing through several mechanisms: (i) scavenging of free radicals and reactive oxygen species (ROS), (ii) promoting wound contraction, and (iii) increasing formation of capillary vessels and proliferation of fibroblasts (Akiyama et al., 2001; Bakondi et al., 2004 ; Fernandez et al., 2002).

Carbopol® is an essential material used for wound dressings synthesis. Traditionally, it has been used in pharmaceutical products to control the viscosity of gels, lotions, and creams. Resultantly, drug delivery systems through transdermal and wound dressings derived from biomaterials have been using Carbopol (Renuka et al., 2012 ; Tang et al., 2007). Carbopol's application for topical dressing delivery have the ability to improve swelling and controlled release properties of the scaffold. Wound dressing must maintain water loss from the wound in a controlled manner to maintain the moist wound environment for better healing. It is biocompatible in nature and mucoadhesive poly(acrylic acid) polymer (Baljit et al., 2013).

1.3. Aim and objectives of the study.

The aim of this study is to develop phase morphing Tannic Acid Carbopol – Scaffold for targeted chronic wound application. The following objectives will be undertaken to fulfil the above aim:

Objective 1: To synthesise a scaffold using carbopol and tannic acid material with the ability to completely dissolve in wound fluid/PBS.

Objective 2: To evaluate the physicochemical and mechanical (tensile) properties of the scaffold using Fourier Transformation Infrared (FTIR) spectroscopy, XRD, and Texture Analyzer.

Objective 3: To evaluate thermal and morphological properties using Differential Scanning Calorimetry (DSC) and Scanning Electron Morphology (SEM).

Objective 4: In vitro evaluation of bioactive release profile via the UV–Vis spectrophotometer.

Objective 5: To evaluate the biodegradation of scaffold in PBS solution employing nanozeta-sizer equipment for evaluation of zeta-potential.

1.4. Overview of the dissertation

Chapter 1: will introduce the dissertation rationale with brief overview of wounds, wounds dressings and limitations associated with available treatments. The focus is mainly on chronic wounds and hydrogel-scaffold as the dressing of desires for chronic wounds management. Also, study aims and objectives including motivation are also outlined.

The successful design of chronic wound dressings depends on an understanding of several factors.

Chapter 2: presents a literature review on the understanding of several factors that determines the successful design of wound dressings for treatment of chronic wounds. It reviews classes of wounds, describes the skin process to heal wounds and associated challenge to successfully which limits healing. In addition, details on available options for treatments of wounds is provides and with best with challenges available to successfully to heal the wound. Finally, several physicochemical factors required to design effective wound treatments are detailed.

Chapter 3: describes the design, materials and methods utilized in the synthesis of the carbopol – Tannic Acid hydrogel scaffold. It details evaluation on the characterization of the carbopol, TA and scaffold, using the Fourier Transformation Infrared (FTIR), Differential Scanning Calorimetry (DSC), the degree of crystallinity using XRD, tensile strength, and Zeta-potential. The cumulative drug release profile was determined by dissolution and UV (Ultraviolet) Spectroscopy.

Chapter 4: provides a conclusion of the study and insight future recommendations in which the study can be enhanced.

CHAPTER 2 CRITICAL REVIEW OF HYDROGEL BASED WOUND DRESSINGS APPLIED IN THE TREATMENT OF CHRONIC WOUNDS

2.1 Introduction

The main primary protector of the human body that is capable of safeguarding the body from infections caused by microorganisms and external environmental damage is known as the skin (Jingjing et al., 2021). Of all the body parts/tissues, the skin is the primary protector, which is definitely the most exposed to damages and easily prone to injuries, abrasions, and burns (Boateng et al., 2015). The very same skin sometimes fails to protect the body and become susceptible to these damages, thus resulting into wounds which require healing (Boateng et al., 2015; Mayet et al., 2014).

Wound healing has become a global medical concern as wounds are contributors of several health and financial challenges. Several challenges which include the increase incidence of type II diabetes, obesity and chronic from types of wounds are the cause of pain and discomfort to patients and a greater disadvantage to the quality of patients' lives (Bohl et al., 2002; Boateng et al., 2015). Thus, these challenges have prompted the requirement of a more therapeutic-effective but yet cost-effective wound dressings. However, because of socio economic challenges, some millions of patients tend to incur a large cost of finances trying to treat chronic and painful wounds. Furthermore, at most one out of five diabetic foot ulcers result in amputation and death from surgical, diabetic, pressure ulcers and burn wounds (Bohl et al., 2002; Roy et al., 2015).

Restoration of tissues of the injured skin is the most important desired therapeutic effect because it results in a wound healing. The restoration and/or healing process is the complex process and involves numerous inter-related molecular as well as biological task to achieve full restoration of tissues (Kasuya et al., 2014). During normal conditions, the wound healing process takes place through four stages that overlaps. The overlapping stages include remodeling, inflammation, proliferation, and migration. The phase at which the skin surface is reforming and regaining its tensile strength is considered as a wound healing phase (Aulton et al., 2013; Mayet et al., 2014).

Since ancient times, dressing which were extensively used are described as traditional wound dressings. Dressing's (traditional dressings) treatment procedures were design with the main objective of being protective dressing materials and to as best as possible maintain the wound dry through allowing exudate to evaporate (Aulton et al., 2013). Furthermore, they should avoid dehydration infections occurrence of the wound. However, some of the traditional dressings have limitations as in some cases they encourage scar tissue formation and

generate new lesion once replaced (Miguel et al., 2001). Therefore, traditional dressings were assumed passive products with limited roles in wound healing process. Fortunately, researchers have discovered that wound can now heal expediently and victoriously in an environment that is moist than dry (Aulton et al., 2013).

The discovery of wounds healing faster and successfully in an environment that is moist has broaden the understanding of advanced wound dressings and their influences in successful healing to an extend that more research focus in now on designing best possible effective dressings (Aulton et al., 2013). With current discoveries, types of wound dressings have been designed with properties to promote wound environment that is moist as well as properties to enhance the healing process through prompting cell proliferation and adhesion (Miguel et al., 2001). Furthermore, what makes advanced wound dressings desirable is because they are biodegradable and non-toxic in their properties, and promote wound exudates absorption, and prevent dehydration of the patient (Dhivya et al., 2021). New advanced dressings have become available in an extreme number over the recent years, and their formulation was around the basis of designing an optimum environment for wounds treatment. However, despite the discoveries of advances wound dressing, their most limitation is their inability to have a singly dressing that is best suitable to treat and manage all types of wounds during all healing phases (Aulton et al., 2013).

Chronic wounds are mostly problematic and are generally difficult to completely treat, especially on the elderly and bedridden (Aulton et al., 2013). An expeditious repair of homeostatic physiological conditions is a desired requirement to achieve a complete repair, or otherwise, a steady and incorrect repair might result in serious wound damages. Damages like onset of infection, injuries to the circulatory system, loss of skin, occurrence of skin diseases, loss of hair and glands and death of tissues in severe cases are some of the possible damages associated with a slow and incorrect wound repair (Boateng et al., 2015). The damages from these chronic wounds are definitely compromising the quality of patient's live and finances, and places a big burden to the already compromised healthcare resources. A successful design of a best possible wound dressing is to prohibit wound related damages. Other advanced newly dressings are formulated to directly deliver wound-healing drugs and/or agents to the site affected (Aulton et al., 2013).

The successful design of chronic wound dressings depends on an understanding of several factors. Factors such as (i) the human anatomy and physiology; (ii) wound healing process, (iii) the patients' conditions in terms of the wound type, health, environment, and social circumstances, (iv) cascade of wound dressing and (v) the effect that the physicochemical

properties of the various dressing materials have on the wound-healing process are of cruciality in the successful design of dressing material in treating of acute and chronic wound (Aulton et al., 2013; Baljit et al., 2013). Therefore, in this review, a brief overview of human skin (anatomy and physiology) will be discussed. A broad overview of classification in types of wounds (acute and chronic) at various stages of healing, and processes involved in the healing of wounds will be is provided. As for treatment of these types of wounds, a cascade of wounds dressing options will be provided, including properties required to enable successful and effective management and treatment in acute and chronic wounds. Some limitations and drawbacks have been noted in treatment of wounds in general. That is, limitations, complications and factors effecting the effective healing of chronic wounds will also be provided in this review. The review will help researchers to successfully design and develop advanced wound dressings which are effective for treating chronic wound.

2.2 Brief overview of the physiology and mechanism of action of the native skin

The defence layer of the human body, which can defend the body form external damages from the environment and microbial infections is known as the skin (Zhang et al., 2020). There are three layers(i.e., the dermis, epidermis, and hypodermis) which mainly make up the skin as per figure 2.1**.** The human skin can sometimes be subjected to defects and breakings of the skin tissue and result to a wound. These defects and breakings generally result from mechanical and thermal damages or caused by underlying physiological or medical state in existence. The damages then compromise the integrity of the skin and limit its capability to safeguard the human body against external infections (Mayet et al., 2014). Other tissues of the skin can be compromised by the extent of these damages. The damages can extend as far as to other structures of the body such as subcutaneous tissue, vessels, nerves, tendons, muscles and as well to the bones to cause death (Boateng et al., 2015, Velnar et al., 2009; Thu et al., 2012). About 265 000 deaths occurs yearly from skin burns or lack of wounds therapeutic treatments as reported by the World Health Organization (WHO), (Das et al., 2016; Khorasani at el., 2018).

The skin is remarkable in its ability to repairs itself from injuries naturally. However, when the damages are large and severe, skin's abilities are limited and compromised. An intervention in a form of proper and instant wound area coverage with an optimal dressing device or any adequate dressing is required to assist the skin protection and speed-up wound healing. Ultimately, the primary goal of wound care management is immediate wound coverage and protection (White et al., 2006). Many factors are involved and need to be taken into consideration to encourage and guarantee a successful wound management in the wound healing process (Mayet et al., 2014).

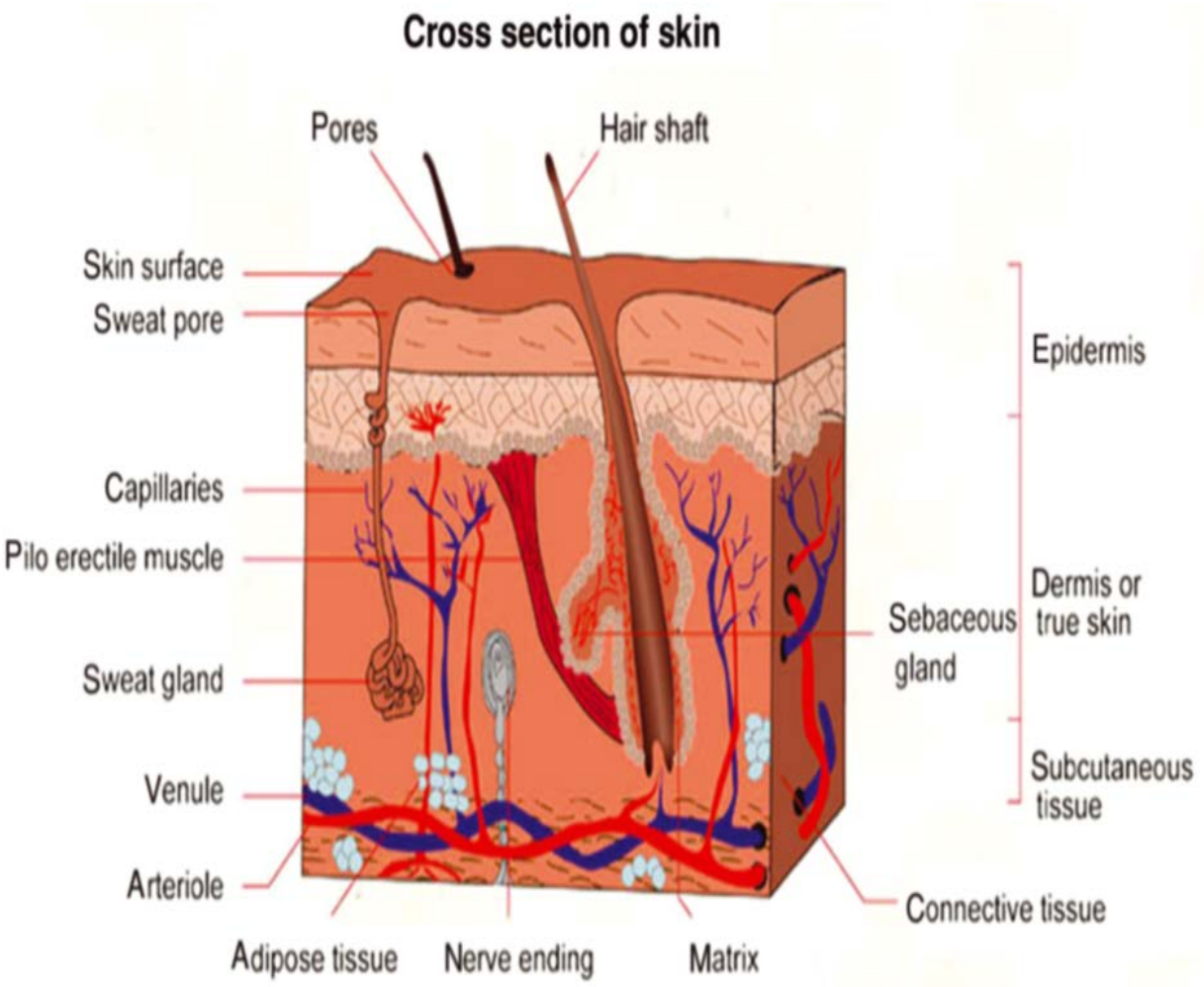

Figure 2-1 The anatomy of skin with main components which comprises of the epidermis, dermis, and sub-dermal layer which makes up a complete skin (Mayet et al., 2014).

The following section discusses classification in types of wounds (acute and chronic wounds) based on the healing process and healing rate will be discussed. Special attention is given to common chronic wounds and their associated complications, management, and their compromises to the quality of patients' lives and enormous financial burdens are also discussed. The key elements involved in the stages and processes of wound healing will be discussed to outline stages of wound healing and possible complications encountered at each phase of wound healing process.

2.3 Classification of types of wounds and management of chronic wounds

Damages to the skin may results in several types of wounds, and their categorizations are decided in accordance with, the affected area of the skin; their healing process and periods; and as well as the affected number of layers of the skin figure 2.1. Wounds have main two types of classes/categories. They can be categorized as either acute or chronic wounds.

Categorization is on the basis of their nature of the repairing process, thus wounds can become acute when the healing process and rate takes less than 8 weeks and chronic when the healing process and rate takes longer than 8-12 weeks (Aulton et al., 2013). The process of wound healing and the rate at which it occurs can be affected by various conditions. Both local and systemic conditions such as lack of blood flow to the wound area and diabetic condition delays and disrupts the healing process. Microbial infections from environmental conditions also disrupts the healing process of several types of wounds. Surgical, bite, pressure ulcers, burn and diabetic wounds are examples of wounds that can be affected by bacterial infections, thus, delaying and disrupting the healing process resulting into a chronic wound (Roy et al., 2015).

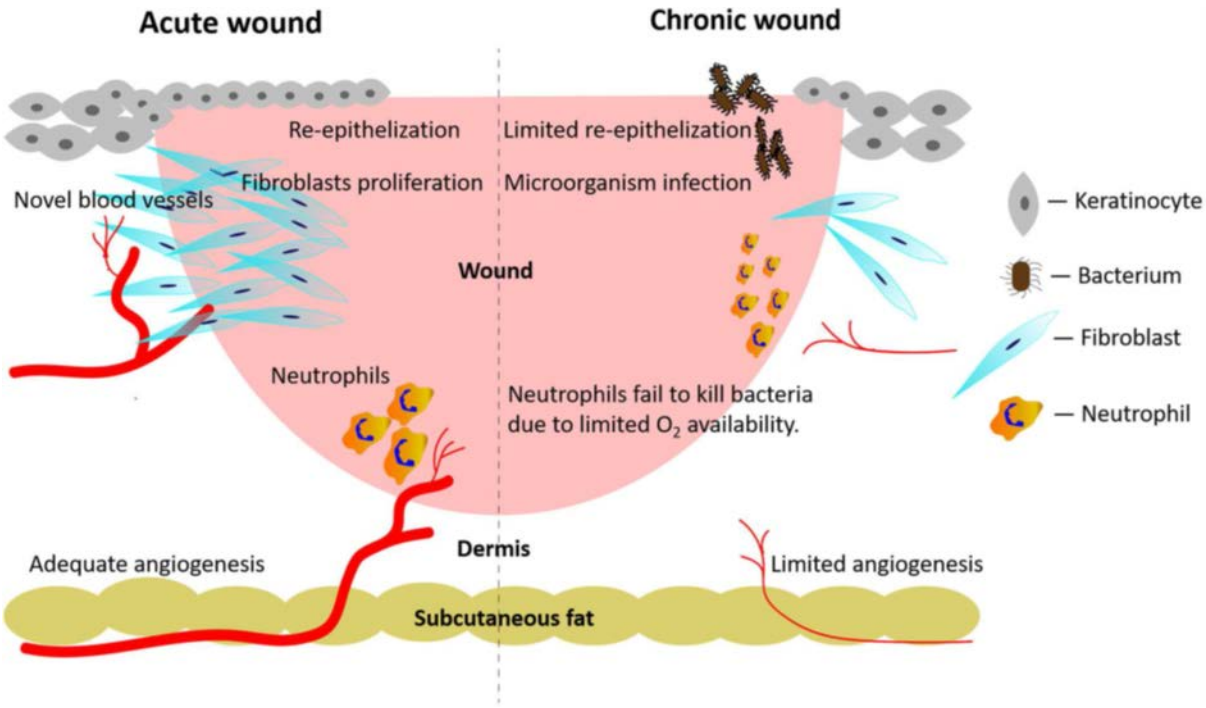

Figure 2-2 Pathological mechanisms active in acute wounds and chronic wounds, respectively. Acute wounds (Left Side): an adequate angiogenesis promotes re-epithelialization, fibroblasts' proliferation, and neutrophils' anti-infection activities. Chronic wounds (Right Side): persistent local bacterial infections hinder the formation of novel blood vessels. In turn, the restricted angiogenesis hampers fibroblasts' proliferation and the neutrophils' anti-infection activities (Wang et al., 2020).

The perceptive understanding of cutaneous burns and wound healing requires thorough comprehension and knowledge with regard to the function of the skin, its anatomy and

physiology (Mihm et al., 1976). Abnormalities may occur during the healing process, which could result in longer healing times and/or turn the wound from acute to a chronic (Aulton et al., 2013). Acute wounds are primarily caused by mechanical injuries which rises from external factors such as tears and abrasions. Also, burns and chemical injuries arising from sources such as corrosion, radiation, electricity, chemical and thermal source courses acute wounds. Whilst chronic wounds form from skin tissue injuries which heals steady and /or do completely heal within 12 weeks (Bohl et al., 2002 ; Gurtner et al., 2008). Chronic wounds are promoted by factors such as infections, diabetes, or radiation exposures (Dovi et al., 2003).

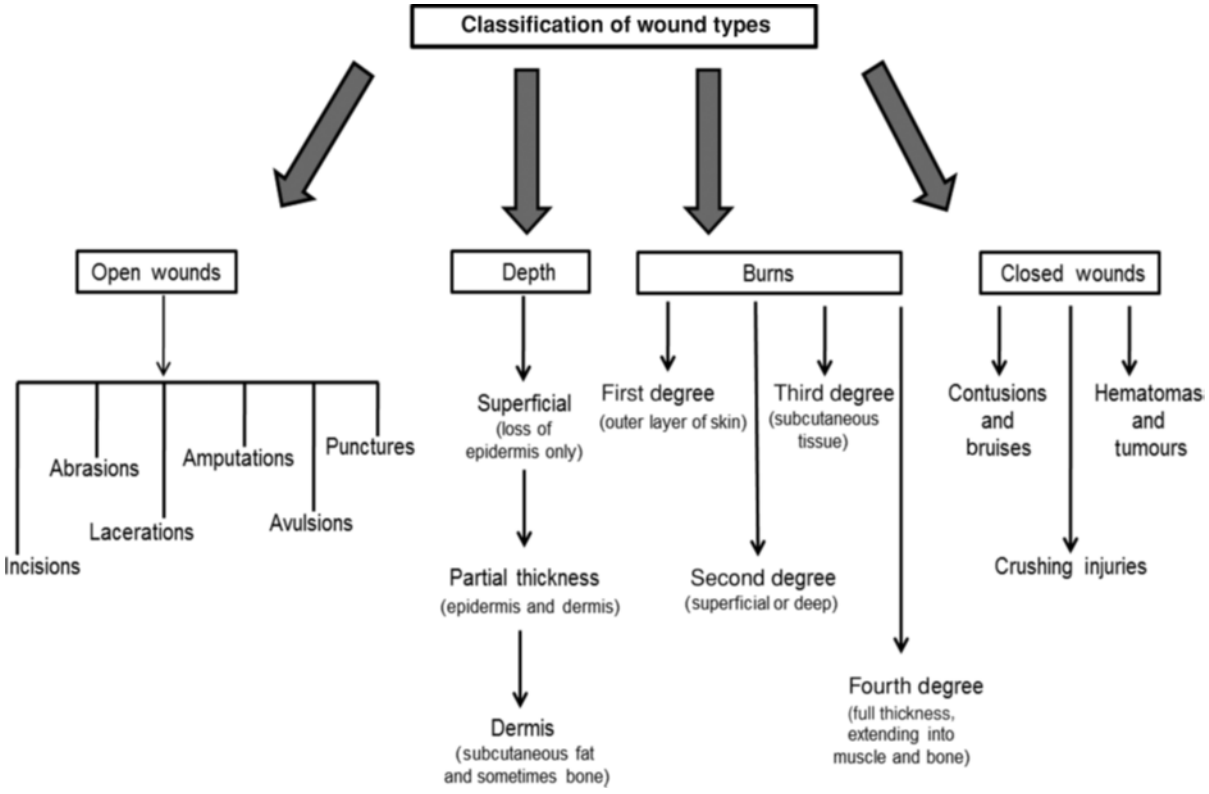

Figure 2-3 Further classification of various forms of wounds (Mayet et al., 2014).

2.3.1 Chronic wounds and associated drawbacks in their management and healing

Developed continents such as Europe have about 4 million patients suffering from chronic wounds every year, requiring 25%–50% of acute hospital beds. Furthermore, the costs associated with chronic wound treatments represent at least 4% of the national annual budget (Rossella et al., 2022). Chronic wounds have significantly become a burden to the population's health and socioeconomics to an extent that they have become a topic of interests. Worldwide these types of wounds form/occur as a result of conditions like severe contaminations,

diabetes, leading to ulcers, and tumors. Because chronic wound takes too long (more than 12 weeks) to heal, the possibility of their reoccurrence is inevitable (Mayet et al., 2016).

Patients which suffer chronic wounds most, are those that suffers with feet diabetics compared to other diabetics at other body parts. This is because feet are continuously in walking functions and further aggravations because of stubbornness of microbial infection, to sympathetic nerve dysfunctions (Akash et al., 2020). Approximately one out of six diabetic patient suffers from chronic foot wounds and one out of five diabetic foot ulcers result into amputation (Akash et al., 2020; Bohl et al., 2002). This is each year of almost one million of patients with diabetic patients undergoing lower limb amputation (Boulton et al., 2005). As chronic wounds prolong to heal, they often become heavily contaminated, usually resulting in significant loss of skin tissues that affects vital body pats like nerves, joints, and bones. Furthermore, impaired wound healing can lead to an excessive production of exudates that can cause maceration of healthy skin tissue around the wound (Cutting et al., 2002). Such wounds fail to heal because of repeated trauma to the injured area or underlying physiological conditions such as diabetes, persistent infections, poor primary treatment, and other patient-related factors (Boateng et al., 2015).

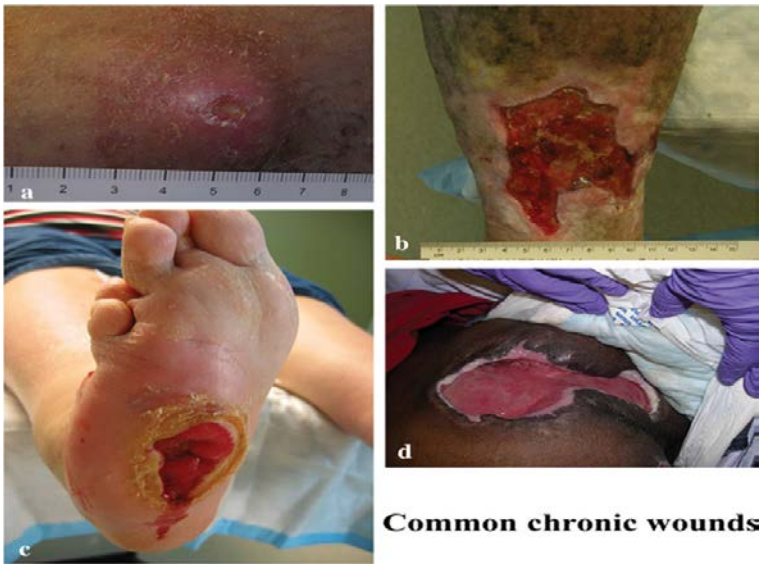

Figure 2-4 (a) Arterial ulcer at the cross malleolus of the leg with sharp margins and a punched out appearance. (b) Venous stasis ulcer with irregular border and shallow base. (c) Diabetic foot ulcer with surrounding callus. (d) Pressure ulcer in a paraplegic (impairment of motor or sensory function in the lower extremities) patient, causing full-thickness skin loss. Adapted from (Boateng et al., 2015).

Management of chronic wounds employing a system that can deliver an active pharmaceutical ingredient to the wound site following a controlled fashion can improve patient's therapeutic outcomes and compliance. More especially when patients have to undergo frequent changes of wound dressing because of a long required treatment (Langer et al., 1980). Chronic wound treated by traditional therapeutic dressings cannot improve patient's therapeutic outcomes and compliance as they cause unavoidable bodily scars and physical dysfunctions. As a result, patients tent to experience pain for too long (Wang et al., 2020.). For treatment of local infections, potential dressing useful include bioadhesive and polymeric dressings (synthetic, semisynthetic, or naturally derived), where it may be beneficial to achieve increased local concentrations of antibiotics while avoiding high-systemic doses. Thus, reducing patient exposure to an excess of drug beyond that required at the wound site (Langer et al., 1980). Table 2.1 summaries common chronic wounds experienced in clinical wound therapies and their associated risk and symptoms.

Table 2-1 Most prevalent chronic wounds encountered in clinical wound therapy.

Type of Ulcer	Description	Risk Factors	Symptoms	Reference
Diabetic ulcers	Diabetic foot ulcers (also known as neuropathic ulcers) are a major complication of diabetes mellitus. The most common cause is uncontrolled blood glucose (sugars) over a prolonged period of time. Two other disorders, diabetic neuropathy, and peripheral vascular disease, can also contribute to ulcer formation.	• Uncontrolled blood sugars • Diabetic peripheral neuropathy • Peripheral vascular disease	Diabetic ulcers usually present on the foot at an area of trauma or a weight-bearing surface. The wound bed is commonly dry and may have necrotic tissue or a foul odour. This kind of ulcer may be a small wound area on the outside but can hide an underlying abscess. The skin around the wound commonly has hyperkeratosis. These ulcers are generally painless because of altered sensation or neuropathy.	Lee at el., 2004; Losi at el., 2013; Moira at el., 2013 and Moura et al., 2013.
Venous ulcers	Venous ulcers, also known as vascular or stasis ulcers, develop as a consequence of venous insufficiency. The damaged valves allow blood to pool in the vein, and as the vein overfills, blood may leak out into the surrounding tissue leading to a breakdown of the tissue and development of a skin ulcer. Venous ulcers commonly occur on the sides of the leg, above the ankle and below the knee.	• Deep vein thrombosis • Obesity or poor nutrition • Pregnancies • A family history of varicose veins • Smoking and excessive alcohol use • The lack of physical activity • Aging	The first sign of a venous skin ulcer is skin that turns dark red or purple over the area where the blood is leaking out of the vein. The wound bed is often beefy red and may bleed easily. The ulcer may be painful. Necrotic tissue, slough (yellow, tan, grey, green, or brown) and/or eschar (tan, brown, or black), may also be present. The skin may also become thick, dry, and itchy. Venous ulcers are commonly slow to heal and often require lifetime modifications to prevent re-development.	Boateng at el., 2007; Hareendran at el., 2005 ; Koksal at el., 2003 and Thomas et al., 1996

Arterial ulcers	Arterial ulcers result from a complete or partial blockage in the arteries. They are almost always caused by atherosclerosis. In this pathology, cholesterol or other fatty plaques settle in the arteries causing obstructions that result in poor blood circulation. This poor circulation leads to tissue death and ulcer formation.	• Trauma • Limited joint mobility • Increased age • Diabetes mellitus • High blood pressure • Arteriosclerosis • Peripheral vascular disease	Wounds commonly have minimal drainage and are often very painful. Pain is often relieved by dangling legs and increased when legs are elevated.	Boateng at el., 2015 and Moffatt at el., 2014
Pressure ulcers	Pressure ulcers, also known as decubitus ulcers or bed sores, occur in people with conditions that limit or inhibit movement of body parts that are commonly subjected to pressure, such as the sacrum and heels. A pressure ulcer is an area of skin that deteriorates when the skin is exposed to prolonged pressure. This prolonged and unrelieved pressure restricts blood flow into the area and tissue damage or tissue death results.	• Patients confined to wheelchair or bed • Increased age • Mental or physical deficits that affect their ability to move. • Chronic conditions that prevent. areas of the body from receiving proper blood flow • Fragile skin (patient under steroidal therapy), urinary or faecal incontinence • Malnutrition	A pressure ulcer generally starts as reddened area on the skin and, if the contributing pressure is unrelieved, the ulcer progresses to a blister, then an open sore, and finally a deep crater. This deterioration may occur rapidly. The most common places for pressure ulcers to form are over bones close to the skin, such as the sacrum, heels, elbows, hips, ankles, shoulders, back, and back of the head. Pressures sores are categorized from stage I (earliest signs) to stage IV (worst) according to severity and the treatments depend on the wound stage.	Miller at el., 2009; Eccleston at el., 2007; Chang at el., 1995 and Chang at el., 1998

2.4 Mechanism underlying wound healing and associated complications in chronic wounds

Wound healing is a dynamic interactive process that involves parenchymal cells, extracellular matrix (ECM), blood cells and soluble mediators (Mayet et al., 2016). The repair of wound starts immediately after the infliction of damage (Kasuya t el., 2014). This section discusses the key elements involved in the stages and processes of wound healing. The inflammation stage occurs straight away after the injury; followed by the proliferation of new tissue; and then maturation (remodelling) of the dermal tissue to regain tensile strength as outlined in figure 2.5**.**

2.4.1 Inflammation phase of wound healing and associated complications

The first phase which responses to an injury is called inflammation phase (Aulton et al., 2013). This phase prepares the injured area to begin the process to heal by prompting the wound, causing it to be painful and swell (Mallefet et al., 2008). Bleeding will take place at the injured area to ensure toxic wastes are removed and homeostasis stars triggered by exudate components. The clotting phases is triggered by platelets, resulting in coagulation and fibrin network formation. Clotting also encourages phagocytosis, vasodilation, vasodilation, and phagocytosis whereby release of serotonin and histamine takes place. Bacteria and dead cells are then engulfed as phagocytes enters the wound. Bleeding stops through the clotting mechanism caused by liberation of platelets and formation of aggregates (Boateng et al., 2008). This phase of healing normally takes place between few minutes and 24 hours of skin injury resulting in the wound being in pain, red in colour, moist and inflamed (Aulton et al., 2013). It is however discovered that too much inflammation might setback the process of wound healing, because prolonged edema disturbs progression to the next step of proliferation (Kasuya t el., 2014)

2.4.2 Proliferation phase of wound healing

Proliferation phase involves epidermal cells proliferating at the border of wound beyond the actively migrating cell. This phase occurs and forms granulation tissue at the wound area just in 2 days to 3 days after the injury. The effect of macrophages and fibroblasts is known as granulations, which continuously provide growth factors important to vitalize fibroplasias and angiogenesis (Adam et al., 1999). However, the dysregulation of fibroplasia leads to sclerotic disease and also provides an implication to understand wound healing (Kasuya t el., 2014).

2.4.3 Remodelling (maturation) phase of wound healing

About three weeks post injury, the remodelling stage is triggered. This stage is the final stage with the aim to form large tensile strength of dermal tissue whereby new collagen molecules

are formed (Aulton et al., 2013). Maturity of the collagen is reached at the wound site as it is dispatched in the outside the cell space in which firm cross-links are formed. The collagen will overtime gain stability and strength, but the injured skin will never regain full properties of its tissue (Sibbald et al., 2003). This takes place 3 weeks after injury and the wound repairs is up to 2 years. The timescale for wound repairs is from about 3 weeks to 2 years. Usually, the tensile strength of the final scar reverts up to 70 and 90% of that of the pre-injured tissue (Aulton et al., 2013).

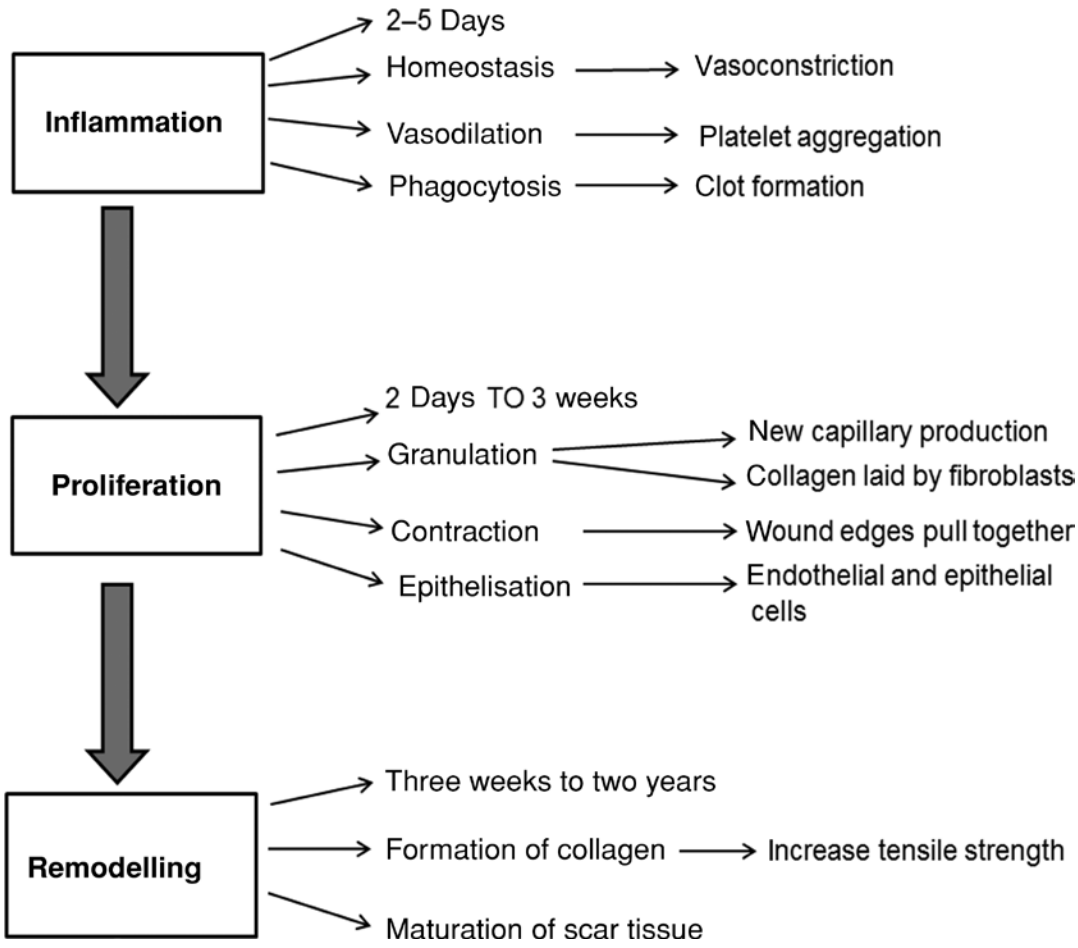

Figure 2-5 Phases and factors involving wound healing process. The process consists of inflammation, proliferation, and remodelling. Multiple factors accelerate and delay wound healing. Several signaling pathways work co-dependently (Mayet et al., 2016).

2.4.4 Factors affecting the healing rate and process of chronic wounds

Complications and abnormalities may occur during the healing process, which could result in longer healing times and/or turn the wound from acute into a chronic wound. This is despite the fact that, healing process is a natural and predicated process in nature (Aulton et al., 2013).

Both local and systemic conditions such as lack of blood flow to the wound area and diabetic condition delays and disrupts the healing process (Roy et al., 2015). Table 2.2. summaries conditions with effects in the healing rate and process resulting in complications and abnormalities. For example, poor treatment as well as bad local, environmental, or systemic conditions can turn a wound from acute to chronic at any stage of healing process. Also, factors such as, the health status of the patient, and bad nutritional status disrupts the healing process and inhibit the ability of the body to fight infections and recover. Surgical, bite, pressure ulcers, burn and diabetic wounds are examples of wounds that can be affected by bacterial infections, thus, delaying and disrupting the healing process (Roy et al., 2015).

Table 2-2 General patients' health, environment, and social circumstances factors affecting wound healing process.

Factors	Effects in the healing process and rate	References
Smoking	Decreases oxygen delivery to the wound due to vasoconstriction and coagulation of small blood vessels. Poor surgical apposition.	Aulton et al., 2013
Mobility	In this pathology, cholesterol or other fatty plaques settle in the arteries causing obstructions that result in poor blood circulation. This poor circulation leads to tissue death and ulcer formation.	Boateng at el., 2015
Compromised circulation	Inadequate cellular oxygenation and perfusion leads to impaired wound healing, triggering wound maceration, and delayed healing.	Rodriguez at el., 2008
Advancing age	Elderly patients have less effective immune system, resulting in decreased resistance to pathogens. Potential problem in healing arises from skin changes, slower metabolism, and chronic health conditions such as circulatory problems and diabetes.	Aulton et al., 2013
Obesity	Excess local mobility, such as over a joint. Excess tension placed on the wound and decreased vascularity of adipose tissue delays healing. Mobility may be reduced.	Boateng at el., 2015
Nutritional status	Deficiencies in protein, vitamins (especially ascorbic acid) and minerals impair the inflammatory phase and collagen synthesis and thus prolong healing times.	Aulton et al., 2013

2.5 Wound dressings options and classification in treatment of wounds

Wound treatment materials are available in numerous varieties or categories. Commonly wounds dressing are classified into types, traditional and advanced wound dressing (Figure 2.6). They can be categorised by the function of the material used at the wound are (e.g absorbent, adherent and occlusive dressings), the different material used in synthesising the dressing materials (e.g polyurethane, collagen, silicone, and collagen dressing types), and/or

the physical nature of the dressing (e.g film, gel, or foam dressings). Also, other dressings are referred to as primary dressings due to their functionality to make contact with the wound area or injured skin directly. Whilst secondary dressings are the type of dressings which makes direct contact with the primary dressing. Lastly, other dressings are those that are incorporated with pharmaceutical agents like antibacterial agents and growth factors. These types of dressing are still the newly and infancy. The above varieties of wounds dressing types are summarised in table 2.3. Below, a variety of available types of dressing are discussed in more details, including their functions and limitations (Aulton et al., 2013).

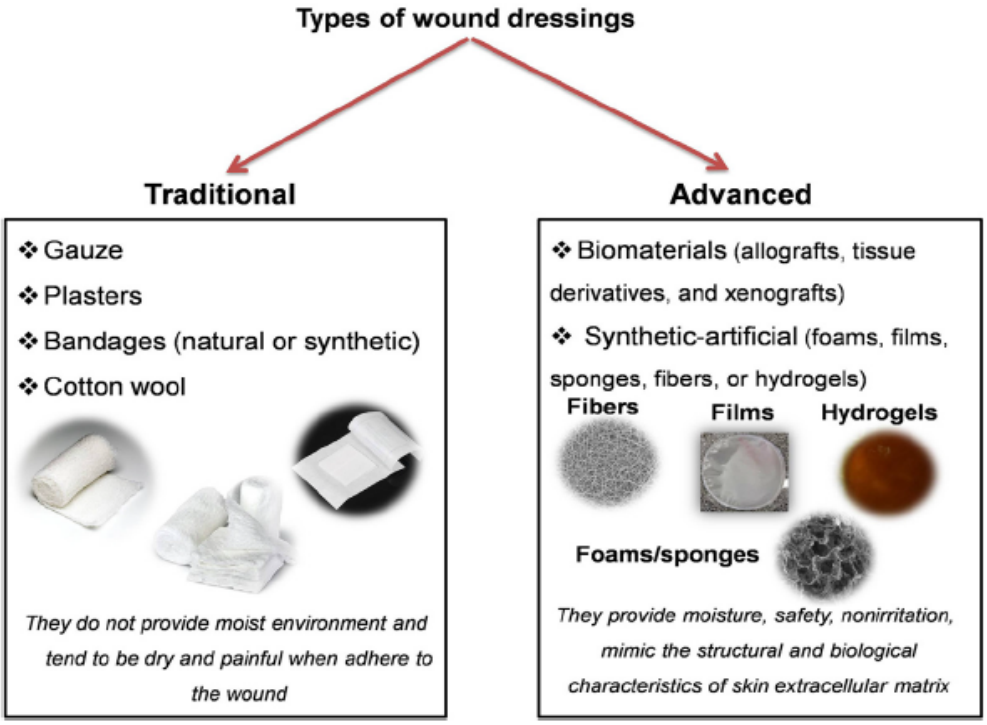

Figure 2-6 An overview of traditional and advanced wound dressings (Bulbul at el., 2022).

2.5.1 Traditional wound dressings and associated limitations in wounds treatments

Traditional wound treatment dressing products have been in use for centuries and these dressings are still in use to date. Each dressing having a specific role as either primary or secondary dressing. These traditional dressings include, cotton wool, gauze, lint, and synthetic or natural bandages (Aulton et al., 2013). Their principle for managing the wound area is simply, they engulf the wound, conceal it, and keep it dry and clean. The dressings are basically used to protect the wound from contaminants. However, these materials' applications in the past for wound management are known to be passive product and their use in wound healing is encouraged very little. Drug delivery to the wound area using traditional wound material is ineffective mostly because they absorb fluids very fast, and during the process,

their rheological characteristics is lost and become mobile. The less encouraged traditional wound dressing types include topical bioactive agents in the form of creams, ointments and for drug delivery (Boateng et al., 2015).

Uses of traditional dressing material are specific to the functions they are able to perform as compared to the use of advanced dressing material like colloids and hydrogel in which each of the dressing material can play a bigger and crucial functions in wound healing process (Mayet et al., 2016). Some of the traditional dressings that have limits in their effective functions of healing performance include cotton wool and gauze. These dressings make the wound painful when cleaned or removed because as the fluid evaporated the wound, they become adhesive. Thereafter, the wound healing process is delayed when evaporation of fluids is encouraged, thus, leaving the wound area dehydrated and painful. In addition, the composition of gauze dressing includes interwoven fibres of polyester and cotton, and its compositions causes great discomfort to the patient as they become embed and loose under the skin (Boateng et al., 2015; Slaughter at al., 2009).

It is for the above findings that the use of solid dressing material in the case of exudative wounds is preferred as better management of exudate is provided and residence of the dressing at the wound site is prolonged (Boateng et al., 2015). The use of traditional wound healing agents is outweighed and less preferred over the use of advanced modern dressings which encourage a moist environment, prevent contamination by bacteria, ensure gaseous exchange that is effective, and inhibit wound emaciation (Slaughter at al., 2009). Therefore, the use of traditional wound dressings for treatment of chronic wounds and most general wounds is being replaced by the more recently developed advanced wound dressings described in this review. In treatments of chronic wounds, therapeutic strategies that are commonly used are chemical approaches like agonists drugs, growth factors and/or inhibitors of critical signaling pathways (Wang et al., 2020). Other recent research fields of interest that might play a key role in the healing of chronic wounds includes fields of nanotechnology and stem cells (Jiang et al., 2018; Bhattacharya et al., 2019).

2.5.2 Advanced wound dressings and associated limitations

The main hypothesise of wound treatment are for the wound to heal as quickly as possible, with less pain as possible, minimum discomfort and little scarring the patient (Langer et al., 1980). These wound treatments hypothesise are achieved when there is minimal scar tissue formation after treatment, there is reduction in production of necrotic tissue and prevention of microbial invasion to the wound. Due to limitations in traditional wound dressings, there has been a shift from simple traditional material to specialised advanced material with specific

properties. Advanced wound materials such as polymers and hydrocolloids are incorporated with various components in optimised proportions (Slaughter at al., 2009). Current treatment options for advanced wound dressings fall under many classification criteria which classify their properties and functions in wound healing, table. 2.3. (Boateng et al., 2015).

Other, advanced materials are formulated with the aim of having a biological activity to release bioactive materials (drugs) induced in the addressing form. These dressings are designed to have biological activity either on its own or by the release of bioactive constituents (drugs) incorporated within the dressing. Whilst traditional dressings such as cotton wool and quaze do to not play a therapeutic role to the wound. Other types of dressings are combined with active pharmaceutical agents such as antibacterial agents or wound debridement agents. Dressing induced with human Platelet Derived Growth Factors (PDGF) has been discovered to treat chronic diabetic ulcers (Aulton et al., 2013). The incorporated drugs can play an active role in the wound healing process either directly as cleansing or debriding agents for removing necrotic tissue, or indirectly as antimicrobial drugs, which prevent or treat infection or growth agents (growth factors) to aid tissue regeneration (Boateng et al., 2015).

Recent greater findings on the underlying cellular and molecular abnormalities that prevented smooth wound healing has revolutionised concept of chronic wounds treatment. An understanding of wound material mechanical, pharmacokinetic and pharmacological properties has provided a novel approach to assist in natural healing and promote greater effects of advanced therapies (Wang et al., 2020; Slaughter at al., 2009). Advanced materials comprising of both natural and synthetic polymers have also been discovered with the ability to control drug delivery at the wound area (Wang et al., 2020). With the controlling degree of swelling, cross-linking density, and degradation rate, delivery kinetics can be tailored according to the desired drug release schedule (Slaughter at al., 2009). Drug release from polymeric designs is controlled by one or more physical processes including, (1) eventual degradation/erosion of the polymeric system, (2) diffusion of drug through the polymer matrix, (3) swelling to form a gel, and (4) hydration of the polymer by fluids (Boateng et al., 2015). The control release formulations can provide not only analgesia for a longer duration, but can also provide slow antibiotic release according to the requirement of the wound. Controlled release of antibiotic ensures a microbe-free environment, and analgesic ensures painless recovery of the patient (Baljit et al., 2016).

2.5.3 Classification of wound dressings

Current treatment options for wound dressings fall under various classes, classified according to their roles, the type of wound applied on or the physicochemical properties of the dressing

(Aulton et al., 2013). Dressings such as films, scaffolds, ointments, creams, and gels are classified by the physical properties (White et al., 2006). Other dressing materials can be classified according to their function at the wound area, namely antibacterial, absorbent, debridgement, adherent or occlusive. Also, classification by the materials used to design dressing material such as chitosan, collagen, alginate, and hydrocolloid can be used for classification of wound dressings (Lipsky et al., 2009). Furthermore, another criterion to classify include advanced modern wound dressings, traditional wound dressings, skin replacement products and wound healing devices. Primary and secondary dressings differentiation is used to differentiate between the primary wound dressing which makes direct contact with the injured skin and secondary which makes direct contact with the primary dressing respectively. Island dressings materials that contain an outer adherent portion with an inner absorbent are classifies as island dressings (Thu et al., 2012; Bowler et al., 2001). Table 2.3 below summarises classification of wounds materials according to their physical properties and application at the wound area (Slaughter at al., 2009).

2.6 Physical characteristics of Ideal properties of advanced wound dressings

The successful design of chronic wound dressings depends on an understanding of the physicochemical properties of the various dressing materials. Evaluation of wound dressing's mechanical and physicochemical properties take high priority when designing and choosing the ideal wound dressing. Most importantly, dressing mechanical strengths, and other properties associated with mechanicals should align with mechanical properties of the natural skin (Bulbul at el., 2022). Major properties of advanced wound dressings include, tensile strength, stress-relaxation rate, stress stiffening effects, elastic modulus, stiffness, viscoelasticity, and more. The composition and physical properties of wound dressing influence its ultimate performance. There are essentially four types of interrelated specifications for wound dressings, discussed in detail below: (Aulton et al., 2013; Bulbul et al., 2022).

2.6.1 Structural Properties

Which define the composition and structure of a dressings. These properties of wound dressings include surface topology, porosity, pore sizes, and organization of inner frames (Wang et al., 2020). The early monographs for dressings consisted mainly of structural specifications with limit tests for potential contaminants. Most dressings monographs were removed from pharmacopoeias when dressings were classified as medical devices from medicines (Aulton et al., 2013).

2.6.2 Performance Specifications

Which characterize one or more functions of dressings. Although the success of a new dressing is ultimately determined by its clinical performance, laboratory testing can provide a rapid estimation of how a dressing will function under certain conditions. The specific property to be characterized in the laboratory will follow nature of wound dressing type and type of wound.

Table 2-3 The key properties of common types of wound dressings.

Form	Materials/Polymers	Bioactives/Other Agents	Application	Reference
Traditional wound dressings	Cotton wool; Gauze and cotton bandages	Can be used in conjunction with hydrogels and hydrocolloids	Primary and secondary dressings	Asbill et al., 2000 and Aulton et al 2013
Hydrocolloids	Gel forming agents such as Elastomers ,gelatine, pectin, and adhesives	Active agents such as local anaesthetics	Clinically useful due to adhesion to both moist and dry sites as well as provision of an optimal wound environment	Boatang et al., 2008 and Aulton et al 2013
Wound healing films and semi-permeable films	Co-polymers, omopolymers and plasticised polymers both natural and synthetic	Wound healing active incorporates such as growthfactors, stabilisers and anti-microbials	Primary and secondary dressings, in conjugation with foams, hydrogels or hydrocolloids. In addition, semi-permeable films may promote enhanced gaseous exchange and a moist wound environment	Aulton et al 2013; Eming et al., 1999 Rossi et al., 2013
Wound healing foams	Natural polymers and polysaccharide. Solid matrix with dispersed gas particles	Pain relieving, anti-bacterial and anti-inflammatory therapeutic agents	Controlled drug delivery and as a matrix for cell growth	Galeano et al., 2003; Falanga. 2005
Hydrogels	Synthetics and natural polymers, e.g., polyvinylpyrrolidine and methacrylates	Anti- inflammatory, anti-microbials and local anaesthetics	Primary dressing having swellable and hydrophilic properties	Kootstra et al., 2003 and Aulton et al 2013

Multi-layered dressings	Natural and synthetic polymers	Active agents such as local anaesthetics, anti-microbials and anti-inflammatories	Multi-characteristic dressings that may provide therapeutic properties simultaneously such as adhesion, absorption, mechanical support and strength and a moist wound environment	Wei at el., 2004
Lyophilised waters	Synthetic polymers: Polyurethane	Antibiotics and growth factors	Adhesive and absorptive dressings	Morghimi et al., 2009; Lee at el 2001
Electrospun nanofibers mats and scaffolds	Natural, electroconductive and synthetic polymers such as	Active drugs such as anti-inflammatories, antibiotics and antiseptics	Primary and secondary dressings mimicking the properties of the	Lawrence. 1994

It is now well discovered that a moist wound environment provided by wound exudate encourages more rapid wound healing, although excess exudate may cause maceration of the wound and surrounding area. There are a number of physical tests that reflect this function by determining the absorbing properties of dressings. Such absorbency tests investigate parameters such as fluid retention, moisture-vapour transmission rate and fluid handling. Fluid affinity tests are used for dressings such as hydrogel that also donate moisture in order to promote autolytic debridement (Aulton et al., 2013; Bulbul et al., 2022).

Laboratory test to determine performance properties of wound dressings
(Eghbalifamet et al., 2020, Namviriyachote at el., 2020; Aulton et al., 2013).

- **Fluid Absorbency**
  - The standard laboratory methods used to assess absorbency focus mainly on the dressing constituency (i.e., hydrocolloid, alginate, or hydrogel).
  - Although these methods enable comparison of the fluid handling properties of structurally similar dressing and provide useful quality control tests, the test are specific to individual dressing groups and the fluid handling properties of different dressing groups, (e.g., alginate versus hydrocolloid, cannot be compared).
- **Fluid Affinity**
  - This test investigates a dressing's ability to donate moisture to, or absorb liquid from, standard substrates. It is largely applicable to amorphous hydrogel dressings which have the ability both to donate and absorb wound exudates.
  - The ability to donates fluid helps to facilitate the rehydration of dry necrotic tissue to promote autolytic debridement.
  - A high fluid affinity also helps absorb excess wound exudate and liquefied tissue debris once autolytic debridement has taken place.

2.6.3 Mechanical properties of dressings.

Mechanical properties of dressings are key because all dressing is of good quality if they are flexible and durable enough to accommodate the stresses caused by distortion or movement when they are applied to different areas of the wound. Mechanical test also provides useful structural parameters in quality control. Properties such as tensile strength (films), compression (foams ad hydrogel sheets) and rheological properties (rehydrated gels) are widely investigated (Venkatachalam et al., 2012; Aulton et al., 2013).

2.6.4 Laboratory test to determine Mechanical Properties of Dressings

(Wang et al., 2020; Venkatachalam et al., 2012; Aulton et al., 2013)**.**

- **Tensile Properties Test**

- During a tensile test, a sample of film dressing is stretched until it breaks. The applied force and displacement (amount of stretch) are measured. From that, (tensile strength, percentage strain at break, and the elastic modulus) can be calculated.
- These parameters are used to provide information about the film's flexibility and rigidity.
- The parameters are useful un assessing the suitability of a film dressing for application to different parts of the wounds.

- **Compression test**
  - For dressings of greater thickness than films, (e.g., foam and hydrogel sheets) while may be able to take up large volumes of fluid but not retain the fluid even under light pressure, hardness tests can be employed.
  - Test can be used to compare the fluid handling properties of these products under varying levels of pressure.
- **Rheological test**
  - Rheological properties such as apparent viscosity, elasticity, and viscoelasticity are useful for characterizing rehydrated hydrocolloid or alginate gels, and amorphous hydrogels.
  - Optimization of rheological parameters can be an important quality control toll and help meet the requirements of such dressings, such as their ability to fill a wound and be pain free on removal.

2.6.5 Safety and acceptability specifications

To ensure that the dressing is safe and acceptable when used appropriately. The biocompatibility and its general safety profile are also significant factors that can affect the performance of the wound dressing and its ability to induce proper healing (Bulbul et al., 2022). Test specifically designed to ensure that wound healing dressings products are safe and acceptable include assessment of bioadhesion, microbial performance, odour, irritancy, and sensitivity. The tests are described below (Aulton et al., 2013).

2.6.6 Laboratory test to determine safety and acceptability specifications

(Bulbul et al., 2022; Aulton et al., 2013)**.**

- **Bioadhesive strength**
  - Bioadhesion is defined as the force required to detach a sample from the surface of a wound.
  - The mechanical and in-vitro bioadhesive strength properties are tested using texture analyser equipment.

- The test measures the force require to detach the dressings from the surface of model excised skin.

- **Odour Control Test**
  - Some types of wounds produce noxious odours caused by the number of volatile agents produced by microorganisms.
  - One of the tests available to test odour adsorbing dressing uses specialised apparatus to examine the ability of different dressings to prevent the passage of volatile amine when applied to a wound.

2.7 Conclusion and Future perspective

Nearly everyone has suddenly suffered an open skin wound as a consequence of a cut, burn, diseases (e.g., diabetes) or surgical interventions. It will take an acute wound few weeks to heal, however with chronic wounds the wound remains unhealed for a long time due to the complex healing process required for effective healing. In this review, the focus was on the type of wounds, traditional and advanced wound dressings, physical characterization of wound dressings, and ideal properties for the wound dressings. There are types of wound dressing from traditional to advanced dressings. For treatment of normal wounds, traditional dressings such as gauze, plaster, lint, bandages, and cotton pads are used. To improve chronic wound healing proves, advanced dressings such as films, foams, hydrocolloids, hydrogels, alginates, hydrofibers, composites, as well as antimicrobial, bioactive, and tissue-engineered dressings are excellent for advanced dressings for wound healing. A variety of characterization methodologies are used to design effective advanced dressings. Wound dressings should possess mechanical properties such as strength and physical properties such as film thickness, drug release, porosity measurements, swelling behavior, stability studies and/or degradation, are essential and significant for the outcome and development of advanced wound dressings. An ideal dressing to treat chronic wounds should possess important characteristics that enable it to; remove excess wound exudate; cost-effective; ensure a moist environment at the wound site; permit thermal insulation; minimizing scar formation; allow nutrient and gaseous transmissions; conform to the wound surface; facilitate, when necessary, debridement; increasing the epidermal migration and helping the angiogenesis; protect wound from bacterial infections; relieve the wound pain; be acceptable to the patient, easily removed, cost-effective, nontoxic, lint-free, nonadherent, nonallergic, and sterilizable, breathable, biodegradable, biocompatible, elastic, comfortable, and conforming. Therefore, future studies should undoubtedly continue for the development of wound dressings with the abovementioned properties.

CHAPTER 3 DEVELOPMENT OF PHASE MORPHING TANNIC ACID AND CARBOPOL SCAFFOLD FOR TARGATED CHRONIC WOUNDS APPLICATION

3.1 Introduction

Hard to heal wound which results in chronic wounds affect more than 40 million patients globally and represent a severe growing burden for the healthcare systems, with annual costs expected to exceed $15 billion by 2022. Only in Europe, 4 million patients suffer from chronic wounds every year, requiring 25%–50% of acute hospital beds. Furthermore, the costs associated with chronic wound treatments represent at least 4% of the national annual budget, subdivided in 15%–20% for materials, 30%–35% for healthcare providers and more than 50% for hospitalization (Rossella et al., 2022). Based on etiology, the Wound Healing Society (WHS) classifies chronic wounds into four categories: pressure, diabetic, venous, and arterial insufficiency ulcers (Eming at el., 2014). Bacterial colonization usually occurs in chronic wounds and it is considered as a primary cause of chronic inflammation (Bowler at el., 2002). Unless there is effective solution in treatment of chronic wounds, these costs will continue to increase to due to the number of patients affected growing yearly from 6.5 million, given the increasing prevalence of diabetes and other chronic diseases that may affect wound healing (Brem at el., 2007). Because of these experienced burdens in the quality of patient's life and finances, it is no surprise, then that wound healing has received a great deal of attention, both from a basic science standpoint and a business perspective.

Given the rise of chronic wounds and the morbidity associated with them, wound management and care aids became increasingly important (Han at el., 2017). These aids are available in a variety of physical forms such as hydrogels, xerogels, tissue engineering, hydrocolloids, skin scaffolds, films, lint, and gauzes (Boateng at el., 2008; Lloyd at el., 1998). Within the pharmaceutical and biomedical arena, there has been great interest for the use of sponges and foams for wound dressing applications, controlled drug delivery, and within the tissue engineering field, as a matrix for cell growth (Queen at el., 1987). Advanced pharmaceutical formulations such as foam dressings, scaffolds, hydrogels, and films have been designed in order to distribute therapeutic substances to exudating wounds in a controlled release manner (Mayet et al., 2016). These formulations may contain pain-relieving, anti-bacterial and anti-inflammatory therapeutic agents. Drug-delivery systems such as foam dressings have high exudate absorbing properties and thus can be used effectively for exudate wounds, as it has been shown to have advantages in treating chronic low-to-high exudating wounds in comparison with dressings of poor absorbing properties (Sussman at el., 2010).

A skin scaffold is a network that supports and holds living tissue together (Chung et al., 2007). In cases where chronic wounds are not able to regenerate independently, it is the creation of the scaffold that will trigger and promote the natural sequence of healing events through mechanical support to the development of neotissue (Mayet et al., 2016). The scaffold is naturally produced by the body when a wound occurs or after injury, also, they may be designed and synthesised as a tissue substitute to speed up the healing process. Synthetic and natural polymers which are biodegradable and compatible have been used for the development of scaffolds for tissue engineering. Scaffolds of optimal activity should mimick the biological function as well as the structure of the skin's extracellular matrix (ECM), thus regulating cellular activity and maintaining mechanical support (Chung et al., 2007).

The ideal scaffold requires several chemical and structural features: (i) the desired shape, mechanical strength and volume contained within a three dimensional architectural structure (ii) minimisation and prevention of immune and inflammatory responses by the insurance of a biodegradable and compatible chemical composition of products and at the surface of the wound site, (iii) a well interconnected open pore structure and highly porous scaffold that allows for optimal tissue in-growth and high cell seeding density and (iv) provision of sufficient support to impaired tissue until its full re-growth by a finely tuned in pattern of the degradation rate of polymeric scaffold. Techniques such as the application of nanotechnology will ensure the fabrication of a biodegradable scaffold for directing a series of tissue regeneration processes in a more active manner promoting faster wound healing (Khil et al., 2003; Mayet et al., 2016).

With the economic and patient care impacts of chronic wound healing, it comes as no surprise that the field of wound healing research in scaffolds and hydrogels is incredibly active. Implanted scaffolds and injected hydrogels are two typical biomaterial scaffold-based LDDSs. The common features of these two delivery systems include the sufficient loading capacity, adjustable long-term release profiles, and the ability to mimic natural tissues for enhancing immune responses (Wang at el., 2017). Implanted scaffolds are often postoperatively utilized for eliminating residual tumor cancer cells and preventing tumor recurrence (Ren at al., 2028). With the pre-designed physicochemical and biological properties, implanted scaffolds can not only directly alter immune microenvironment for inhibition of tumor formation, but also provide a proximate site for immune-cell interaction, expansion, and dispersion both in vitro and in vivo (Wolf at el., 2019).

3.2 Materials and methodology

3.2.1 Materials

Tannic acid (TA) and Carbopol 940 (Sigma-Aldrich,St. Louis, MO, USA) were used as received. Phosphate Buffer Tablet: (provided by (Sigma-Aldrich, St. Louis, MO, USA). All chemicals were used without further purification, and deionized water was used for all experiments (Baljit S., et al 2013).

3.2.2 Synthesis of tannic acid-carbopol scaffold.

Synthesis of the scaffold dressing was carried out by pouring tannic acid (0.5% w/v) dropwise into Carbopol (0.5% w/v) solution, (solution of Phosphate Buffer Solution (PBS) while stirring at low speed at 25 °C (Figure 3.2)**.** Carbopol was stirred for 1hr at 150 rpm and TA stirred for 10min at 150 rmp. The mixed solutions were stirred at 150 rmp for 20-minute time period. The homogenised solutions of 25ml of TA and Carbopol were transferred into the 90mm Petri plates and placed in a freezer (FreeZone 2.5, Labconco, Kansas City, Missouri, USA) at -80 °C for a 24-hr time. The freezing step was followed by the lyophilisation process (Free zone 12 lyophiliser, Labcono, Kansas City, USA) for 24 hours to eliminate entrapped solvent to form scaffolds (Baljit et al., 2013; Mndlovu et al., 2019 ; 2023).

3.2.3 Evaluation of surface morphology of the scaffolds

Morphology of the scaffold samples were examined by a scanning electron microscope (SEM) (Phenom™ Scanning Electron Microscope, FEI Company, OR USA), employed at a 15 kV voltage for the evaluation of porosity, surface roughness, and particle size of films of the scaffolds. Before analysis, all formulations were mounted on aluminum stubs using a carbon adhesive tape and samples were coated with carbon/gold palladium in a 2:1 ratio (Mndlovu et al., 2023). Samples were prepared by freeze drying to remove access water particles after been frozen at -80°C. According to Zhang and co-workers (2011), the process of freeze drying also greatly influences the morphology of the microscopic images obtained due to the formation of ice nuclei (Mayet at el., 2016). Thus, in order to preserve better morphology, the scaffolded samples were first frozen at -80°C, (Figure 3.2).

3.2.4 Physicochemical characterization of the synthesised scaffolds by FTIR Spectroscopy

FTIR analysis which identifies absorption bands based on vibrational molecular transitions was conducted to characterize starting materials and the developed scaffold. FTIR parameters were set as follows; using PerkinElmer Spectrum 2000 ATR-FTIR (PerkinElmer 100, Llantrisant, Wales, UK), spectrometer fitted with a single-reflection diamond MIRTGS detector was employed at a wavenumber range of 4000–650 cm−1 (Baljit et al., 2013).

3.2.5 Thermophysical property analysis by differential scanning calorimetry studies (DSC)

DSC is used to observe the physical as well as chemical interaction of polymers used in the formulation. The thermophysical properties of the scaffolds were evaluated using a differential scanning calorimeter(DSC) (Mettler Toledo, DSC, STARe System, Swchwerzenback, ZH, Switzerland). DSC measurements were taken for all starting material and finial product samples weighing between 3-10 mg, materials were sealed in aluminum crucibles and heated over a temperature range of 0–400 ^{0}C at a heating rate of 10°C/min under N2 atmosphere. DSC curves were plotted as heat flow against temperature (Mndlovu et al., 2019). Calibration of the DSC modulus was done in respect to enthalpy and temperature. Thermoanalysis of the samples was carried out with regard to the glass transitions, melting points, chemical reactions, and phase change temperatures of the polymeric system (Mayet at el., 2016).

3.2.6 Determination of Degree of Crystallinity by X-Ray Diffraction (XRD)

XRD is used to provide information regarding the crystalline, semicrystalline, or amorphous structure of the formulation. The crystal properties are incredibly significant for the drug release and diffusion behavior since crystalline drugs cannot be dissolved reaching maximum therapeutic concentration (Poonguzhali at el., 2017; Siafaka at el., 2016). For the establishment of the crystallographic interactions occurring in the TA and Carbopol and upon crosslinking to final product, X-ray diffraction spectra were recorded on the benchtop MiniFlex 600 (Rigaku, Japan) diffractometer using CuKα radiation at 40 kV and 15 mA. The 2θ scan range was selected between 3–90 degrees at a scan rate of 10 degrees/minute (Mndlovu et al., 2023).

3.2.7 Determination of tensile strength mechanical properties of the scaffolds

The mechanical properties of the scaffold were determined via the tension analysis with the aid of the Texture Analyzer (TA.XT plus, Stable Microsystems, Surrey, UK). The scaffolds were cut into a rectangular shape and the surface area was calculated. The tests were acquired at a 1% strain rate with 70% strain at room temperature (25 ^{0}C). The tensile strength was obtained from the highest stress point of the stress vs. strain graph (Mndlovu et al., 2023). The properties of the polymers were measured by fixating film samples between two brackets placed 3 cm apart and a tension force of 0.5mm/s applied to the system to determine the force at breaking point, Figure 3.1. This approach was employed to generate force-distance profiles to determine the tensile strength and work of extensibility which can be computed from the peak tensile force required.

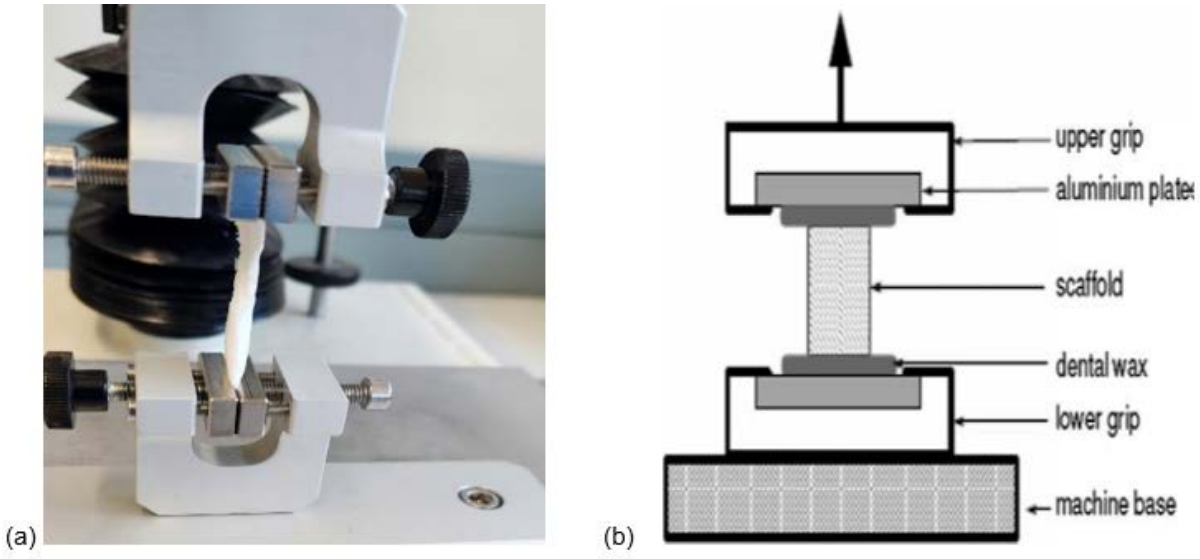

Figure 3-1 Schematic illustrating the textural analysis procedure employed to cause a break or fracture in the scaffold. Fig 3.1 (a), lab illustration. Fig 3.1 (b) adopted from (AI-Munajjed at el., 2008).

3.2.8 *In vitro* drug release studies of scaffold containing TA

The *In vitro* drug release profile from drug loaded scaffold was determined in different mediums of triplicate for statistical significance. The release study was carried out in 100 ml PBS (pH 7.4) using the SnakeSkin dialysis tube (3.5 K MWCO, 22 mm, Separation Scientific SA (Pty) Ltd) diffusion technique, and maintained at 37 ^{0}C stirring at 30 rpm in an orbital shaking incubator (LM-530-2, MRC Laboratory Instruments Ltd Hahistadrut, Holon, Israel) for 336 h. Aliquots (4 ml) were withdrawn at predetermined time points (1, 2, 4, 6, 8, 24, 48, 72, 120, 168 and 336 h) from the release medium and replaced with an equivalent 4ml volume of fresh PBS to maintain sink conditions. All sampling was performed in triplicate for statistical significance. The amount of TA encapsulated in the scaffold and released at predetermined time points in the PBS medium was determined at 265 nm via a UV–Vis spectrophotometer (Mndlovu et al., 2023). The complete release was done by weigh 300mg in 100ml PBS overnight to allow complete release of TA through low starring at 15rmp. An aliquot was analyse using UV-VIS spectrophotometer to determine the amount of TA encapsulated. Calculations of the concentration of drug released were performed utilizing the calibration curve and plotted in figure 3.7. To determine the mass of the drug released, and percentage thereof, equation.1 was used:

$$\text{Mass of drug released (mg)} = \frac{\text{Concentration of drug} \times \text{volume of dissolution medium}}{1000}$$

Equation 1 Calculation of mass of TA released from the Carbopol-TA scaffold encapsulated within the scaffold.

3.2.9 Assessment of degradation using zeta potential of Carbopol - TA scaffold

A Zetasizer NanoZS instrument (Malvern Instruments (Pty) Ltd., Worcestershire, UK) was employed in quantifying the particle surface charge of pristine and partial crosslinked polymers. The materials (Carbopol, Tannic Acid and Scaffold) were prepared using the orbital shaking incubator (LM-530-2, MRC Laboratory Instruments Ltd Hahistadrut, Holon, Israel) maintained at 37 ^{0}C, and set at a speed of 25 rpm. In this method, 25mg of materials were weighed into vials containing phosphate-buffered saline (PBS) 5 ml (pH 7.4). The rate of degradation was measured after 0 1, 2 3, 4 and 7th day. The degree of degradation was obtained via the use of plotting a graph. The technique works by following the electric potential in the double artificial layer. The obtained point where the diffuse and the stern layers are bonded gives insights on the stability of the colloidal dispersions, thereby indicating the repulsion degree among adjacent particles charged in a dispersion. The prepared carbopol-TA scaffolds were sonicated in distilled water and filtered before suspended in the cell for analysis of zeta potential at 25 °C (Mndlovu et al., 2019, and 2023).

3.2.10 Statistical analysis

Everything was done in triplicates, and all tests were represented as averages and standard deviation.

3.3 Results and Discussions

3.3.1 Fabrication of composite scaffolds

Synthesis of polymers was performed with the casting method, (Figure 3.2). From the experimental observations, it was noted that films of white to yellow were produced due to the presence of cross-linkage by tannic acid. When lower polymeric concentrations were used and the degree of crosslinking was minimal, films tend to have weaker mechanical properties, are more white in colour. Upon an increase in polymeric concentration and crosslinker a yellowish colour is more dominant in films and mechanical properties are improved.

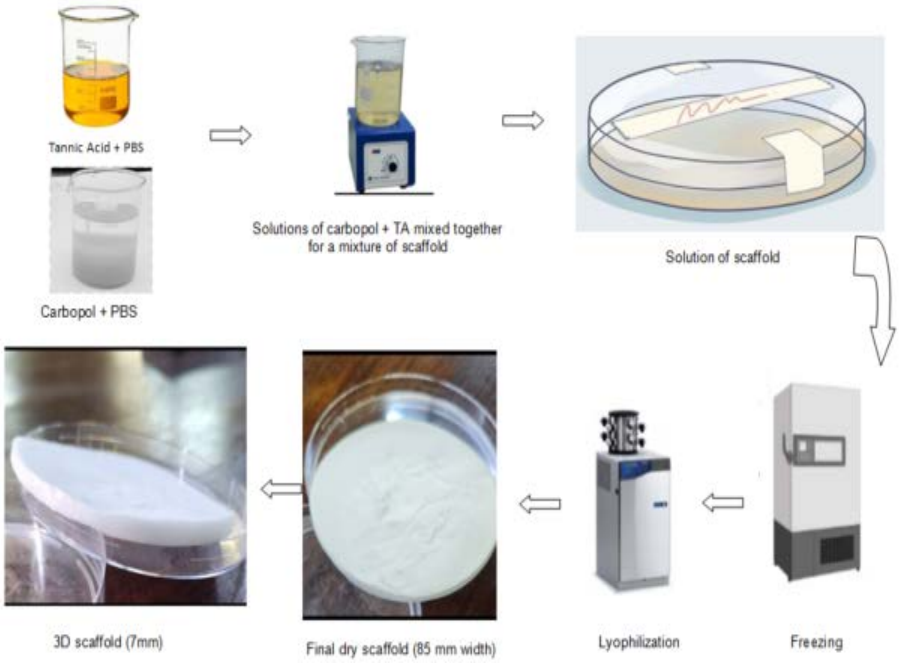

Figure 3-2 Schematic representation of scaffold synthesis of carbopol and tannic acid, and characterization of these polymers.

3.3.2 Scanning electron microscopy analysis of the scaffolds

Scanning electron microscopic was performed to evaluate the morphological characteristics of the scaffolds and provide some details on the morphology in various combinations. The images were obtained in order to determine the morphologies and microstructure of the lyophilized matrix at different sample angles. The SEM images of the scaffolds are presented in parts (a) scaffold, (b) scaffold, (c) scaffold, and (d) scaffold respectively (Figure 3.3). From the morphologies images obtained Figure 3.3 a, b, c and d scaffold formation of porous sites and fibrils can be observed where interconnecting pores dure to crosslinking of TA. Figure 3.3 c and d demonstrated a greater domain of fibrillogenesis with the presence of a slight fibril structure as demarcated. This was attributed to interactions occurring between water molecules and hydrophobic groups in the structure (Mayet at el., 2016). The images displayed various aspects of the scaffolds such as the porosity and the presence of smaller particles on the surface of the scaffolds. These pores factors have a prominent role in the four key elements of wound-healing process; cell proliferation, function, and migration during the healing of wounds, as reviewed and discussed at section (2.4. Mechanism underlying wound healing and associated complications in chronic wounds).

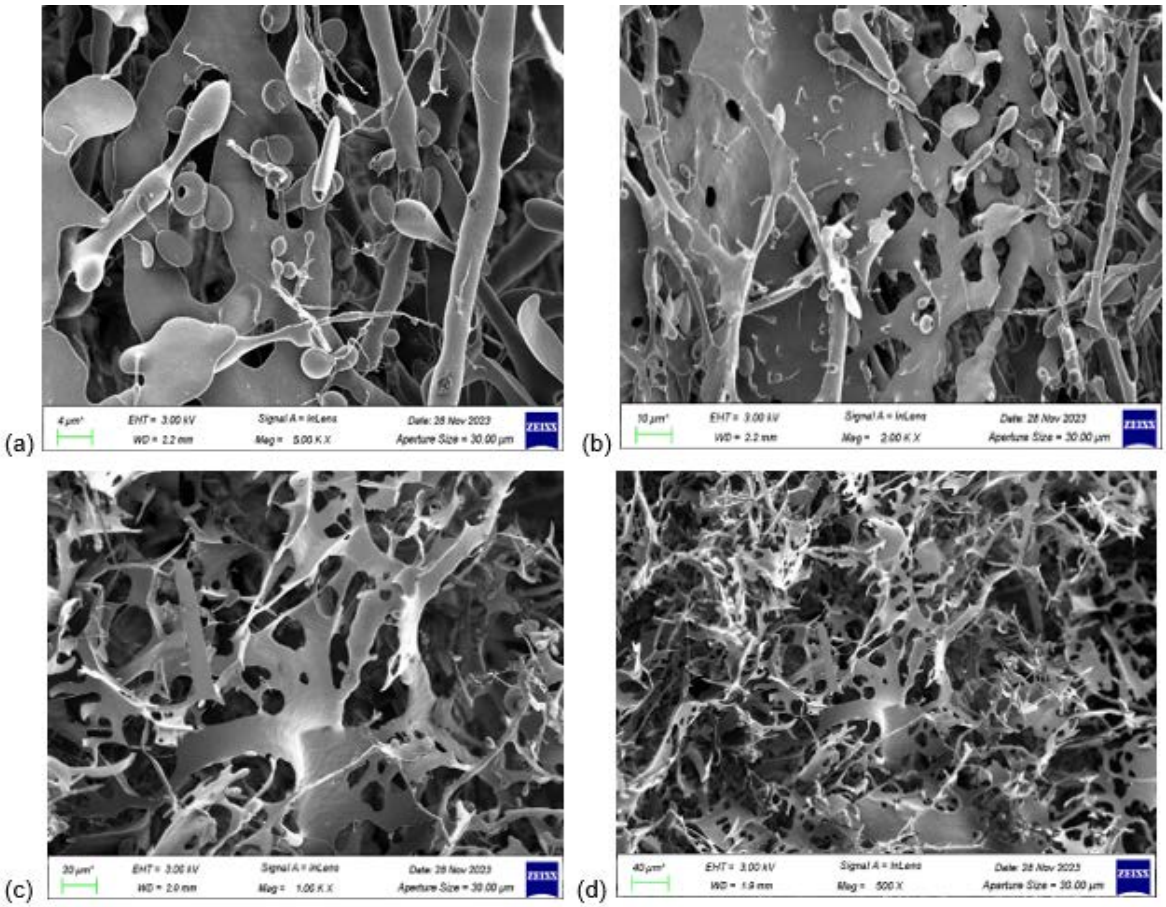

Figure 3-3 Scanning electron micrographs of carbopol and tannic acid cross-linked scaffold at 500, 1000, 2000 and 5000X magnification.

3.3.3 Physicochemical characterization of the synthesised by FTIR spectroscopy

FTIR study is one of the handful and significant techniques in the tissue engineering and drug delivery field since it can depict interactions between the polymeric matrix and the active molecules. Such interactions can affect the release rate, degradation, and overall in vitro and vivo performance of the dressing. FTIR spectra, it can be visualized whether the formulation causes an important change in the molecular structure of any component or not. This change can alter the chemical stability or safety of the formulation (Abdel-Mohsen at el., 2020; Bulbul at el., 2020).

The characteristics and compositions of the starting materials and scaffold were evaluated for their partial-crosslinking and interpolymer complexation reactions. Figure 3.4 shows FIIR spectra of the materials. The FTIR spectrum on the materials shows a broad absorption band between 3500 and 2900cm $^{-1}$ of TA and scaffold (due to O-H stretching) and a slightly narrow band between 3000 and 3300 cm $^{-1}$ carbopol (due to O-H stretching). A sharp band at 1526 cm $^{-1}$ for all materials (due to C-H stretching), and bands between 1510 cm $^{-1}$ and 1000 cm $^{-1.}$ (C-O bending and stretching). The results observed were found to be similar to those

concluded in studies by Baljit et al., 2016. The FRIR results indicate the presence of carbopol, and TA, TA as a crosslinker in the polymer metrics. Formation of new ethereal linkages in the polymer during the cross-linking reaction is indicted by clear peaks between 1700 – 1500 cm^{-1}. (Baljit et al., 2013; Mndlovu et al., 2019). These peaks occur at higher vibrational frequencies and are associated to the degree of polymer network formation as well as crosslinking resulting in a change within the structural environment. The presence of the crosslinker promotes the band formation as is indicated in the FTIR spectra, resulting in stretching and conjugation within the structure and bending between CH bonds in the aromatic ring. Formulations displaying greater band intensities corresponded to a degree of crosslinking.

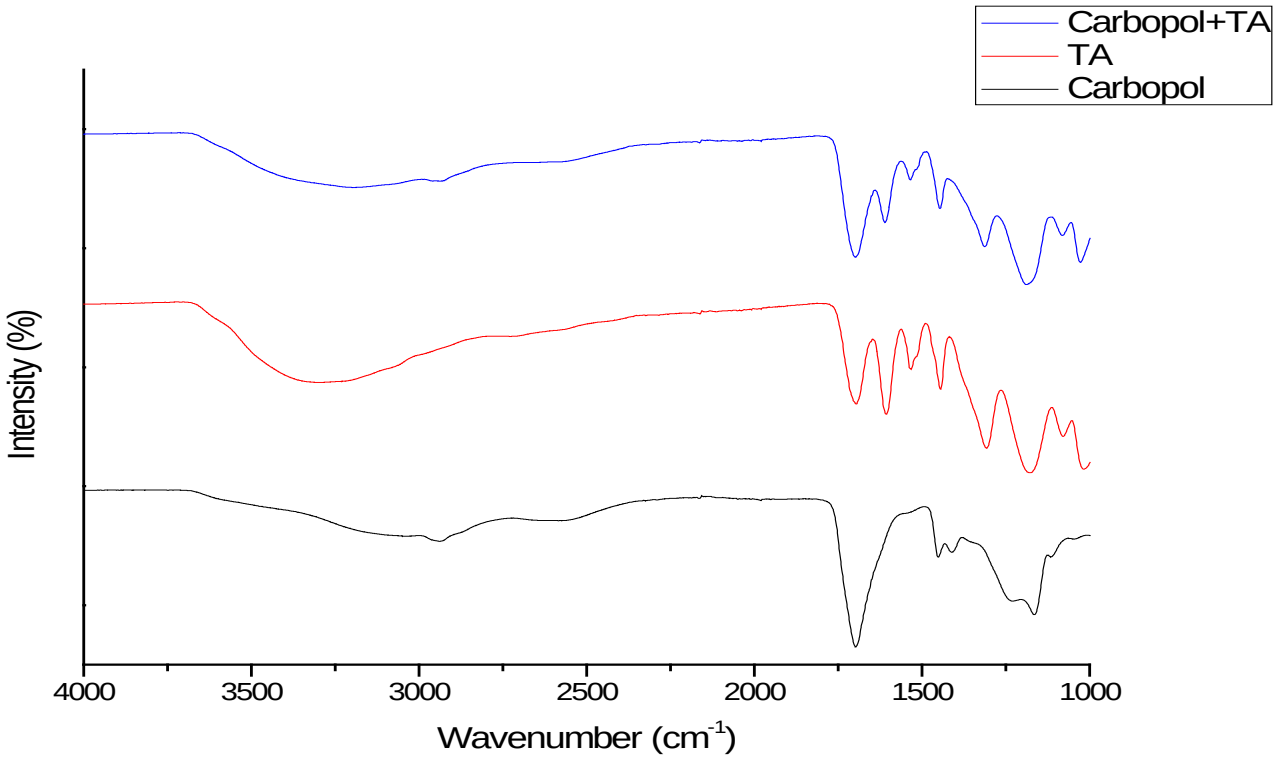

Figure 3-4 The characterization of the carbopol-TA and synthesis scaffold using FTIR spectroscopy.

3.3.4 Thermophysical characterization by differential scanning calorimetry (DSC) studies

Carbopol-TA and scaffolds were analysed by Differential Scanning Calorimetry (DSC), for evaluation of glass transition temperature and melting point. Figure 3.5 present the DSC curves of the polymers. Data was collected using the Mettler Toledo DSC-1 STARe system, heated from 0-400°C, at a heating rate of 10°C per minute, resulting in thermograms of heat flow versus temperature. The DSC findings showed a shift on the exothermic behaviour towards the low temperatures on the polymers. The exothermic behaviour of all the materials

observed between 120 and 290 °C is representative of the thermal degradation of the main chains of Carbopol and/or Tannic Acid (Mndlovu et al., 2019). The DSC thermograms of the various films showed a glass transition temperature (Tg) ranging between 275 and 300°C. This was related to a change in heat capacity when a transition occurred due to network formation and crosslinker concentration. The partially crosslinked scaffold showed a decreased degradation temperature which is due to the partial-crosslinking and the hydrogen bond deformation.

The DSC analysis was used to observe the physical as well as chemical interaction of sample (Carbopol-TA) used in the scaffold formulation. This is so that the scaffold formulation maintains its physical and chemical structure integrity to ensure storage stability as well as functional performance which are important for quality and efficacy in wound treatment. The partial crosslinking decreased the degradation temperature range and this is indicative of controlled thermal degradation by partially crosslinking polymers for controlled scaffold stability.

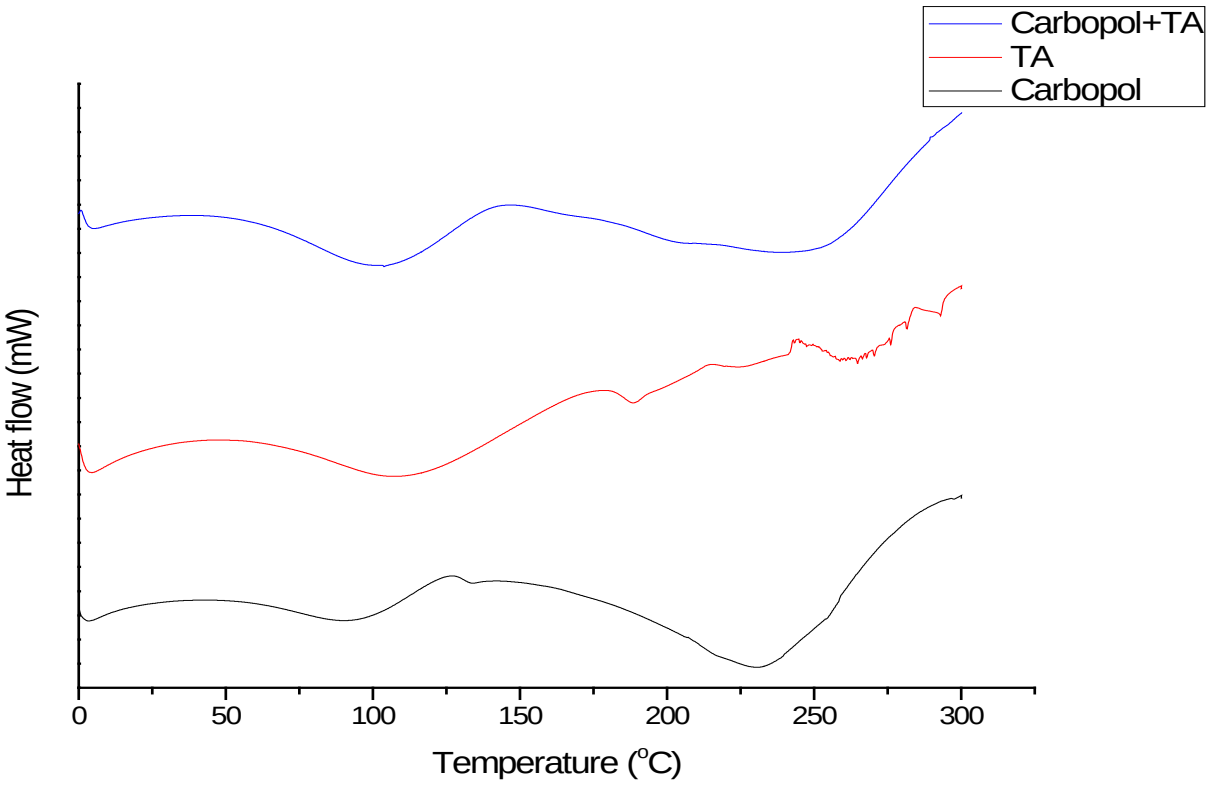

Figure 3-5 The thermophysical property analysis evaluated via DSC.

3.3.5 Degree of Crystallinity determination by X-Ray Diffraction (XRD)

XRD patterns of the polymer were recorded to study any change in the crystalline structure of the polymer. As shown in Figure 3.6, X-ray diffraction (XRD) pattern of scaffold and carbopol exhibited a characteristic saccharide amorphous peak at a 2θ of 20° and a semi-crystalline amorphous peak at 25° (Mndlovu et al., 2019). TA has a sharper peak compared to pure scaffold and carbopol. The scaffold and carbopol showed a smooth semi-crystalline peak at 2θ = 25° and 2θ = 21.5°, respectively. These peaks were more amorphous than the one on TA therefore indicating a slight phase change during partial crosslinking. This observation is made with observed new carbopol-TA scaffold bond formation which decreases the interactions among polymeric chains and consequently diminishes the semicrystalline nature of the natural carbopol (Baljit et al., 2016).

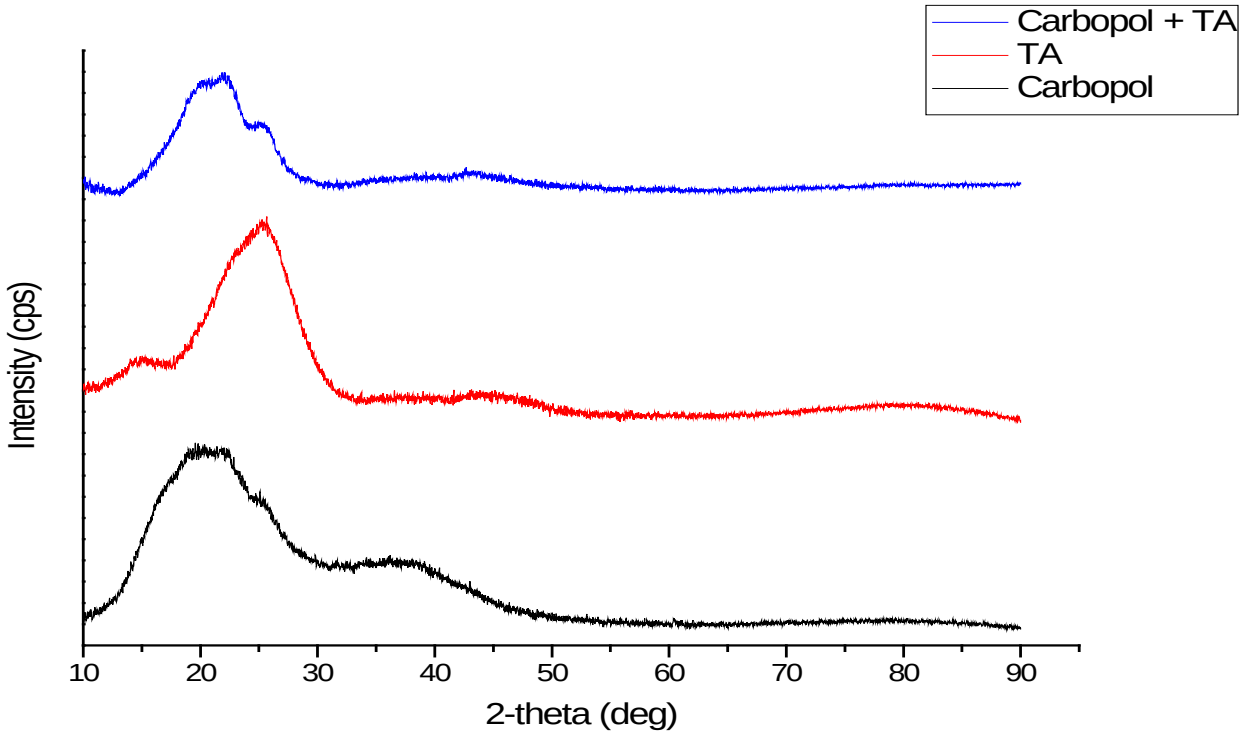

Figure 3-6 Determination of the degree of Crystallinity evaluated via XRD.

3.3.6 Tensile strength measurements of scaffolds

The tensile strength is measured for mechanical properties. Wound dressings should ideally present elasticity to follow skin movements and resistance to mechanical abrasion (Okur at el., 2019). The tensile strength of wound dressing should be enough to tolerate the dressing replacement and handling during the wound healing time (Fahimirad at el., 2019). It was reported that an ideal wound dressing must show a tensile strength ranging from 2.5 to 35 MPa in healthy conditions (Gomes at el., 2019). The mechanical properties of the wound

dressing could majorly impact the wound-protective function. It has been proven that the ideal dressing should present flexibility and resistance to ruptures or cracks when applied even if it is used locally to cover dermal wounds or as internal wound support (Vedakumari at el., 2017, Rezvanian at el., 2017; Thomas at el., 2020). Flexibility is required to overcome strains and stresses on body and only a flexible dressing could sustain various mechanical stresses without breaking (Baljit et al., 2013).

The tensile and mechanical properties of the carbopol was investigated by textural analysis and shows that the results of tensile strength of the scaffold were highly very flexible in nature and have tensile strength 1.5E-7 MPa. Thus, the scaffold's lack of plastic deformation (brittle failure) was indicated by the scaffold tensile strength ranging below the range of 2.5 to 35 MPa and breaking very sharply without elongation when it was pulled apart.

3.3.7 Ultra Violet graph demonstrating wavelength (λmax) for the scaffold at 265 nm.

The λmax of TA was found at 265.0 nm. Based on the λmax, the calibration curve was obtained (Figure 3.7). This curve confirms that there is a linear correlation between concentration of the drug and the absorbance (R2 = 0,9944) – i.e., an increase in the concentration of the drug results in an increase in the respective absorbance (Abbas et al., 2017).

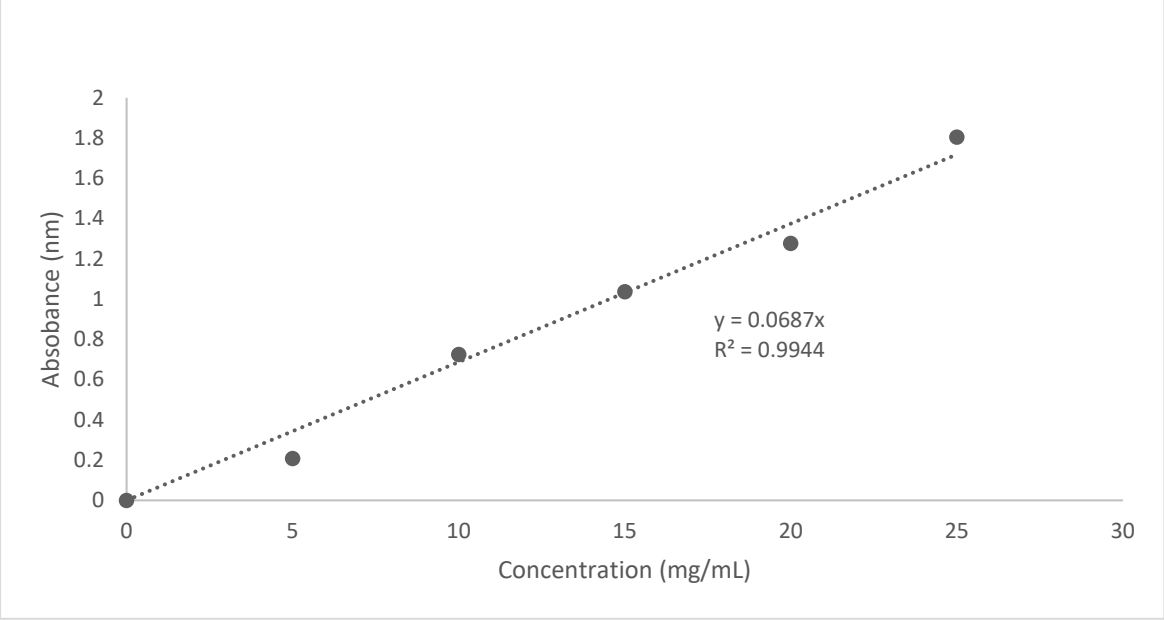

Figure 3-7 Calibration curve of utilizing maximum wavelength (λ_{max}) of 265 nm.

3.3.8 *In vitro* drug release analysis of the scaffold containing Tannic Acid

Studies have investigated the potential of antioxidant activity of Tannic Acid (TA). TA is the simplest and principal hydrolyzable tannin that has astringent, antioxidant, antimicrobial, antiviral and anti-inflammatory properties (Fernandez et al., 2002). It is believed that tannins can promote wound healing through several mechanisms: 1) scavenging of free radicals and reactive oxygen species (ROS), 2) promoting wound contraction, and 3) increasing formation of capillary vessels and proliferation of fibroblasts (Akiyama et al., 2001; Bakondi et al., 2004; Fernandez et al., 2002).

UV–Vis absorption properties of molecules allow molecules, complexes, and formulations to be evaluated for various properties such as bioactive release, degradation, and complexation (Mndlovu et al., 2023). The drug release profile of tannins from scaffold was determined by analysis of the release medium at pre-determined time points over a period of 350 hours and the results of release profiles of the drug is presented in Figure 3.8. The release profile of the scaffold was determined in triplicates. The encapsulation efficiency was 100% and drug loaded was 50%. The drug release from the scaffold occurred with an initial short burst effect for up to 24% of release within 48 hrs, then had a gradual release up to 7th day. These ensure initial fast and a later slow and sustained drug release at the site of application. The initials burst release ensure immediate enough bioavailability to trigger therapeutic effects. Then, the sustained release formulations can provide not only analgesia for a longer duration, but can also provide slow antibiotic release according to the requirement of the wound. Controlled release of antibiotic ensures a microbe-free environment, and analgesic ensures painless recovery of the patient. Antimicrobial scaffold with antioxidant and controlled drug release potential can be a suitable wound management material (Baljit et al., 2016).

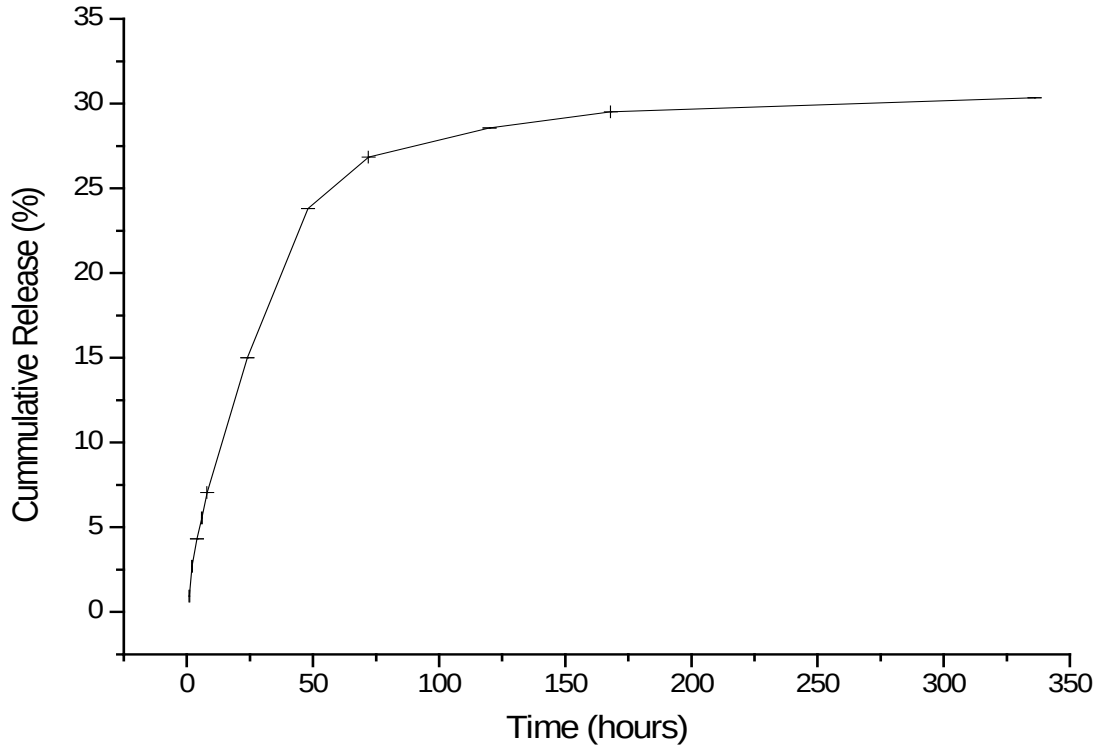

Figure 3-8 Release profile from drug loaded carbopol-TA scaffold films in different medium at 37 °C.

3.3.9 *In vitro* biodegradation of the polymers and scaffold by zeta potential evaluation

Zeta Potential measurements offer insights into the surface charge and stability of the scaffold over time. The surface charge analyses are important as they can gives insight in the particle's stability and potential antimicrobial properties. This is in terms of the ionic interaction that may exist between microorganisms, drugs, and polymers.

Carbopol, TA and scaffold were evaluated for zeta potential employing the Zetasizer NanoZS analyser (Malvern Instruments Ltd. UK). The observed zeta potential for carbopol, TA and scaffold displayed with (Figure 3.9). Carbopol and TA displayed the highest surface charges of between -20 and -35mV, which decreased after partial-crosslinking to -15mV of the scaffold. The decrease on the surface charge signifies the crosslinking of the polymers using TA as a crosslinker. Furthermore, the high surface charge of the partially crosslinked polymers inferred that there was partial-crossliking since the crosslinking did not decrease the surface of charge of the polymers to be below -10 mV. Therefore, the scaffold had a zeta potential that was above -10 mV up to the 7th day, which confirm the high stability as reported by Orlowski and co-workers which have reported that, high positive or negative zeta potential values indicate

the high stability of colloids (Orlowski at el., 2018). The stability scaffold therefore confirms less rate of degradation which is an excellent quality property ensure up to 7th day of release and efficacy.

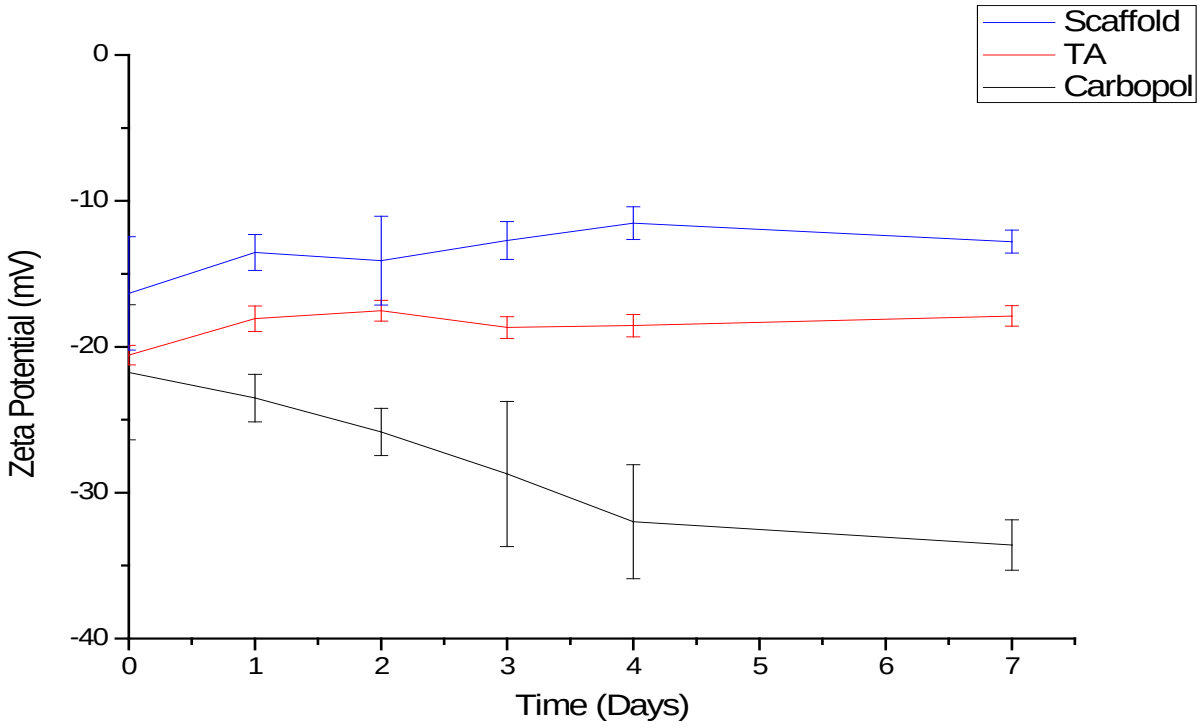

Figure 3-9 Degradation graph of carbopol tannic acid and synthesised scaffold by zeta-potential.

CONCLUSIONS AND RECOMMENDATIONS

4.1 Conclusion and recommendations

Approaches to chronic wound healing that are common today are typically based on simple traditional wound care regimens involving debridement, cleaning, and application of moist dressings. More advanced dressings such as topical gels and scaffolds containing growth factors have resulted in enhanced healing rates in some clinical studies, however, on the whole these treatments are difficult to design, synthesise and are often too expensive for application to large, chronic wounds. Thus, the successful design of chronic wound dressings depends on an understanding of several factors. Factors such as (i) the human anatomy and physiology, (ii) wound healing process, (iii) the patients' conditions in terms of the wound type, health, environment, and social circumstances, (iv) cascade of wound dressing and (v) the physicochemical properties of the various dressing materials. In this present study, a phase morphing tannic acid and carbopol scaffold for targeted chronic wound application was successfully developed and the formulation of the composite scaffolds was investigated. TA was innovately employed as a crosslinker and bioactive. SEM analysis confirmed the spherical shape of the scaffolds. Physicochemical characterizations of the Scaffold were performed using FTIR, DSC, and XRD – these confirmed the presence of T.A (as a crosslinker and bioactive) within the scaffold's structures. The mechanical properties of the scaffolds through tensile strength were not improved and broke very sharply without elongation when it was pulled apart. UV/Vis spectroscopy (λ = 265 nm) confirmed the linear correlation between the concentration of the drug and absorbance. Th drug release profile of TA as both a crosslinker and a bioactive allowed for a high burst release in 48 hrs, enabling therapeutic effects (antimicrobial, antiviral anti-inflammatory, and antioxidant properties) of TA to be prompt at the initial stages of wound healing. The bust drug release profiles was followed by controlled release. The release was slower and more sustained at average of 30% up to the 14^{th} day. The burst and controlled release profile at all stages were complemented by the stable scaffold. The scaffolds displayed a stable profile of up to the 7^{th} day when degradation analyses were performed through zeta-potential evaluation. The use carbopol and TA produced desirable results of physicochemical properties for wound dressing applications in treatment of chronic wounds.

Further studies with *in vivo* models should be conducted to assess this as a potential novel therapeutic outlet for the treatment of chronic wounds. Mechanical properties could be conducted to assess desirable tensile range for scaffolds, and if this is a limitation in design and application of scaffolds in wound treatments. Furthermore, cell viability studies can be conducted.

REFERENCES

Abbas, N., Arshad, M.S., Hussain, A., Irfan, M., Ahsan, M., Rasool, M.F. and Rehman, M.H., 2017. Development and validation of a spectroscopic method for the simultaneous analysis of miconazole nitrate and hydrocortisone acetate in pharmaceutical dosage form. Tropical Journal of Pharmaceutical Research, 16(2), pp.413-420.

Abdel-Mohsen AM, Abdel-Rahman RM, Kubena I, Kobera L, Spotz Z, Zboncak M, et al. Chitosanglucan complex hollow fibers reinforced collagen wound dressing embedded with aloe vera. Part I: preparation and characterization. Carbohydr Polym 2020;230:115708.

Adam singer A, Richard AF, Clark MD. 1999. Cutaneous wound healing. New Engl J Med 341:738–746.

Al-Munajjed A, Hien, Kujat R, Gleeson J.P, Hammer J. (2008). Influence of pore size on tensile strength, permeability, and porosity of hyaluronan-collagen scaffolds. J Mater Sci: Mater Med (2008) 19:2859–2864

Akash, M. S. H., Rehman, K., Fiayyaz, F., Sabir, S., and Khurshid, M. (2020). Diabetes-associated infections: development of antimicrobial resistance and possible treatment strategies. Arch. Microbiol. doi: 10.1007/s00203-020-01818-x. [Epub ahead of print].

Ali B.H, Ziada A, Blunden G. 2009. “Biological effects of gum arabic: a review of some recent research.” Food Chem. Toxicol. 47, 1–8.

Al-Yahya A.A, Al-Majed A.A, Gado A.M, Daba H, Al-Shabanah O.A, El-Azab A.S, Abd-Allah A.R.A. 2009. “Acacia senegal gum exudate offers protection against cyclophosphamide-induced urinary bladder cytotoxicity.” Oxid. Med. Cell. Longev. 2, 207–213.

Asbill C.S, Mickmack B.B. 2000. Percutaneous penetration enhancers: Local versus transdermal activity. Pharmaceutical Science Technology Today 3, 36–41

Aulton E.M, Taylor K.M.G. 2013. The design and manufacture of medicines. 4. 699 – 709

Bakker, K., Apelqvist, J., Lipsky, B. A., Van Netten, J. J., and International Working Group on the Diabetic (2016). The 2015 IWGDF guidance documents on prevention and management of foot problems in diabetes: development of an evidence-based

global consensus. Diabetes Metab. Res. Rev. 32(Suppl. 1), 2–6. doi: 10.1002/dmrr.2694

Baljit S, Sushma S, Abhishek D. 2013. “Design of antibiotic containing hydrogel wound dressings: Biomedical properties and histological study of wound healing.” International Journal of Pharmaceutics. 82-91

Bhattacharya, D., Ghosh, B., and Mukhopadhyay, M. (2019). Development of nanotechnology for advancement and application in wound healing: a review. IET Nanobiotechnol. 13, 778–785. doi: 10.1049/iet-nbt.2018.5312

Boateng J, Catanzano O. 2015. Advanced Therapeutic Dressings for Effective Wound Healing—A Review. Vol. 104, 3653–3680

Boateng JS, Matthews KH, Stevens HN, Eccleston GM. 2008.Wound healing dressings and drug delivery systems: A review. J Pharm Sci 97(8):2892–2923

Bohl S, Kristyn S, Joseph L, Paula B, Jennifer L.W, Laura A, Poole W. 2002. “Effects of nitric oxide releasing poly (vinyl alcohol) hydrogel dressings on dermal wound healing in diabetic mice.” Original research articles. 10:286–294.

Boulton, A. J., Vileikyte, L., Ragnarson-Tennvall, G., and Apelqvist, J. (2005). The global burden of diabetic foot disease. Lancet 366, 1719–1724. doi: 10.1016/S0140-6736(05)67698-2

Bowler P.G, Davies B. 1999. The microbiology of infected and noninfected leg ulcers. International Journal of Dermatology. 38, 573–578

Bowler PG, Duerden BI, Armstrong DG. 2001. Wound microbiology and associated approaches to wound management. Clin Microbiol Rev 14(2):244–269.

Brem H, Stojadinovic O, Diegelmann RF, et al. Molecular markers in patients with chronic wounds to guide surgical debridement. Mol Med. 2007;13(1–2):30–9.

Broderick N. 2009. Understanding chronic wound healing. Nurse Practitioner 34(10):16–22.

Broudriot U, Dersh R, Griener A, Wendorff J.H. 2006. Electrospinning approaches toward scaffold engineering—A brief overview. Artificial Organs 30,785–792

Bulbul E,O, Okur M.E, Oku N.U, Siafaka P.I. 2022. Traditional and advanced wound dressings: physical characterization and desirable properties for wound healing

Chang KW, Alagoof S, Ong KT, Sim PH. 1998. Pressure ulcers–randomised controlled trial comparing hydrocolloid and saline gauze dressings. Med J Malaysia 53:428–431.

Chao Y, Chen Q, Liu Z. 2020. Smart injectable hydrogels for cancer immunotherapy

Chung HJ, Park TG. 2007. Surface engineered and drug releasing pre-fabricated scaffolds for tissue engineering. Adv Drug Deliv Rev 59:249–262.

Clark D.T., Gazi M.I., Cox S.W., Eley B.M., Tinsley G.F. 1993. “The effects of acacia arabica gum on the in vitro growth and protease activities of periodontopathic bacteria.” Journal of Clin. Periodontol. 20, 238–243.

Cutting KF, White RJ. 2002. Maceration of the skin and wound bed. 1: Its nature and causes. J Wound Care 11(7):275–278.

Daniel C.Roy, Seth T, David M.B, Nicole L.W, Sandra C.B, Luke R.B, Justin M.S, Robert J.C. 2015. “Ciprofloxacin-Loaded Keratin Hydrogels Prevent Pseudomonas aeruginosa Infection and Support Healing in a Porcine Full-Thickness Excisional Wound.” Advances in wound care 4, 8, 457-468

Das, S., and Baker, A. B. (2016). Biomaterials and nanotherapeutics for enhancing skin wound healing. Front. Bioeng. Biotechnol. 4:82. doi: 10.3389/fbioe.2016.00082

Dovi, J. V., He, L. K., and DiPietro, L. A. (2003). Accelerated wound closure in neutrophil-depleted mice. J. Leukoc. Biol. 73, 448–455. doi: 10.1189/jlb.0802406

Eccleston GM. 2007. Wound dressings. In: Aulton ME, editor. Pharmaceutics: The science of dosage form design. 3rd edition. UK: Churchill Livingstone. pp 264–271.

Eghbalifam N, Shojaosadati SA, Hashemi-Najafabadi S, Khorasani AC. 2020. Synthesis and characterization of antimicrobial wound dressing material based on silver nanoparticles loaded gum Arabic nanofibers. Int J Biol Macromol;155:119_30.

Eming S.A, Whitsitt J.S, He L, Krieg T, Morgan J.R, Davidson J.M. 1999. Particle-mediated gene transfer of PDGF isoforms promotes wound repair. Journal of Investigative Dermatology. 112, 297–302

Fahimirad S, Ajalloueian F. Naturally-derived electrospun wound dressings for target delivery of bio-active agents. Int J Pharm 2019;566:307 - 28.

Falanga V. 2005. Evaluation of bilayered cell therapy for full thickness excision wounds: A multicenter, prospective, randomized, controlled trial. Wound Repair Regeneration. 13, A4-A27

Galeano M, Deodato B, Altavilla D, Squadrito G, Seminara P, Marini H. 2003. Effect of recombinant adeno-associated virus vector-mediated vascular endothelial growth factor gene transfer on wound healing after burn injury. Critical Care Medicine. 31, 1017–1025

Gomes Neto RJ, Genevro GM, Paulo Ld A, Lopes PS, de Moraes MA, Beppu MM. Characterization and in vitro evaluation of chitosan/konjac glucomannan bilayer film as a wound dressing. Carbohydr Polym 2019;212:59_66.

Gurtner GC, Werner S, Barrandon Y, Longaker MT. 2008. Wound repair and regeneration. Nature 453(7193):314–321.

Hareendran A, Bradbury A, Budd J, Geroulakos G, Hobbs R, Kenkre J, Symonds T. 2005. Measuring the impact of venous leg ulcers on quality of life. J Wound Care 14:53–57.

Jiang, S., Li, S. C., Huang, C., Chan, B. P., and Du, Y. (2018). Physical properties of implanted porous bioscaffolds regulate skin repair: focusing on mechanical and structural features. Adv. Healthc. Mater. 7: E1700894. doi: 10.1002/adhm.201700894

Jingjing S, Jiankang L, Jiaheng L, Kun Z, Jingan L. 2021. “Hydrogel preparation methods and biomaterials for wound dressing.” Life 11, 2-19

Kasuya A, Tokura Y. 2014. Attempts to accelerate wound healing. Journal of Dermatological Science 76 (2041) 169-172

Khil M.S, Cha D.I, Kim H.Y, Kim I.S, Battaria N. 2003. Electrospun nanofibrous polyurethane membranes as wound dressings. Journal of Biomedical Materials Research B Applied Biomedicals. 67, 675–679

Khorasani, M. T., Joorabloo, A., Moghaddam, A., Shamsi, H., and MansooriMoghadam, Z. (2018). Incorporation of ZnO nanoparticles into heparinised polyvinyl alcohol/chitosan hydrogels for wound dressing application. Int. J. Biol. Macromol. 114, 1203–1215. doi: 10.1016/j.ijbiomac.2018.04.010

Koksal C, Bozkurt AK. 2003. Combination of hydrocolloid dressing and medical compression stocking versus Unn’s boot for the treatment of venous leg ulcers. Swiss Med Wkly 133:364–368.

Kootstra N.A, Verma I.M. 2003. Gene therapy with viral vectors. Annual Review in Pharmacological Toxicology. 43, 413-39

Langer R. 1980. Polymeric delivery systems for controlled drug release. Chem Eng Commun 6:1–48.

Lawrence J.C. 1994. Dressings and wound infections. American Journal of Surgery. 167, 21S-24S

Lee KY, Mooney DJ. 2001. Hydrogels for tissue engineering. Chem Rev 101:1869–1879.

Lee P.Y, Chesnoy S, Huang L. 2004. Electroporatic delivery of TGFbeta1 gene works synergistically with electric therapy to enhance diabetic wound healing in db/db mice. Journal of Investigative Dermatology. 123, 791–798

Lipsky BA, Hoey C. 2009. Topical antimicrobial therapy for treating chronic wounds. Clin Infect Dis 49(10):1541–1549.

Lloyd LL, Kennedy JF, Methacanon P, Paterson M, Knill CJ. 1998. Carbohydrate polymers as wound management aids. Carbohydr Polym 37:315–322.

Losi P, Briganti E, Errico C, Lisella A, Sanguinetti E, Chiellini F, Soldani G. 2013. Fibrin-based scaffold incorporating VEGF- and bFGF-loaded nanoparticles stimulates wound healing in diabetic mice. Acta Biomaterilia. 9, 7814–7821

Mallefet P, Dweck AC. 2008. Mechanism involved in wound healing. Biomed Scientist 7:609–615.

Mayet N. Pillay V, Choonara Y.E, du Toit LC, Kumar P. 2016. “Inflammatory dependent bioresponsive smart transdermal delivery system incorporating suspended nanofibrous mats as a platform for wound healing”. 35 – 41

Mayet N. Pillay V, Choonara Y.E, Kumar P, Lomas KT, Charu T du Toit LC, Pillay Viness. 2014. A Comprehensive Review of Advanced Biopolymeric Wound Healing Systems”. 2211 – 2226

Mihm M.C, Soter N.A, Dvorak H.F. 1976. “The structure of normal skin and the morphology of atopic eczema.” The Journal of Investigative Dermatology. 67, 3, 305-312.

Miller M.S. 2009. Use of topical recombinant human platelet-derived growth factor-BB (becaplermin) in healing of chronic mixed arteriovenous lower extremity diabetic ulcers. Journal of Foot Ankle Surgery. 38, 227–231

Miyoshi M, Kavaizoe T, Igawa H.H, Tabata Y, Ikada Y, Suzuki S. 2006. Effects of bFGF incorporated into a gelatin sheet on wound healing. Journal of Biomaterials Science Polymer. 16, 893–907

Mndlovu H, du Toit L C, Kumar P, Marimuthu T, Kondiah P, Choonara Y E and Pillay V 2019 Development of a fluid-absorptive alginate-chitosan bioplatform for potential application as a wound dressing Carbohydr. Polym.

Mndlovu H, du Toit LC, Kumar P and Choonara YE. 2023. Tannic acid-loaded chitosan-RGD-alginate scaffolds for wound healing and skin regeneration

Moira L.I.F, Dias A.M.A, Carvalho E, Sousa H.C. 2013. Recent advances on the development of wound dressings for diabetic foot ulcer treatment- A review. Acta Biomaterialia. 9, 7, 7093-114

Morghimi H.R, Makmalzadeh B.S, Manoti A. 2009. Enhancement effect of terpenes on silver sulphadiazine permeation through third degree burn eschar. Burn. 35, 1165–1170

Moura L.I.F, Dias A.M.A, Carvalho E, de Sousa H.C. 2013. Recent advances on the development of wound dressing s for diabetic foot ulcer treatment—A review. Acta Biomaterialia. 9, 7093–7114

Namviriyachote N, Muangman P, Chinaroonchai K, Chuntrasakul C, Ritthidej GC. 2020. Polyurethanebiomacromolecule combined foam dressing containing asiaticoside: fabrication, characterization and clinical efficacy for traumatic dermal wound treatment. Int J Biol Macromol;143:510-20.

Okur U, Hokenek N, ME, Ayla S, Yoltas A, Siafka P.I. 2019. An alternative approach to wound healing field; new composite films from natural polymers for mupirocin dermal delivery. Saudi Pharm J 2019;27:738_52.

Orlowski P, Zmigrodzka M, Tomaszewska E, Ranoszek-Soliwoda K, Czupryn M, Antos-Bielska M, Szemraj J, Celichowski G, Grobelny J & Krzyzowska M. 2018. Tannic acid-modified silver nanoparticles for wound healing: the importance of size, International Journal of Nanomedicine, , 991-1007.

Paul W, Sharma C.P. 2004. Chitosan and alginate wound dressings: A short review. Trends in Biomaterials. Artificial Organs. 18, 18–23

Patzke G.R, Krumeich F, Nesper R. 2002. Oxidic nanotubes and nanorods—Anisotropic modules for a future nanotechnology. Angewandte Chemie International Edition. 41, 2446–2461

P.G. Bowler, Wound pathophysiology, infection and therapeutic options, Ann. Med. 34 (2002) 419–427.

Poonguzhali R, Basha SK, Kumari VS. Synthesis and characterization of chitosan-PVP-nanocellulose composites for in-vitro wound dressing application. Int J Biol Macromol 2017;105:111_20.

Queen D, Evans JH, Gaylor JDS, Courthey JM, Reid WH. 1987. Burn wound dressings—A review. Burns 13:218–228.

Ren L, Lim YT. Degradation-regulatable architectured implantable macroporous scaffold for the spatiotemporal modulation of immunosuppressive microenvironment and enhanced combination cancer immunotherapy. Adv Funct Mater 2018;28. 1804490.

Renuka M, Nishadh P, Jigar S, Tejal M. 2012. “Mucoadhesive wound healing film of doxycycline hydrochloride.” Int. Journal. Drug Dev. Res. 4, 128–140.

Rezvanian M, Ahmad N, Mohd Amin MCI, Ng S-F. Optimization, characterization, and in vitro assessment of alginate-pectin ionic cross-linked hydrogel film for wound dressing applications. Int J Biol Macromol 2017;97:131_40.

Riley RS, June CH, Langer R. 2019. Delivery technologies for cancer immunotherapy.

Rodriguez PG, Felix FN, Woodley DT, Shim EK. 2008. The role of oxygen in wound healing: A review of the literature. Dermatol Surg 34(9):1159–1169.

Rossella L, Monica B, Gianluca, C Valeria C. 2022. Wound dressing products: A translational investigation from the bench to the market. 3, 82-200.

Rossi S, Faccendini A, Bonferoni M.C, Ferrari F, Sandri G, Del Fante C, Perotti C, Caramella C.M. 2013. —Sponge-likell dressings based on biopolymers for the delivery of platelet lysate to skin chronic wounds. International Journal of Pharmaceutics. 440, 207–215

S. Dhivya, V.V. Padma, E. Santhini, Wound dressings–a review, BioMedicine 5 (2015) 22

S.A. Eming, P. Martin, M. Tomic-Canic, Wound repair and regeneration: mechanisms, signaling, and translation, Sci. Translational Med. 6 (2014) 265sr266.

S.P. Miguel, Sónia P. Miguela,1, Rosa S. Sequeiraa,1, André F. Moreiraa, Cátia S.D. Cabrala,António G. Mendonçaa,b, Paula Ferreirac, Ilídio J. Correiaa, An overview of electrospun membranes loaded with bioactive molecules for improving the wound healing process. 139 (2019)1-22

Satish, L., Santhakumari, S., Gowrishankar, S., Pandian, S. K., Ravi, A. V., and Ramesh, M. (2017). Rapid biosynthesized AgNPs from Gelidiella acerosa aqueous extract mitigates quorum sensing mediated biofilm formation of Vibrio species-an in vitro and in vivo approach. Environ. Sci. Pollut. Res. Int. 24, 27254–27268. doi: 10.1007/s11356-017-0296-4

Sherman RA, Wyle F, Vulpe M. 1995. Maggot therapy for treating pressure ulcers in spinal cord injury patients. J Spinal Cord Med 18:71–74.

Sibbald GR, Orsted H, Schultz GS, Coutts P, Keast D. 2003. Preparing the wound bed 2003, Focus on infection and inflammation. Ostomy Wound Manag 49:24–51.

Slaughter BV, Khurshid SS, Fisher OZ, Khademhosseini A, Peppas NA. 2009. Hydrogels in regenerative medicine. Adv Mater 21(32–33):3307–3329.

Sussman G. 2010. Understanding wound films. Wound Int 1:4.

Tang C, Yin L, Yu J, Yin C, Pei Y. 2007. “Swelling behavior and biocompatibility of carbopol-containing superporous hydrogel composites.” Journal of Appl. Polym. Sci. 104, 2785–2791.

Thomas D, Nath MS, Mathew N, R R, Philip E, Latha MS. Alginate film modified with aloe vera gel and cellulose nanocrystals for wound dressing application: preparation, characterization and in vitro evaluation. J Drug Deliv Sci Technol 2020;59:101894.

Thomas S, Fear M, Humphreys J, Disley L, Waring M. 1996. The effect of dressings on the production of exudate from venous leg ulcers. Wounds 8:145–149.

Thu HE, Zulfakar MH, Ng SF. 2012. Alginate based bilayer hydrocolloid films as potential slow-release modern wound dressing. Int J Pharm 434(1–2):375–383.

Thu HE, Zulfakar MH, Ng SF. 2012. Alginate based bilayer hydrocolloid films as potential slow-release modern wound dressing. Int J Pharm 434(1–2):375–383.

Trent JT, Kirsner RS. 2003. Wounds and malignancy. Adv Skin Wound Care 16(1):31–34.

Vedakumari WS, Ayaz N, Karthick AS, Senthil R, Sastry TP. Quercetin impregnated chitosan_fibrin composite scaffolds as potential wound dressing materials—fabrication, characterization and in vivo analysis. Eur J Pharm Sci 2017;97:106_12.

Velnar T, Bailey T, Smrkolj V. 2009. The wound healing process: An overview of the cellular and molecular mechanisms. J Int Med Res 37(5):1528–1542.

Wang C, Ye Y, Hu Q, et al. Tailoring biomaterials for cancer immunotherapy: emerging trends and future outlook. Adv Mater 2017;29. 1606036.

Wapnir R.A, Sherry B, Codipilly C.N, Goodwin L.O, Vancurova I. 2008. “Modulation of rat intestinal nuclear factor NF-_B by gum arabic.” Dig. Dis. Sci. 53, 80–87.

Wei C.J. 2004. Connexins and cell signaling in development and disease. Annual Review in Cell and Development Biology. 20, 811–838

White RJ, Cooper R, Kingsley A. 2001. Wound colonization and infection: The role of topical antimicrobials. Br J Nurs 10(9):563–578.

White RJ, Cutting K, Kingsley A. 2006. Topical antimicrobials in the control of wound bioburden. Ostomy Wound Manage 52(8): 26–58.

Winkler L.W. 1888. Die Bestimmung des in Wasser gelösten Sauerstoffen. Ber. Dtsch. Chem. Ges. 21, 2843–2855.

Wolf MT, Ganguly S, Wang TL, et al. A biologic scaffold-associated type 2 immune microenvironment inhibits tumor formation and synergizes with checkpoint immunotherapy. Sci Transl Med 2019;11:eaat7973.

Yang C, Blum NT, Lin J, Qub J, Huang P. 2020. Biomaterial scaffold-based local drug delivery systems for cancer immunotherapy

Yu Wang, U Armato, and J Wu, Targeting tunable physical properties of materials for chronic wound care, 2020, 8, 584.

Zhang H, Liu YX, Chen GP. 2020. Vesicular antibodies: immunotherapeutic silk inverse opal particles for post-surgical tumor treatment.

Zhang H, Liu YX, Chen GP. 2020. Vesicular antibodies: immunotherapeutic silk inverse opal particles for post-surgical tumor treatment.