# DEVELOPMENT OF A HYDROGEL WOUND DRESSING SYSTEM FOR THE APPLICATION OF BURN WOUNDS INJURIES

A Dissertation submitted to the Faculty of Health Sciences, University of the Witwatersrand, in fulfilment of the partial requirements for the degree of Master of Science in Medicine with specialization in Pharmaceutical Affairs.

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**DECLARATION**

I, Leshania Pillay, 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 as a research component at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.

Signature

On this 26th day of July 2024

**DEDICATION**

In embarking on this academic journey, I found myself enveloped in a tapestry of support, encouragement, and wisdom from individuals that I hold dear to my heart. Expressing gratitude feels inadequate compared to the depth of appreciation I have for those who have illuminated my path with their kindness and guidance.

To my academic peers, your scholarly wisdom and constructive feedback have shaped this thesis into a testament of rigorous inquiry. Your steadfast commitment to excellence has inspired me to push the boundaries of my intellectual capabilities.

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**ABSTRACT**

Burn injuries, representing a significant public health concern, necessitate innovative treatments to enhance healing, mitigate infections, and minimize long-term scarring. This study focused on the design, synthesis, and evaluation of a novel hydrogel wound dressing system, incorporating tannic acid into a carboxymethylcellulose-gelatin matrix, aimed at optimizing the healing process of acute burn wounds. Utilising an interdisciplinary approach, the research investigated the hydrogel's physicochemical properties, biocompatibility, biodegradability, and therapeutic efficacy through a comprehensive suite of analytical techniques including Fourier Transform Infrared Spectroscopy (FTIR), Differential Scanning Calorimetry (DSC), Scanning Electron Microscopy (SEM), and *in vitro* NIH 3T3 cytotoxicity assays. The findings revealed that the scaffold demonstrated desirable characteristics such as enhanced mechanical stability and effective tannic acid release profile without exhibiting cytotoxic effects to NIH 3T3 fibroblast cells. These properties are critical for creating a moist healing environment, preventing wound infection, and promoting tissue regeneration. The study's outcomes suggest that the developed hydrogel wound dressing system holds considerable promise for advancing burn wound care, offering a scientifically grounded and potentially cost-effective solution for improving patient outcomes. Further *in vivo* studies and clinical trials are warranted to validate its efficacy and safety for clinical application.

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| | |
|---|---|
| AI | Artificial Intelligence |
| CMC | Carboxymethylcellulose |
| DSC | Differential scanning calorimetry |
| ECM | Extracellular matrix |
| FTIR | Fourier Transform Infrared Spectroscopy |
| MTT | 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide dye |
| PBS | Phosphate buffered saline |
| SEM | Scanning Electron Microscopy |
| TA | Tannic acid |
| UV | Ultraviolet |

# Chapter 1 BACKGROUND AND INTRODUCTION

## 1.1 Introduction

The skin barrier is extremely important for human life. It physically protects the human body against external factors such as infectious agents, chemicals, systemic toxicity, and allergens. Internally, the skin helps to maintain homeostasis and protects against increased loss of water from the body (Kanwar, 2018). Burns are the most extensive form of soft tissue injury, occasionally causing extensive and deep wounds and death. Due to excessive scars and skin contractures, burns can cause severe psychological and emotional distress (Cubison et al., 2006).

Burn injuries are a major public health problem in terms of morbidity and long-term physical and psychosocial disability, especially in lower-income communities in developing countries such as South Africa. Approximately 3,2 % of South Africans are affected by thermal burns annually (Burrows et al., 2009). Studies have shown that the most common causes of burns injuries in South Africa are heat-induced thermal burns injuries (Allorto et al., 2009; Daffue et al., 2018). Thermal burns are sub-classified as either wet thermal injuries such as scalds from boiling water or as dry thermal injuries such as mild flame-induced burn injuries (Tiwari, 2012).

Burn wounds can be classified into different categories depending on the layers of skin and tissues involved in the burn. Superficial burns usually affect the epidermis which is the outer layer of the skin. Superficial burns usually cause skin redness whilst no blisters are formed. Partial thickness burns usually involve both the epidermis and the dermis and are characterised by redness, blisters and may be swollen and painful. Full thickness burns affect all layers of the skin and may affect the muscle and bone. These types of burns may appear white or charred and no pain may be felt if the nerves are damaged (Tiwari, 2012; Wasiak et al., 2013; Wounds International, 2014).

Healthy, intact skin has a slightly acidic pH of 4.0 to 6.0. This is an important aspect of the skin's barrier function because it can regulate the bacterial population and prevent infection (Sharpe et al., 2009). When a wound occurs, the acidic environment and pH of the skin are destroyed, exposing the pH of the more neutral underlying tissues (pH 7.4). With successful healing and epithelial regeneration, the skin becomes acidic again. Acute wounds have a more

neutral pH. In the process of acute wound healing, various factors such as hypoxia and increased production of lactic acid causes the pH to drop. An acidic pH environment is considered beneficial because it increases the proliferation and migration of fibroblasts and regulates bacterial colonization (Jones et al., 2015). The treatment of burn wounds has a significant impact on wound healing time. Ensuring wound treatment that promotes healing affects the long-term quality and appearance of the scar and minimizes the risk of burn wound infection. If the wound becomes dry or infected, superficial and partial thickness wounds can develop into deeper burn wounds (Wasiak et al., 2013).

A study at the emergency department of a hospital in KwaZulu-Natal showed that 65,6 % of patients had not received any form of first aid prior to admission for burn wound injuries (Fiandeiro et al., 2015). The lack of such treatment can have a detrimental effect and incur long-term costs and financial burdens on the patients, their family as well as the healthcare system. The cost implications of lacking first aid in burn wound injuries are profound, affecting patients, their families, and the healthcare system at large. The absence of immediate first aid can lead to more extensive treatments, such as debridement and skin grafting, significantly increasing overall treatment costs (Outwater and Van Braekel, 2020). The prolonged and painful recovery process for severe burn injuries necessitates long-term care and rehabilitation, including repeated hospital visits, physiotherapy, and psychological support, contributing to higher cumulative costs (Biswas et al., 2020). The strain on healthcare systems is further exacerbated in mass casualty scenarios, impacting resource allocation and operational efficiency (Hughes et al., 2021). Families, particularly in low- and middle-income countries, face substantial financial burdens due to direct medical costs and lost income from prolonged recovery periods (Stokes and Johnson, 2017). Effective and easily accessible first aid treatments, such as hydrogel dressings with cooling properties to minimise tissue damage, can reduce the severity of burns and associated medical expenses (Goodwin et al., 2015). Comprehensive first aid education and public awareness campaigns are crucial to mitigating these costs, emphasizing the necessity of widespread availability of first aid resources (Fiandeiro et al., 2015; Goodwin et al., 2015).

This study aims to design and develop an effective and novel tannic acid loaded, carboxymethylcellulose-vanillin-gelatin wound dressing system for the optimal healing of a burn wound. This system would provide desirable clinical and pharmaceutical properties such as promoting connective tissue proliferation, collagen synthesis, providing a suitably moist environment that prevents the wound from drying out, providing a protective barrier to prevent hypothermia and to prevent exposure of the open wound to the external environment. Furthermore, this hydrogel wound dressing would possess innate antimicrobial properties, have

adequate mechanical properties, allow for extended usage without signs of cytotoxicity thereby reducing the frequency of wound dressing changes, to allow for easy removal from the wound site without inflicting pain or to prevent further damage and timeous wound closure (Aelenei et al., 2009; Cubison et al., 2006; Stoica et al., 2020; Sun et al., 2012).

### 1.2 Study rationale and motivation

While several methods have been validated for the treatment of burns, several limitations necessitate research on novel and effective strategies. An ideal burn wound dressing should exhibit the following features: a) It should allow efficient gaseous exchange; b) prevent bacterial penetration; c) absorb wound exudate while minimising adherence to the surface wound; d) stimulate wound healing; and e) be non-toxic, elastic and biodegradable (Caló and Khutoryanskiy, 2015). Most wound dressings lack the necessary therapeutic effects required to target all aspects associated with the process of satisfactory healing (Koehler et al., 2018a).

In current practice, cold (running) water and Burnshield® burn wound dressings are used as a primary wound cover for emergency burn care in South Africa (Allorto, 2020; Jonkheijm et al., 2013). Burnshield® is composed of gauze- dressing infused with 1 % tea tree oil, 96 % purified water (aqua) and a gelling mixture (Levy et al., 2016). It requires the application of a non-absorbent bandage such as a crepe bandage to keep the gel-dressing in place to the wound area, which may limit the efficacy of the dressing if a non-absorbent bandage is unavailable. Burnshield® has been reported to cause allergic contact dermatitis to a patient who suffered a first-degree burn injury (Storan et al., 2016).

Hydrogels, synthesised from hydrophilic polymers, possess the unique ability to absorb and retain large amounts of water, which closely mimics the water-rich environment of human tissues (Arabpour et al., 2024). This feature is pivotal for their application in various biomedical fields, including wound healing and drug delivery. Among these are hydrogel scaffolds, especially when derived from natural polymers such as gelatin, have significantly advanced the field of wound care (Negut et al., 2020). Gelatin is particularly valued for its biocompatibility, non-toxic nature, affordability, and impressive water absorption capabilities, making it an ideal candidate for wound dressing technologies (Ndlovu et al., 2021). Despite these advantages, the mechanical strength of natural polymers often falls short of the requirements for certain medical applications. This challenge is addressed by combining them with synthetic polymers, enhancing the structural integrity of the hydrogels while providing adjustable degradation rates to suit specific biomedical needs (Koehler et al., 2018b).

In the specific context of treating burn wounds, research has increasingly focused on hydrogel scaffolds that incorporate gelatin and carboxymethyl cellulose (CMC). These materials have

been shown to significantly accelerate the healing process, with gelatin-based scaffolds demonstrating a potential to revolutionize wound care (Ndlovu et al., 2021). CMC-based hydrogels, and especially formulations including sodium carboxymethyl cellulose (Na-CMC), have also been highlighted for their effectiveness in burn treatment, reflecting the broader utility of CMC in wound care applications (Ozyilmaz et al., 2023). The use of vanillin as a crosslinking agent in these hydrogels enhances their mechanical and barrier properties, underlining the importance of crosslinkers in improving the functionality of biofilms (Tomadoni et al., 2019).

However, a critical limitation of these hydrogel systems is their intrinsic lack of antibacterial activity, a gap that is bridged by incorporating bioactive substances such as tannic acid (TA). TA not only stabilises collagen and elastin within the extracellular matrix but also offers antioxidant, anti-inflammatory, and antibacterial properties. These attributes significantly bolster the wound healing efficacy of hydrogel scaffolds, highlighting the transformative potential of integrating bioactive compounds into hydrogel formulations for enhanced therapeutic outcomes (Kaczmarek, 2020; Liu et al., 2024).

Compositionally and mechanically, hydrogel scaffolds are similar to the natural extracellular matrix of the skin, from a chemical and mechanical perspective, thus it can serve as supporting material for cells during tissue regeneration and as a carrier in delivering a therapeutic agent/active pharmaceutical ingredient (Jones et al., 2015).

Through the strategic combination of natural and synthetic polymers, and the incorporation of bioactive compounds like TA (see Table 1-1 below for comparison), the development of hydrogel scaffolds for wound care is poised for innovation. This approach not only mitigates existing limitations but also paves the way for creating more effective, multifunctional wound healing therapies, showcasing the dynamic potential of hydrogel technology in biomedical applications.

**Table 1-1 Table comparing the polymers and bioactive used for the synthesis of the scaffold.**

| Property | CMC | Gelatin | Tannic acid | Refs. |
|---|---|---|---|---|
| **Biocompatibility** | Highly biocompatible, favouring its use in direct contact with human tissues, facilitating integration without significant adverse immune responses. | Known for excellent biocompatibility, mimicking the natural extracellular matrix to promote cell growth and wound healing. | Natural compound with high biocompatibility, minimizing the risk of allergic reactions and adverse responses. | (Deng et al., 2024; Liu et al., 2024; Ndlovu et al., 2021) |
| **Antibacterial Activity** | Acts as a physical barrier against infections, crucial for burn wounds. | No known antibacterial properties. | Significant, preventing infection in susceptible burn wounds. | (He H et al., 2024; Ibrahim et al., 2022; Kaczmarek, 2020) |
| **Antioxidant Properties** | Low antioxidant activity, potentially enhanced in formulations. | Limited inherent capacity, often boosted with additives. | Potent antioxidant capabilities, essential for reducing oxidative stress and promoting cellular repair in wound sites. | (Deng et al., 2024; Ndlovu et al., 2021; Zhang et al., 2024) |
| **Astringent Effects** | Lacks inherent astringent properties, focuses on hydration. | Mild astringent effects beneficial for wound care | Strong, aiding tissue contraction and protection. | (Liu et al., 2023; Ndlovu et al., 2021; Supriya et al., 2023) |

| | | | | |
|---|---|---|---|---|
| **Wound Healing Promotion** | Supports a moist environment for healing. | Accelerates healing through cell proliferation. | Promotes rapid healing via cell proliferation and migration. | (Akhlaq et al., 2024; Liu et al., 2024; Shahmohammadi et al., 2024) |
| **Moisture Retention** | High, maintaining an optimal healing environment. | Ensures a moist environment beneficial for healing. | Adequate, with tailored formulations for wound moisture. | (Kaal et al., 2005; Liu et al., 2024; Shahmohammadi et al., 2024) |
| **Drug Delivery System** | Suitable for therapeutic agent embedding and controlled release. | Delivers drugs directly to wounds, improving healing. | Facilitates controlled drug release for wound management | (Kaczmarek, 2020; Karami et al., 2022; Li Y et al., 2024) |
| **Cost-Effectiveness** | Economical production, providing a cost-effective material for wound care. | Value enhanced by multifunctional healing properties. | Economical due to plant- based origin, effective for hydrogels. | (He H. et al., 2024; Supriya et al., 2023; Szabó et al., 2023) |
| **Natural Source** | Derived from cellulose, offering an eco-friendly option. | Sourced from animal collagen, supporting natural healing methods. | Plant-derived, aligning with sustainable material sourcing. | (Akhlaq et al., 2024; Ndlovu et al., 2021; Supriya et al., 2023) |

## 1.3 Aim and objectives

The purpose of this study was to design and synthesize a biocompatible and biodegradable, hydrogel system for a potential treatment of acute superficial burn-wounds.

The following objectives were conducted and completed in the study:

**Objective 1**: To synthesize a biocompatible tannic acid loaded hydrogel employing solution gelation approach.

**Objective 2**: To evaluate the chemical and physical properties of the hydrogel scaffold and its starting materials employing FTIR, and DSC.

**Objective 3**: To determine the mechanical properties of the hydrogel scaffold employing the texture analyser equipment.

**Objective 4**: To assess the surface morphological properties of the hydrogel scaffold utilising SEM analysis.

**Objective 5**: To determine tannic acid drug release from the scaffold *in vitro* utilising UV-Vis spectrophotometer.

**Objective 6**: To undertake *in vitro* biodegradation analysis employing the Zeta Sizer to determine the change in zeta potential of the scaffold over time.

**Objective 7**: To evaluate the cytotoxicity of the hydrogel scaffold and starting materials by using the NIH 3T3 mouse embryonic fibroblast cell line.

## 1.4 Overview of this research report

**Chapter 1**: Serves as the introduction of the dissertation and describes pathophysiology of burn wounds and the challenges faced by individuals who incur these burn wounds. Discussion on current therapies for acute burn wounds and the challenges including cost implications and access to treatment. Furthermore, this chapter briefly presents hydrogel systems as novel drug delivery platforms for the treatment of acute burn wounds and advantages thereof. Literature from previous studies involving hydrogels is presented as evidence for the efficacy of hydrogels use in wound healing. Finally, the aim and objectives of the study are outlined in this chapter.

**Chapter 2**: A critical review of the significant role of pH in the healing of burn wounds, exploring how pH influences the effectiveness of various types of wound dressings on the healing process. It highlights the importance of maintaining an optimal pH environment for enhancing

cellular functions, enzyme activity, and controlling microbial proliferation, which are pivotal for tissue repair and regeneration. The chapter highlights clinical evidence supporting the use of pH-modified dressings in improving healing outcomes and discusses the challenges and future directions in the development and application of these dressings. Emphasizing the integration of smart hydrogels, nanotechnology, and biosensors, it advocates for further research and innovation in dressing technologies to optimise burn wound care.

**Chapter 3**: Describes the design, materials and methods used, as well as results and discussion for the synthesis of the hydrogel scaffold. It explains the characterization conducted utilising the FTIR, DSC, SEM and Texture Analyser. The drug release profile was determined by dissolution and UV/Vis Spectroscopy. Degradation studies were performed using Zeta Potential analysis. Cell viability studies were also conducted utilising MTT assay for proof of concept and confirmation of non-toxicity to physiological tissue.

**Chapter 4**: This study culminates by proving through the means of “proof of concept” that this scaffold is a leap forward in the treatment of acute burn wounds, offering an alternative to the current paradigms in wound dressing technologies. Future *in vitro*, further biodegradation studies, pH/thermo-responsive abilities are recommended.

## CHAPTER 2 : CRITICAL REVIEW OF PH INFLUENCED PARAMETERS WHICH HAVE AN EFFECT ON BURN WOUND HEALING AND APPLICATIONS

### 2.1 Introduction

The management of burn wounds remains a paramount challenge in clinical practice, necessitating interventions that accelerate healing, prevent infections, and minimising long- term scarring. Among the myriad of factors influencing the healing trajectory, the pH of the wound environment has garnered significant attention for its pivotal role in modulating biological processes essential for tissue repair and regeneration. The pH level within wound environments is a critical determinant of enzymatic activities, microbial proliferation, and the functionality of key cellular constituents involved in the healing process (Figure 2.1) (Schreml et al., 2010). This understanding has catalysed a shift towards the development of wound care strategies that consider pH modulation as a central tenet for optimizing healing outcomes.

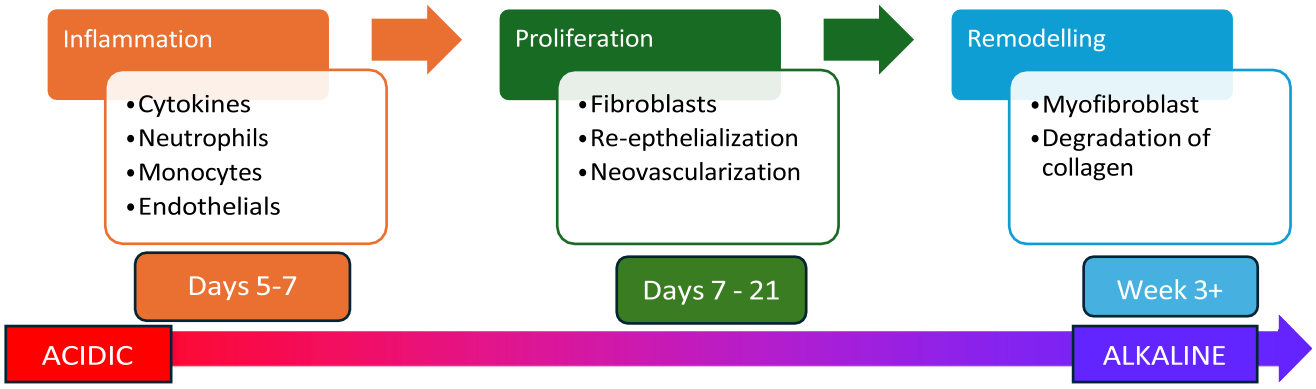


**Figure 2-1 The wound healing phases to attenuate fibrosis and scarring. Image adapted from (Schreml et al., 2010).**

The interplay between wound pH and healing dynamics is complex and multifaceted. An acidic wound environment is traditionally associated with enhanced antimicrobial properties and is considered conducive for the initial inflammatory phase of wound healing. This phase is crucial for setting the stage for subsequent tissue repair and regeneration (Gethin, 2007.; Schreml et al., 2010). Conversely, the progression to later stages of healing has been observed to benefit from a neutral to slightly alkaline pH, which supports fibroblast proliferation, collagen deposition, and ultimately, wound closure (Percival et al., 2014). These insights have underpinned the rationale for the development of pH-modified wound dressings, which aim to provide an optimal healing milieu through the dynamic modulation of wound pH (Schreml et al., 2010).

Recent advancements in wound care technology have led to the introduction of smart wound dressings capable of pH-responsive behaviour. These innovative dressings not only protect the wound from external contamination but also actively participate in the healing process by adjusting the wound pH in accordance with the healing stage (Derwin et al., 2021). The development and application of such dressings represent a significant leap forward in personalised wound care, offering the potential for improved healing rates, reduced infection risks, and better overall outcomes for patients with burn injuries.

Despite the promising developments in pH-modified and smart wound dressings, the literature presents a fragmented picture of their efficacy and mechanisms of action. This literature review aims to consolidate current knowledge on the significance of pH in acute burn wound dressings. It will explore the biological underpinnings of the influence of pH on wound healing, evaluate the development, and performance of pH-modified dressings, and scrutinize the clinical evidence supporting their application in burn wound management. Through a comprehensive synthesis of available research, this review seeks to elucidate the role of pH management in advancing wound care practices and highlight future directions for research and clinical application.

### 2.1.1 The role of pH in wound healing

The treatment of burn wounds presents a complex challenge in medical care, requiring strategies that not only address immediate tissue damage but also prevent infection and optimise healing. The selection of appropriate wound dressings is crucial for managing burn injuries. Dressings not only serve to protect the wound from external contamination but also create an environment conducive to healing. The pH properties of these dressings play a significant role in their effectiveness.

Wound healing is a dynamic process, involving a series of coordinated phases that include inflammation, proliferation, and maturation. The pH level of the wound environment plays a pivotal role at each stage, influencing cellular functions, enzyme activity, and microbial proliferation. The pH of the wound environment can significantly affect the behaviour of cells critical to the healing process, such as fibroblasts and keratinocytes. A slightly acidic pH has been shown to enhance fibroblast proliferation and collagen synthesis, which are essential for

tissue repair and strength. Schneider et al., demonstrated that an acidic environment could accelerate wound closure by stimulating keratinocyte migration (Schneider et al., 2007).

Enzymatic processes crucial for wound healing, including those involved in debridement and remodelling, are pH-sensitive. A study by Gethin found that an optimal acidic to neutral pH range is vital for maintaining enzymatic activity that aids in cleaning the wound and facilitating the proliferation phase (Gethin, 2007). The wound's pH can influence the susceptibility to infection, with an acidic environment inhibiting bacterial growth. A study by Percival et al. highlighted the antimicrobial properties of acidic wound conditions, which can reduce the risk of infection and promote a safer healing environment (Percival et al., 2014).

Research by Ono et al. stresses the diagnostic potential of pH monitoring in burn wounds. Their study demonstrated that an elevation in wound pH could precede visible signs of infection in second-degree burns, suggesting that pH monitoring might serve as an early, non- invasive indicator of infection, critical for timely intervention and management (Ono et al., 2015). A study by Sharpe et al. also provided valuable insights into the relationship between wound surface pH, healing time, and burn depth. Their findings indicated that wounds with a lower pH at the time of the second dressing change were more likely to heal without the need for grafting, suggesting the potential of pH measurement as a prognostic tool in burn wound care (Sharpe et al., 2009).

## 2.2 Types of burn wound dressings and their pH characteristics

### 2.2.1 Alginate based wound dressings and their impact in burn wounds

Alginate dressings, known for their natural origin from seaweed and their gel-forming capabilities upon contact with wound exudate, have been shown to maintain an optimal pH for healing, absorbing moisture and facilitating tissue regeneration effectively (Aderibigbe and Buyana, 2018). Recent studies have further explored the pH sensitivity of these dressings and their impact on wound healing. Recent literature has introduced innovations in alginate-based dressings, enhancing their pH sensitivity and wound healing properties (Maghsoudi et al., 2024).

Sadeghi-Aghbash et al. developed alginate-based dressings for burn wounds, incorporating ciprofloxacin and zinc oxide. These dressings demonstrated pH-sensitive drug release, with increased release at higher pH levels, and displayed excellent wound healing capabilities in terms of wound closure and tissue regeneration (Sadeghi-Aghbash et al., 2024). This highlights the importance of pH-sensitive formulations in optimizing the therapeutic outcomes

of alginate dressings. Another study by Hautmann et al. designed a composite wound dressing by combining an electrospun fleece with a multilayer film. This innovative dressing showcased pH-sensitive bonding and was studied for its wound healing properties, presenting comparable tensile strength to traditional alginate dressings. The incorporation of pH-sensitive elements into the dressing design exemplifies the advancements in creating more effective wound care solutions (Hautmann et al., 2024). Modern alginate dressings are becoming increasingly effective in managing wounds by incorporating advanced materials and technologies, such as drug release mechanisms and structural modifications, promoting faster healing, and improving patient outcomes.

### 2.2.2 Silver based wound dressing's impact on wound's pH

Silver sulfadiazine is recognised for its broad-spectrum antimicrobial activity, which is crucial in preventing wound infections. However, the mechanism by which silver sulfadiazine potentially alters the wound's pH, and its implications for healing remains a critical area for careful consideration. Atiyeh et al. previously highlighted the potential for silver sulfadiazine to delay healing processes by affecting the wound's pH balance, suggesting a need for a judicious approach to its use (Atiyeh et al. 2007).

### 2.2.3 Foam based dressing's impact on burn wound pH

Foam dressings are highly valued in burn wound care for their ability to meet the complex requirements of wound healing. They ensure the wound remains at an ideal temperature, supporting tissue repair, as Jones et al. have emphasized the importance of thermal regulation (Jones et al., 2002). Additionally, these dressings adeptly manage moisture by absorbing excess exudate while keeping the wound moist, thus preventing tissue dehydration and reducing maceration risk (Chaganti et al., 2019). Oxygen exchange facilitated by foam dressings is crucial for cellular respiration and inhibiting anaerobic bacteria, enhancing wound microenvironment health (Sarheed et al., 2016).

A pivotal aspect of foam dressings is their ability to maintain balanced wound pH, essential for optimal enzyme function and tissue oxygenation, critical for healing processes (Schneider et al., 2007). An acidic environment helps deter bacterial colonization, reducing infection chances (Gethin, 2007). Landén et al. point out that pH impacts healing by affecting cellular function and matrix formation (Landén et al., 2016).

A study by Raepsaet et al. further enhances this discussion, providing a systematic review of the effectiveness of foam dressings in treating complex wounds. Their comprehensive analysis of outcomes and measurement instruments underlines the clinical significance and

versatility of foam dressings in managing various wound types, emphasizing the dressings' role in achieving optimal healing conditions through moisture management, thermal regulation, and pH balance (Raepsaet et al., 2022).

Foam dressings emerge as a versatile tool for burn wound management, excelling in thermal insulation, moisture regulation, and facilitating gas exchange. Their capacity to maintain appropriate wound pH, corroborated by the findings of Cutting, Schneider et al., Gethin and Raepsaet et al., underscores their critical role in promoting conducive healing environments. This body of evidence highlights the necessity of employing suitable wound care strategies to enhance patient outcomes in burn wound management (Cutting, 2008; Gethin, 2007; Raepsaet et al., 2022; Schneider et al., 2007).

### 2.2.4 Honey based dressing’s impact on burn wound pH

Honey-based dressings, renowned for their natural acidity, have been utilised for their ability to create a conducive environment for wound healing. This environment not only promotes enhanced antimicrobial activity but also supports the overall healing process. Molan and Rhodes discussed the multifunctional benefits of honey in wound care, including its role in pH modulation, which is crucial for managing wound environments effectively. Honey's natural acidity helps to lower the pH of the wound environment. A lower pH is associated with reduced presence of protease activity, increased oxygen release, and enhanced fibroblast activity, all of which are beneficial for wound healing. The antimicrobial properties of honey, attributed to its low pH, high osmolarity, and the presence of hydrogen peroxide and other bioactive compounds, play a significant role in preventing wound infections (Molan and Rhodes, 2015).

### 2.2.5 Hydrogels dressing’s impact on burn wound pH

Hydrogel dressings are favoured for their cooling effect and ability to maintain a moist healing environment. These dressings, which often have a slightly acidic to neutral pH, support cell migration and proliferation, critical for the early stages of wound healing (Martin and Nunan, 2015).

Smart hydrogels are an innovative class of hydrophilic polymer networks that can undergo significant and reversible changes in their structure and properties in response to external stimuli. Unlike conventional hydrogels, which maintain consistent properties, smart hydrogels react to variations in environmental conditions such as temperature, pH, or the presence of specific ions or molecules. This responsiveness enables smart hydrogels to transition between states of swelling and deswelling, altering their volume, porosity, and mechanical strength accordingly. The capacity for such dynamic behaviour makes smart hydrogels highly versatile

and valuable in applications ranging from drug delivery systems, where they can control the release of therapeutics, to tissue engineering scaffolds that adapt to biological environments, and even as actuators in soft robotics (Caló and Khutoryanskiy, 2015; Qiu and Park, 2001).

Smart hydrogels represent a breakthrough in wound dressing technology, with their ability to change physical properties in response to the pH of the wound environment. These hydrogels are engineered to provide optimal moisture and mechanical support, which are crucial for different stages of wound healing. Zhang et al. demonstrated that smart hydrogels could accelerate wound closure and reduce infection rates by maintaining an acidic environment conducive to early-stage healing and gradually adjusting to support later stages of tissue regeneration (Zhang et al., 2023). Cao et al. highlighted the potential of self-regulating hydrogels that adjust their stiffness in response to pH changes, promoting cell migration and proliferation essential for wound healing (Cao et al., 2023).

Recent research into pH-sensitive hydrogels has illuminated their significant potential across a variety of biomedical applications, particularly in the realm of controlled drug delivery and tissue engineering. Studies have demonstrated that these hydrogels' responsiveness to environmental pH changes can be finely tuned for precise drug release in specific bodily regions characterized by distinct pH levels. For instance, Salih and Alshrefi explored the pH- sensitive swelling behaviour and drug release kinetics of chitosan-modified hydrogels, uncovering the pivotal role of cross-linking agents in modulating these hydrogels' stability and responsiveness for targeted drug delivery (Salih and Alshrefi, 2024). Similarly, Kalmer et al. investigated how polymer composition affects the rheological and mechanical properties of Eudragit L100-55/gelatin-based hydrogels, revealing their capability to act as pH-sensitive drug delivery systems that can adapt to the gastric pH medium (Kalmer et al., 2024).

Hu et al. introduced a low-cytotoxicity fluorescent probe within hydrogels for real-time pH measurement, demonstrating the probe's consistent sensitivity across a range of pH values (Hu et al., 2024). Nabipour et al. developed a bio-based hydrogel for pH-responsive drug delivery, showing promise for controlled release under both physiological conditions and the acidic environment of tumour cells, highlighting its potential for targeted cancer therapy (Nabipour et al., 2024). A comprehensive review by Gong et al. on cellulose-based intelligent- responsive hydrogels emphasized their capability to adjust proton capture or release in response to different pH levels, underlining the adaptability of pH-sensitive hydrogels in drug delivery applications (Gong et al., 2024). These studies show the versatility and importance of pH-sensitive hydrogels in advancing medical science, offering innovative solutions for precision medicine through localised and controlled therapeutic agent delivery.

A noteworthy advancement comes from Pinto e Souza et al. who developed pH-sensitive wound dressings using PVA/PAA hydrogels and bioactive glass nanoparticles doped with cerium and cobalt. These materials showcased pH-responsive behaviour, allowing for controlled release of therapeutic ions, and highlighting the potential of such dressings in bioactive wound care (Pinto e Souza et al., 2024).

Ribeiro et al. modified polyvinyl alcohol-based electrospun mats with naturally-derived halochromic molecules for potential applications in wound healing monitoring. This innovation permits the bioactive dressing to assist in monitoring healing progression through pH sensitivity, without necessitating frequent dressing removal and thereby preventing potential wound disturbances (Ribeiro et al., 2023). Liu et al. introduced a bioinspired natural shellac dressing for rapid wound sealing and healing. This type of bioactive shellac-based wound dressing showcased superior mechanical strength, biocompatibility, and pH sensitivity, marking a significant step forward in the development of efficient wound care solutions (Liu et al., 2023).

These studies illustrate the dynamic progress in the development of bioactive dressings that not only provide antimicrobial and anti-inflammatory action but also adapt to the wound's healing needs through pH-sensitive mechanisms. This approach enhances the overall healing process, from the initial inflammatory phase to later stages of tissue regeneration, by maintaining an optimal wound environment. The various wound dressings have been compared in Table 2-1 below.

**Table 2-1 Types of Burn Wound Dressings**

| Dressing Type | Material Responsible for pH Sensitivity or Control | pH Sensitivity or Control | Advantages | Disadvantages | Reference |
|---|---|---|---|---|---|
| **Standard Hydrogel** | Not specified. | Not specified. | Maintains moist healing environment, cooling effect. | Limited absorption capacity. | (Martin and Nunan, 2015) |
| **Smart Hydrogels** | pH-sensitive polymers | Adjusts to wound pH. | Supports cell migration and proliferation. | Complex manufacturing. | (Cao et al., 2023; Jia et al., 2023) |
| **Alginate** | Calcium and sodium ions in alginate (general knowledge, not from provided examples). | Slightly acidic. | High absorption, forms gel with wound exudate. | Can dry out the wound. | (Hautmann et al., 2024; Hu et al., 2024; Sadeghi-Aghbash et al., 2024) |
| **Foam** | Not specified. | Neutral. | Thermal insulation, moisture control. | May not suit all wound types. | (Cutting, 2008) |
| **Honey-based** | Natural enzymes and pH-modifying components in honey. | Naturally acidic. | Antimicrobial properties, promotes healing. | Potential stickiness. | (Molan and Rhodes, 2015) |

| | | | | | |
|---|---|---|---|---|---|
| | (general knowledge, not from provided examples). | | | | |
| **Spray-Type Alginate Hydrogel** | Calcium and sodium ions in alginate. | Slightly acidic. | Promotes healing, Reduces pain. | Specific to initial burn care. | (Choi et al., 2024) |
| **Polyvinyl Alcohol Hydrogel with Nanoclay** | Not specified. | Neutral. | Enhances stem cell adhesion, Prevents dressing sticking to the wound. | Focused on specific wound types. | (Renani et al., 2024) |
| **Oxidized Alginate-Gelatin/Silk Fibroin Hydrogels with Cu-Ag Doped Mesoporous Bioactive Glass** | Cu-Ag doping in mesoporous bioactive glass (general knowledge, not from provided examples). | Slightly alkaline. | Targets enhanced healing and infection control. | Advanced manufacturing required. | (Akhlaq et al., 2024) |
| **Injectable Hydrogel** | pH-sensitive polymers for glucose-responsive insulin delivery discussed in Li et al. (2017). | pH-responsive. | Antimicrobial and anti-inflammatory properties, Ideal for antibiotic-resistant infected wounds. | Requires precise application. | (Li Y. et al., 2024) |

## 2.3 Evidence supporting pH-modified dressings in burn wound care

Optimal wound care strategies are crucial for the effective management of burn injuries, with the pH level of the wound environment playing a critical role in healing outcomes. Recent advancements in pH-modified dressings have sparked significant interest, offering potential improvements in healing efficacy through the modulation of wound pH.

Wang et al., introduces intelligent hybrid hydrogels capable of rapid *in situ* detection and photothermal therapy of bacterial infections, particularly in burn wounds. These hydrogels respond to the presence of bacteria by altering the pH of the environment, which can be monitored in real-time. Upon detection of an infection, the hydrogel facilitates localised photothermal therapy by converting near-infrared light to heat, effectively reducing bacterial load and restoring optimal pH conditions for healing. This innovative approach highlights the critical interplay between pH modulation and infection control in promoting wound healing (Wang et al., 2020).

Derwin et al.'s systematic narrative review consolidates clinical evidence on the impact of various topical agents and dressings on wound pH and temperature. The review underscores the potential of specific dressings and agents, such as Manuka honey and silver dressings, to lower wound pH and improve healing outcomes. Manuka honey, known for its antimicrobial properties, also exerts a modulating effect on wound pH, fostering an environment conducive to healing. The review calls for further research to explore the mechanisms by which these agents influence wound pH and their overall efficacy in acute burn wound care (Derwin et al., 2022).

## 2.4 The role of pH-modified dressings in burn wound care

The therapeutic efficacy of pH-modified dressings in managing burn wounds is underpinned by several key mechanisms. These mechanisms work in conjunction to create an optimal healing environment, addressing the critical aspects of wound repair, including antimicrobial defence, enzymatic activity, and cellular function. A comprehensive understanding of these actions provides insight into the clinical benefits observed with the use of these dressings.

Optimal wound healing requires the activity of various enzymes involved in debridement, angiogenesis, and tissue remodeling. The activity of these enzymes, such as matrix metalloproteinases (MMPs) and collagenases, is pH dependent. An alkaline environment, often observed in chronic wounds, can lead to excessive MMP activity, detrimental to healing. Conversely, pH-modified dressings can help maintain a balanced enzymatic activity by preserving an optimal pH, thus promoting efficient debridement and tissue repair (Schneider et al., 2007).

The healing of burn wounds involves the orchestrated activity of various cell types, such as keratinocytes, fibroblasts, and endothelial cells, which are crucial for processes including re-

epithelialization, collagen deposition, and neovascularization, respectively. These cellular functions are influenced by the wound's pH level, where a slightly acidic to neutral pH is optimal for fibroblast proliferation and keratinocyte migration, thus facilitating effective wound closure and tissue regeneration. Dressings such as pH-modified dressings play a vital role in this context by optimizing cellular functions and maintaining the wound environment within this beneficial pH range (Martin and Nunan, 2015; Zhao R et al., 2016).

### 2.4.1 Antimicrobial properties

Maintaining an acidic environment in wound care using pH-modified dressings has been recognised for its significant antimicrobial activities. This strategy is especially beneficial in managing burn wounds, where the risk of infection is heightened. The acidic conditions created by these dressings are known to be less favourable for the survival and proliferation of bacteria, notably reducing the susceptibility of wounds to infections. Research supports this approach, indicating that pathogens such as *Pseudomonas aeruginosa* and *Staphylococcus aureus*, which are frequently implicated in burn wound infections, show reduced viability in acidic environments (Percival et al., 2014). This antimicrobial effect is pivotal for preventing infections that could otherwise delay the healing process and lead to further complications.

Several studies have further explored innovative approaches and substances that exhibit antimicrobial properties in response to pH changes. For instance, the investigation into the micellization of curcumin for its improved stability and antimicrobial activity in acidic conditions suggests the potential of incorporating natural compounds into dressing formulations (Ryu et al., 2024). Similarly, the development of propolis-infused non-woven materials for nursing pads emphasizes the broad-spectrum antimicrobial sensitivity of various bacterial species to natural extracts, further underscoring the role of pH in enhancing antimicrobial effectiveness (Somogyi Škoc et al., 2024). These findings highlight the ongoing interest in and the importance of leveraging pH sensitivity to augment the antimicrobial capabilities of wound dressings. Focusing on the mechanisms through which pH modification influences bacterial viability and infection risk, these studies contribute to a deeper understanding of how to optimise wound care practices, especially in the context of burn wounds where infection control is a critical concern.

### 2.4.2 Tissue regeneration properties

Recent research, such as the work by Kojima et al., further underscores the significance of pH sensitivity in wound healing. They engineered pH-responsive, self-healing vesicle-type artificial tissues to emulate the natural tissue environment, highlighting the potential of creating optimal healing conditions through pH modulation (Kojima et al., 2024). Similarly, Shi et al. developed

functional hydrogels for delivering the proteolytic enzyme Serratiopeptidase, which aids tissue regeneration. These novel pH-sensitive hydrogels adjust to the wound's pH, emphasizing the crucial role of pH in promoting wound healing and tissue repair (Kamenova et al., 2024). Together, these studies and innovations highlight the consensus in the literature regarding the positive impact of maintaining an appropriate pH environment on cellular function and tissue regeneration in burn wounds. By leveraging the principles of pH modification, researchers and clinicians aim to enhance the efficacy of wound healing interventions, thus contributing to more efficient tissue repair and recovery.

### 2.4.3 Anti-inflammatory properties

The inflammatory response is pivotal in the initial phase of wound healing, as it involves the recruitment of immune cells to clear debris and pathogens. However, prolonged inflammation can impede healing and lead to scar formation. Recent studies emphasize the role of pH- modified dressings in modulating the inflammatory response, potentially curtailing excessive inflammation and encouraging progression to the proliferative phase of healing. This modulation is achieved partly through the impact of pH on cytokine production and immune cell activity, essential in managing the inflammatory response (Kondo and Ishida, 2010). A study by Kolesov et al. on the fabrication of Alginate/Ozoile gel microspheres through the electrospray process highlights the potential of pH-sensitive alginate microspheres in managing oxidative stress and inflammation, showing great promise in the modulation of the LPS-induced inflammatory response in colonic epithelial cells. Such findings highlight the significant potential of pH-sensitive materials in effectively managing inflammatory processes (Kolesov et al., 2024).

Additionally, research by Gustavo Lima et al. explores the modulating effects of jelleine, a family of peptides isolated from the royal jelly of honeybees, on tumour cell growth and the inflammatory process (Gustavo Lima et al., 2024). This study reveals the pH-dependent activities of these peptides, further illustrating the role of pH in controlling inflammation and suggesting potential avenues for new medicines based on natural compounds and their pH- sensitive properties (Gustavo Lima et al., 2024).

These recent advancements underline the importance of pH modulation in the inflammatory response within wound healing contexts. There is a clear pathway toward reducing excessive inflammation, thus supporting the overall healing process and reducing the risk of complications associated with delayed wound healing.

The clinical evidence supporting the use of pH-modified dressings in burn wound care suggests a promising avenue for improving patient outcomes. However, the implementation of these

dressings in clinical practice requires careful consideration of wound characteristics, patient factors, and dressing properties. Future research should focus on identifying the optimal pH range for different wound types and stages of healing, as well as developing new materials and technologies that can provide precise pH modulation.

### 2.5 Limitations and challenges

To date, technological advancements in burn wound care are indeed paving the way for more effective treatments, yet they also introduce several challenges that need to be addressed. Key among these is the cost of dressing development, accessibility, and data privacy concerns, alongside the need for regulatory approvals to bring these innovations to market effectively and safely.

Research into smart 3D structures in skin tissue engineering, such as gas foamed scaffolds, demonstrates the potential for creating advanced wound dressings that cater to specific needs in wound care. However, the development of these technologies poses significant challenges for healthcare systems, emphasising the need for cost-effective solutions (Bianchi et al., 2024). The use of 3D bioprinting technologies for regenerative therapy of skin damage offers exciting prospects for post-burn wound treatment. Despite the advancements, there are still issues that need improvement, particularly concerning the biocompatibility and effectiveness of these constructs in wound healing (Baranovska and Lutsenko, 2024).

Exploration of novel treatments like the Plasma Directed Electron Beam (PDEB™) highlights the potential of new technologies in enhancing wound healing and addressing chronic wounds. PDEB™ has demonstrated efficacy in inhibiting bacterial growth and has shown encouraging safety profiles when applied to human epithelial cells. This technology, particularly the PDEB™ handheld device, has shown promise in military operational medicine and beyond, emphasizing its potential for broad therapeutic use (Bauer et al., 2024). The mechanism by which PDEB™ enhances wound healing involves the application of a directed electron beam to the wound area. This beam generates plasma, which has been found to possess antimicrobial properties, effectively reducing bacterial load in wounds and thereby decreasing the risk of infection (Bauer et al., 2024). The plasma produced by the electron beam promotes tissue regeneration and healing by stimulating cellular responses and increasing the expression of growth factors involved in wound repair (Nguyen et al., 2022).

Cold plasma technology, similar to PDEB™, has been evaluated in various studies and has shown to accelerate wound healing processes, reduce inflammation, and enhance cell proliferation and migration. These properties are crucial for effective wound management and are especially beneficial for chronic wounds that are resistant to conventional treatments (Dubey et al., 2022).

Despite these promising findings, safety and efficacy remain critical challenges that require thorough investigation to ensure these innovations can be safely implemented. Comprehensive studies and clinical trials are necessary to fully understand the long-term effects and to confirm the preliminary positive results observed in early research (Bauer et al., 2024).

These studies collectively underline the complexities involved in advancing burn wound care through technological innovations. Efforts to mitigate these challenges involve not only enhancing the technological aspects but also ensuring these advancements are accessible, cost-effective, and secure, all while navigating the intricate process of regulatory approvals. This multifaceted approach aims to make significant strides in improving patient care in the face of evolving challenges within the field of burn wound management.

## 2.6 Future directions and challenges in the development and application of pH- modified dressings

### 2.6.1 Nanotechnology in pH-modified dressings

The integration of nanotechnology in the development of pH-modified dressings represents a promising area of research, with significant potential to advance wound care. This interdisciplinary approach combines the precision of nanotechnology with the critical need for pH-sensitive interventions in wound healing environments. Notably, nanoparticles, including silver nanoclusters, are under investigation for their ability to offer pH-responsive antimicrobial properties. These innovations promise targeted infection control that becomes active in response to the specific pH changes associated with infections, a crucial advancement for enhancing wound care outcomes (Davies et al., 2017).

The development and implementation of nano-carriers for drug delivery within these dressings signify a leap forward in therapeutic interventions. These nano-carriers are engineered to release healing agents, such as growth factors or antibiotics, in a pH-dependent manner. This specificity ensures that the therapeutic agents are released directly at the wound site and at the optimal time during the healing process, potentially speeding up recovery and improving the efficacy of the healing process (Biswas et al., 2012).

### 2.6.2 Smart hydrogels with integrated sensors

Smart dressings with integrated sensors, particularly those sensitive to pH changes, represent a significant leap forward in personalised wound care. These innovative dressings combine the precision of nanotechnology and the practicality of real-time monitoring to offer dynamic treatment options tailored to the wound's specific needs. The integration of pH sensors and wireless communication capabilities within these dressings allows for continuous, non- invasive monitoring

of the wound environment, providing valuable data to adjust treatment plans effectively. Recent advancements have led to the development of injectable conductive hydrogels that exhibit self-healing properties, motion monitoring capabilities, and targeted bacterial theranostics, making them ideal for bioelectronic wound dressing applications. These hydrogels are particularly noted for their excellent sensitivity, cyclic stability, and pH- responsive behaviour, facilitating intelligent wound monitoring and healing (Shan et al., 2024).

The progress in hydrogel dressings with built-in sensors or intelligent sensing capabilities underscores the trend towards more sophisticated wound care solutions. These hydrogels are prepared with pH-sensitive materials, enabling real-time wound assessment and adding a valuable layer to therapeutic interventions (Jin et al., 2023).

These advancements highlight the potential of smart dressings with integrated sensors to revolutionize wound care. In enabling timely updates on wound status and ensuring interventions are optimally timed, healthcare providers can support healing more effectively, ushering in a new era of personalised, responsive wound management.

### 2.6.3 pH sensitive Films

The creation of multifunctional graphene quantum dot-polyvinyl alcohol-polyglycerol luminescent films has introduced an intelligent pH sensing system suitable for the detection of pH variations in sweat. This technology can extend to health monitoring, facial masks, and medical dressings, offering a versatile approach to smart wound care (Ke et al., 2023). Further innovation is seen in chitosan-based composite film dressings, which not only respond to the wound's pH environment but also feature smart sensing and anti-inflammatory properties. These dressings are designed to accelerate the healing process, especially for diabetic wounds, by integrating pH-sensitive purple cabbage indicators (Li C. et al., 2024).

### 2.6.4 Bioresponsive dressings

Bioresponsive dressings represent a frontier in wound care innovation, designed to interact with the biological components of the wound environment actively. These advanced dressings are engineered to respond to specific biological signals, such as enzymes or cytokines, indicative of the wound's healing phase. By altering their physical structure or releasing therapeutic agents in response to enzymatic activity associated with inflammation or infection, bioresponsive dressings offer adaptive support, minimizing the risk of transitioning into chronic wounds. Guo et al. have developed physical dynamic double-network hydrogels, demonstrating promising stability, elasticity, and bioresponsive properties. These hydrogels combine self-healing, adhesive, and antibacterial capabilities, responding adeptly to pH changes in the wound environment, signifying a leap towards intelligent wound monitoring and healing facilitation (Guo et al., 2023).

### 2.6.5 Electrospun nanofibers

The research by Koohzad and Asoodeh on cross-linked electrospun pH-sensitive nanofibers, adsorbed with Temporin-Ra, illustrates the potential of bioresponsive materials to generate biological functions in response to pathological abnormalities. This study highlights how such materials, through their sensitivity to pH changes, can meet the ideal requirements for an effective wound dressing, promoting wound healing and offering a strategic approach to infection control (Koohzad and Asoodeh, 2023). These advancements emphasise the critical role of bioresponsive dressings in the evolution of wound care, providing a more nuanced and effective treatment approach. Using the capability to dynamically adjust to the biochemical milieu of wounds, these innovative dressings aim to enhance healing outcomes, offering a promising avenue for personalised and efficient wound management.

### 2.6.6 Personalised wound care

The progression towards personalised wound care represents a significant evolution in the treatment of injuries, particularly through the lens of pH sensitivity. The focus is now on developing bespoke solutions that cater to the unique requirements of each patient's wound. This personalised approach leverages advanced diagnostics and algorithms to analyse specific wound conditions, including pH levels, enabling healthcare providers to recommend the most suitable dressing type and treatment regimen. The move towards such customised care aims to enhance healing outcomes by accounting for the distinct characteristics of each wound and patient. It signifies a departure from the one-size-fits-all methodology, embracing a more nuanced understanding of wound management that could lead to shorter healing times and improved patient quality of life. Recent research underscores the importance of integrating diagnostic tools and algorithms with pH-modified dressings to achieve these tailored treatment plans (Percival et al., 2014; Wang et al., 2022).

### 2.6.7 Current innovations in material science

Focusing on the properties of novel materials, researchers are creating dressings that can actively contribute to the healing process, offering tailored responses to the unique conditions of each wound. As these technologies continue to evolve, they hold the potential to revolutionize wound management, making treatments more effective and personalised to the needs of individual patients.

The integration of wearable technologies and sensors into wound dressings is transforming the landscape of patient care by enabling continuous and non-invasive monitoring of critical wound parameters, including pH levels. This shift towards smart wound care solutions facilitates real-time tracking of the healing process, providing valuable insights that can guide treatment decisions.

Recent studies highlight significant advancements in the development of smart wound dressings equipped with sensors, heralding a new era in personalised wound management. Peng et al.

describe wearable sensors that enable the real-time monitoring of wounds by assessing physiological conditions like pH levels, offering insights that can prompt timely adjustments in wound care (Peng L et al., 2024). Wang et al. further emphasize the evolution of intelligent dressings capable of continuous pH monitoring, marking a shift towards dressings that not only monitor but actively contribute to the healing process. Additionally, Wang et al introduce a novel approach with an integrated bilayer microneedle dressing and triboelectric nanogenerator, providing a multifaceted platform for the efficient management of infected wounds (Wang et al., 2024).

Yu et al. present the e-Bandage, using smartphone technology for therapeutic intervention, thereby enhancing treatment efficacy through integrated sensing and actuation capabilities. These breakthroughs highlight the potential of integrating wearable technologies and sensors into wound dressings, enabling continuous, non-invasive monitoring that drives personalised, effective treatment plans, and represents a significant leap forward in the pursuit of precision medicine in wound management (Yu et al., 2024).

### 2.6.8 Potential digital health solutions for enhanced care

The fusion of artificial intelligence (AI) with digital health solutions, particularly in the realm of burn wound care, signifies a monumental shift towards more personalised and efficient treatment methodologies. This integration powers the capabilities of AI to enhance the selection and monitoring of pH-modified dressings, tailoring wound care to the unique needs of each patient.

Research, such as the systematic review by Anisuzzaman et al., illuminates the role of AI in accurately classifying burn wounds through image analysis, a step critical in choosing the correct dressing type (Anisuzzaman et al., 2022). Similarly, the application of machine learning in evaluating burn wounds, as explored by Huang et al., allows for the analysis of data derived from dressings, thereby facilitating the adaptation of treatment plans based on the wound's healing trajectory (Huang et al., 2021).

The dawn of AI applications for remote wound management, demonstrated by the study conducted by Barakat-Johnson et al., introduces a new era of wound care where wearable sensors integrated with pH-modified dressings enable continuous monitoring and assessment. This technology promises to bridge the gap between patients and healthcare providers, offering real-time insights into the healing process (Barakat-Johnson et al., 2022).

Cirillo et al. have shown how deep learning can be employed to predict burn depth accurately, further refining the selection process for wound dressings to optimise the healing environment (Cirillo et al., 2019). Collectively, these advancements highlight the transformative potential of AI and digital health in making burn wound care more responsive, effective, and tailored to the individual circumstances of each patient, thereby improving treatment outcomes and enhancing

patient engagement.

## 2.7 Future perspectives and conclusion

Recent research highlights the pivotal role of pH in optimizing burn wound care, introducing innovative solutions like pH-modified dressings, smart technologies, and digital health platforms that collectively aim to enhance treatment outcomes. Dynamic pH-responsive dressings, which adjust to wound conditions to accelerate healing, alongside bioresponsive materials that release therapeutic agents based on wound signals, represent significant strides towards creating a more conducive healing environment.

Technological advancements have further transformed wound care through the development of smart dressings and wearable sensors. These innovations, equipped with pH monitoring capabilities, facilitate real-time wound assessment, enabling healthcare professionals to tailor treatments to individual wound dynamics. This personalised approach aids in early complication detection and intervention, potentially hastening the healing process.

Material science has also contributed to wound care advancements by introducing stimuli-responsive and nano-structured materials into dressings. These innovations improve drug delivery and antimicrobial properties, responding effectively to the wound environment and enhancing dressing efficacy.

The integration of digital health solutions, utilising AI to analyse data from wearable sensors, promises a future where treatment plans are highly personalised, improving patient engagement and adherence to care protocols. However, challenges like cost, accessibility, data privacy, and regulatory approvals remain barriers to widespread adoption.

In summary, the recent surge in research around the role of pH in burn wound care marks a significant advancement in the field. Harnessing the potential of pH-modified dressings, smart technologies, innovative materials, and digital health solutions, treatment is becoming increasingly efficient and personalised. The successful navigation of existing challenges will be key to fully leveraging these innovations in clinical settings, aiming for improved healing outcomes and enhanced quality of life for burn patients.

# CHAPTER 3 DESIGN AND SYNTHESIS OF A HYDROGEL SYSTEM FOR THE TREATMENT OF ACUTE BURN WOUNDS

## 3.1 Introduction

The burden of acute burn wounds on healthcare systems globally is profound, with South Africa facing distinctive challenges that magnify the impact of these injuries on its healthcare infrastructure. A study by Allorto et al. specifically addresses the healthcare burden of burn injuries in the KwaZulu-Natal Province, South Africa, illustrating the significant strain placed on resources due to the wide spectrum of injury severity managed within the provincial healthcare system (Allorto et al., 2023). Economically, the repercussions of acute burn wounds are extensive, encompassing not only the immediate costs of medical care but also long-term rehabilitation and lost productivity. The financial implications for both the healthcare system and patients are substantial, creating an urgent demand for cost-effective treatment options. A pertinent analysis by Albertyn et al. sheds light on the economic strain burn injuries inflict on South African healthcare, revealing a dire need for interventions that balance clinical efficacy with economic sustainability (Albertyn et al., 2006).

The classification of burn wounds is a fundamental aspect of clinical assessment and directly informs the therapeutic approach. Burns are primarily categorized by their depth into superficial (first-degree), partial thickness (second-degree), and full thickness (third-degree), each presenting distinct characteristics and healing challenges. Superficial burns affect only the epidermis, the outermost layer of skin. Characterized by redness, pain, and slight swelling without blistering, these burns typically heal within 5 to 7 days. Superficial burns are often treated with cooling, analgesia, and minimal wound care, emphasizing the body's natural healing capabilities (Goodwin et al., 2015).

Understanding the classification of burn wounds is crucial for clinicians to determine the most appropriate treatment strategy. This categorisation not only impacts the choice of wound care techniques but also influences decisions regarding the need for surgical intervention, rehabilitation, and long-term scar management. The depth of a burn wound affects healing time, potential for infection, scarring, and the overall treatment plan, highlighting the importance of accurate assessment and individualized patient care (Smolle et al., 2017).

In tackling the challenge of healing burn wounds, an innovative approach called scaffolds is making significant strides in the fields of tissue engineering and regenerative medicine. These scaffolds are specially designed structures that help repair and regenerate damaged tissue, showing promise for more effective burn treatments (Jones et al., 2015). They possess three

main qualities: they're biocompatible, meaning they work well within the human body without causing harm; they're biodegradable, which means they can safely break down and be removed by the body, avoiding the need for additional surgery; and they support the growth and multiplication of cells, acting like the body's own scaffolding to aid in the formation of new tissue (Negut et al., 2020)

One of the key breakthroughs with scaffolds is their ability to be loaded with bioactive molecules, like growth factors and cytokines, that directly contribute to and speed up the healing process, making the repair of burn wounds more efficient (Jones et al., 2015) t. This combination of structure and biology in scaffolds represents a big leap forward in treating burn wounds. They offer treatments that are not only faster but also produce results that look and function more like the original skin, improving patient recovery significantly (Qin et al., 2022).

By creating a 3D environment that closely resembles the body's natural setting, scaffolds can greatly enhance healing, lower the chance of infection, and improve the overall look and function of the healed area for those recovering from burns. This shift in approach is setting a new standard in burn care, paving the way for therapies that could greatly reduce the impact of burn injuries on patients and healthcare systems alike (Negut et al., 2020).

This progress in medical science not only highlights the potential of tissue engineering but also emphasizes a commitment to advancing care for burn victims through innovative research and development (Ndlovu et al., 2021; Negut et al., 2020; Qin et al., 2022; Singh et al., 2023).

This study focuses on the development of an innovative wound dressing system incorporating TA into a CMC-gelatin matrix, which is further crosslinked with vanillin to enhance its therapeutic efficacy for burn wound healing. The designed scaffold aims to leverage the natural properties of its constituents to achieve optimal wound recovery outcomes. Tannic acid, known for its potent antioxidant and antimicrobial activities, plays a crucial role in preventing infection at the wound site and accelerating the healing process (Aelenei et al., 2009). The CMC component contributes to the scaffold's biocompatibility and biodegradability, ensuring that the dressing can be absorbed or easily removed from the wound without causing further damage or discomfort to the patient (Nabipour et al., 2024).

Furthermore, the gelatin in the matrix supports tissue regeneration by promoting cell attachment and proliferation, essential for effective wound repair (Ndlovu et al., 2021). The crosslinking with gelatin not only enhances the mechanical stability of the scaffold but also ensures its integrity over extended periods, reducing the need for frequent dressing changes and thereby improving patient compliance. The degradation of the scaffold post-application facilitates easy cleaning of the burn

wound area, further contributing to patient comfort and compliance with the treatment regimen.

This innovative wound dressing system, therefore, holds promise for significantly improving burn wound care by integrating the therapeutic benefits of tannic acid with the structural and functional advantages of a carboxymethylcellulose-gelatin matrix (Aelenei et al., 2009; Ke et al., 2023; Liu et al., 2024; Mndlovu et al., 2023; Ndlovu et al., 2021; Szabó et al., 2023).

## 3.2 Materials and Methodologies

### 3.2.1 Materials

The following materials: Carboxymethylcellulose (CMC), Tannic Acid (TA), Vanillin, Gelatin, Ethanol (99%), PBS, MTT reagent, Dimethylsulfoxide (DMSO), DMEM High Glucose, PenStrep antibiotics, and Lysozyme were purchased from Sigma–Aldrich (St. Louis, MO, USA). The NIH 3T3 cells were purchased from SEPARATIONS (Johannesburg, South Africa).

### 3.2.2 Synthesis of tannic acid loaded scaffold

The preparation involved dissolving tannic acid, gelatin, and CMC in distilled water under different conditions to ensure complete solubilization (Figure 3.1a). 2% TA was dissolved in deionised water at constant stirring (30 rpm for 30 minutes). Carboxymethyl cellulose (CMC) was dissolved in deionised water at constant stirring (30 rpm for 30 minutes) at a below 0 °C temperature. Gelatin was dissolved in deionised water at constant stirring (30 rpm for 30 minutes) at room temperature (25 °C). A separate solution of vanillin was prepared in ethanol (Figure 3.1b) to prepare a 1 % $^{v}/_{v}$ solution. The solutions of CMC, gelatin, and tannic acid were combined, and the reaction was allowed to continue for 30 minutes constant stirring (30 rpm at room temperature). Subsequently, vanillin solution was added gradually, and the reaction was permitted to proceed for an additional 30 minutes at 30 rpm constant stirring. This step ensures homogeneous mixing and proper crosslinking of the components (Figure 3.1c) (Ndlovu et al., 2021). The final hydrogel solution consists of the following proportions CMC (1 % $^{w}/_{v}$), gelatin (1 %$^{w}/_{v}$) and tannic acid (0,25 % $^{w}/_{v}$). The formed solution was transferred to a petri dish and frozen for 24 hours at -80 °C before being lyophilized for 24 hours (Figure 3.1d). Lyophilization was employed to remove excess water while preserving the porous structure of the scaffold, crucial for maintaining its mechanical integrity and enhancing its bioactivity (Ke et al., 2023).

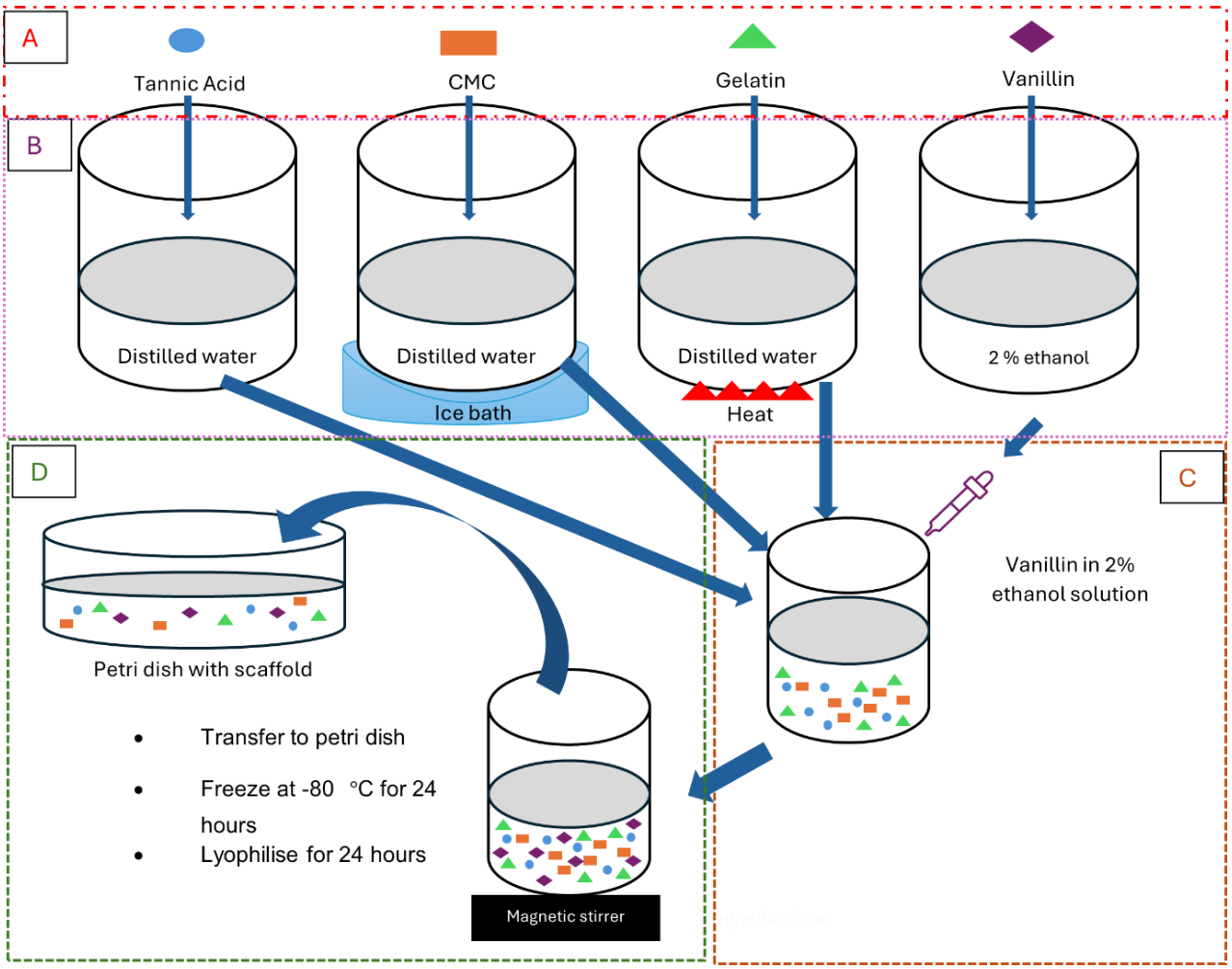


Figure 3-1 Synthesis of the scaffold from weighed amounts of CMC, gelatin, vanillin and tannic acid

### 3.2.3 Chemical analysis of the starting materials and scaffold using FTIR

The FTIR was used in the current study to evaluate the impact of chemical interaction between polymers (CMC and Gelatin), crosslinking with vanillin and to confirm that the drug was loaded to the scaffold. The following samples were prepared and analysed with FTIR, CMC powder, gelatin powder, vanillin, tannic acid, and the scaffold. The Perkin Elmer Spectrum 2000 FTIR Spectrometer was employed, applying a single- reflection diamond MIRTGS detector (Perkin Elmer Spectrum 100, Llantrisant, Wales, UK). Samples of the pristine polymers (CMC and gelatin), crosslinking agent (vanillin), bioactive (TA), and the synthesised scaffold were placed on a diamond crystal and each ran 64 times to minimise signal to noise ratio, in the wavelength range of 4000 – 650 $cm^{-1}$. The FTIR spectrum of all the samples will have the intensity in the y-axis and wavenumber on the x-axis. The FTIR spectrum will allow for analysis of the peaks for each starting material and also peak shift due to the chemical interaction taking place during formation of the scaffold.

### 3.2.4 Thermal analysis of the scaffold and starting materials using Differential Scanning Calorimetry (DSC)

Thermal analysis of the scaffold and starting materials using Differential Scanning Calorimetry (DSC) was employed to evaluate the thermal properties and stability of the materials. This technique helps identify thermal transitions like melting and crystallisation, assess the effects of crosslinking on mechanical strength, and evaluate interactions between scaffold components such as CMC, gelatin, tannic acid, and vanillin. Comparative DSC analyses were performed on the scaffold, starting polymers and bioactive (TA). Data was collected using the Mettler Toledo, DSC, STARe System, (Swchwerzenback, ZH, Switzerland system), heated from 20-400 °C, at a heating rate of 10 °C/minute under a constant flow of $N_2$ gas, resulting in thermograms of heat flow versus temperature. Accurately weighed samples (± 5 mg) were placed into a covered aluminium sample holder containing a central pin hole. DSC curves were plotted as heat flow against temperature. DSC analysis of all samples ensures the scaffold maintains structural integrity under physiological conditions, predicts stability during storage, and verifies functional performance in retaining moisture and providing thermal insulation, crucial for effective burn wound healing.

### 3.2.5 Morphological analysis of the scaffold via SEM

Morphological analysis of the scaffold via Scanning Electron Microscopy (SEM) was used to evaluate the surface characteristics and microstructural properties of the scaffold. SEM provides high-resolution images that reveal detailed topography, composition, and other essential properties at a micro to nanoscale level. The SEM (Phenom™ Scanning Electron Microscope, FEI Company, OR, USA) was employed at a 15 kV voltage for the evaluation of the surface morphology of the scaffolds. Before analysis, all formulations were mounted on aluminium stubs using a carbon adhesive tape and samples were coated with carbon/gold-palladium in a 2:1 ratio. SEM analysis helps in understanding the textural variations and pore structures, which are essential for enhancing cell adhesion, nutrient transport, and overall tissue regeneration. The detailed images obtained from SEM highlight the heterogeneous structures, rough surfaces, and layered compositions that support cell infiltration and mechanical interlocking with the wound bed, ensuring the scaffold acts as a conducive matrix for tissue formation and healing.

### 3.2.6 Mechanical analysis of the scaffold using Texture Analyser

Mechanical analysis of the scaffold using a Texture Analyser was conducted to assess its tensile strength and compression properties, which are critical for ensuring the scaffold's structural integrity and mechanical resilience. The mechanical characteristics of the scaffold, namely the tensile strength, were evaluated through compression and tension tests using the Texture Analyzer (TA.XT plus, Stable Microsystems, Surrey, UK). The scaffold was shaped into a rectangle, and the surface area was computed. These evaluations were conducted at a strain of

70 % at ambient temperature (25 °C). The maximum tensile strength was determined by identifying the peak stress point on the stress-strain curve (Mndlovu et al., 2023). This analysis is essential as the scaffold must endure the physical stresses encountered during the healing process and support cellular activities such as adhesion, proliferation, and differentiation. Compression and tension tests performed by the Texture Analyser provide detailed insights into the scaffold's mechanical characteristics, confirming its suitability for maintaining form and function in burn wound healing.

### 3.2.7 *In vitro* tannic acid release from the scaffold

Conducting the *in vitro* tannic acid release test from the scaffold is used for elucidating the release kinetics of the therapeutic agent, thereby assessing the scaffold's potential efficacy in wound healing applications. Lyophilized scaffold samples were weighed into the dialysis membrane (snake skin dialysis tubing) (3.5KDa) followed by addition of 3 mL phosphate buffered saline (PBS) (pH 7; 37 °C) and placed in the orbital shaker at 37 °C with mechanical stirring (30 rpm). The dialysis membrane containing the scaffold was immersed in a PBS bath volume of 100 mL. Aliquots (3 mL) were withdrawn at predetermined time points (0, 1, 2, 4, 8, 24, 48 and 96 hours) from the release medium and replaced with an equivalent volume of fresh PBS to maintain the sink condition. All measurements were conducted in triplicate. This analysis provides insights into the temporal release profile of tannic acid, ensuring that the scaffold can deliver a controlled and sustained release of the bioactive compound. Such controlled release is essential for maintaining effective antimicrobial and anti-inflammatory activity, supporting continuous wound healing, and reducing the frequency of dressing changes. Furthermore, the *in vitro* release test aids in optimizing the formulation to achieve the desired therapeutic outcomes.

### 3.2.8 *In vitro* biodegradation of the scaffold and starting materials utilising Zeta Potential

Conducting *in vitro* biodegradation analysis of the scaffold and starting materials using Zeta Potential was used to evaluate the stability and degradation characteristics of the scaffold. The scaffold and the starting materials (tannic acid, CMC and gelatin) were weighed into glass polytop vials. This was followed by pouring 5 mL of PBS containing 5 mg/mL lysozyme into the polytop vial. 5 mL of 0.1 M PBS at pH 7.4 was added to the samples and incubated further for 7 days at 37 °C with mechanical stirring (30 rpm). The degree of degradation was measured on days 0, 1, 2, 3, and 7. The zeta potential was determined at each time point using the Zeta sizer Nano-ZS machine (Malvern Instruments, Worcestershire, United Kingdom). All data was expressed as the mean value ± standard deviation of triplicate samples. Zeta Potential measurements offer insights into the surface charge and stability of the scaffold over time in a biological environment. This data can assist in understanding the scaffold's interactions with surrounding tissues and fluids, which can influence its effectiveness in wound healing applications. Tracking the Zeta

Potential during degradation helps assess the scaffold's ability to maintain structural integrity while gradually degrading to support tissue regeneration and healing.

### 3.2.9 *In vitro* cytotoxicity studies of the scaffold and starting materials utilising NIH 3T3 fibroblast cells

Conducting in vitro cytotoxicity studies of the scaffold and starting materials using NIH 3T3 fibroblast cells was critical for assessing the biocompatibility and safety of the materials. This evaluation determines whether the scaffold and its components are non-toxic to living cells, ensuring their suitability for medical applications. Utilising the NIH 3T3 cell line, a well-established model for fibroblast cells, allows for the assessment of potential cytotoxic effects over various concentrations and time points. The MTT assay measures cell viability based on metabolic activity, providing quantitative data on the cytotoxicity of the materials.

The fibroblast cell line NIH 3T3 cells were used to investigate the cytotoxicity of the scaffold using the (3- [4, 5-dimethylthiazol-2yl]-2, 5-diphenyl tetrazolium bromide (MTT) assay. NIH 3T3 cells were grown in Dubelcco's modified eagle medium (DMEM) (Sigma-Aldrich, MO, USA) supplemented with 10% FBS and 1% of the relevant antibiotics (Sigma-Aldrich, MO, USA) in an incubator at 5 % $CO_2$. Cells were seeded overnight followed by treatment of various concentrations of the scaffold in cells containing 100 µl RPMI medium ($1 \times 10^4$ cell $ml^{-1}$) in a 96 well and incubated for 24, 48 and 72 hours. Following incubation, the cell culture medium were aspirated and 10 µL of 2 mg/mL MTT solution was added to the cells, followed by a 2 to 4-hour incubation. To dissolve the formed formazan crystals, 200 µL of DMSO was added per well, and the cells were incubated further for 20 min in the dark at room temperature. Finally, absorbance was measured at 570 nm using a Victor X3 Plate Reader (Perkin Elmer MA, USA). All data was expressed as the mean value ± standard deviation of triplicate samples.

Confirming that the scaffold does not induce significant cytotoxic effects is essential for its application in wound healing, where maintaining healthy cell function and promoting tissue regeneration are primary objectives.

## 3.3 Results and Discussion

### 3.3.1 Synthesis of the hydrogel scaffold

The scaffold was prepared using the method depicted in Figure 3.1. Tannic acid (TA) was selected for its potent antimicrobial and antioxidant properties, which are crucial for preventing infections and promoting healing in burn wounds. A concentration of 0.125 % $^{w}/_{v}$ was chosen based on studies demonstrating its effectiveness in enhancing wound healing and minimizing cytotoxicity (Chen et al., 2019).

Gelatin was chosen for its biocompatibility and ability to promote cell adhesion and proliferation, essential for tissue regeneration. A concentration of 1% w/v was used, balancing mechanical stability and bioactivity (Ndlovu et al., 2021).

Carboxymethylcellulose (CMC) was included for its ability to form hydrogels that mimic the natural extracellular matrix, providing a moist environment conducive to wound healing. A concentration of 1% $^{w}/_{v}$ was used to ensure optimal viscosity and scaffold formation (Rahman et al., 2021).

Vanillin was employed as a crosslinking agent due to its ability to enhance the mechanical properties and stability of the hydrogel scaffold. It was dissolved in a 2% $^{w}/_{v}$ ethanol solution to facilitate its integration into the hydrogel matrix (Tomadoni et al., 2019).

Mixing the polymers thoroughly before adding the crosslinker ensures uniform distribution and interaction between the components, which is essential for forming a stable and functional scaffold. The use of vanillin as a crosslinker improves the scaffold's mechanical properties, making it more robust and durable, which is vital for its application as a wound dressing (Tomadoni et al., 2019).

Lyophilization preserves the scaffold's porous structure, which is important for promoting cell infiltration and nutrient transport (Ke et al., 2023). It also ensures the stability and longevity of the scaffold, making it suitable for long-term storage and use in wound healing applications. Additionally, lyophilization is required for testing the mechanical and chemical properties of the scaffold, ensuring that it meets the necessary criteria for effective wound healing applications (Zhang et al., 2024).

### 3.3.2 Characterization of the synthesised scaffold and starting materials utilising FTIR spectroscopy

Samples of CMC and gelatin polymers, along with the crosslinking agent vanillin and bioactive molecule TA, were analysed using FTIR to verify the existence of functional groups within these substances. The FTIR spectra of gelatin, CMC, vanillin, tannic acid, and the synthesised scaffold are displayed in Figure 3.2.

FTIR analysis of gelatin identified key absorption peaks corresponding to its protein structure. The Amide I band at approximately 1650 $cm^{-1}$, related to C=O stretching, signifies protein secondary structures (α-helices, β-sheets), consistent with literature (Barth, 2007; Muyonga et al., 2004). The Amide II band around 1550 $cm^{-1}$, due to N-H bending and C-N stretching, aligns

with findings by Kong and Yu, reflecting gelatin's conformation (Kong and Yu, 2007). Additionally, peaks in the 3070 - 2900 $cm^{-1}$ range indicate aliphatic chains, aligning with structural characteristics in literature (Valcarcel et al., 2021). These findings validate gelatin's molecular structure and FTIR spectroscopy's role in biomolecular analysis.

FTIR analysis of CMC revealed distinct absorption bands reflective of its molecular structure. A broad peak around 3400 $cm^{-1}$ indicates O-H stretching due to significant hydrogen bonding, a characteristic of polysaccharides. The appearance of bands around 1600 $cm^{-1}$ and precisely at 1620 $cm^{-1}$ highlights the presence of carboxylate (COO-) groups seen in Figure 3.3, indicative of cellulose carboxymethylation as seen in literature (Abu Hassan et al., 2024) . Specific bands at 1424 $cm^{-1}$ and 1327 $cm^{-1}$ are associated with $–CH_2$ scissoring and –OH bending vibrations, respectively, denoting the structural modifications due to carboxymethylation (Meas et al., 2024). The band at 1070 $cm^{-1}$, corresponding to $CH\text{-}O\text{-}CH_2$ stretching, aligns with the structural characteristics of CMC (Khalid et al., 2024).

In the FTIR analysis of vanillin, peaks in the 3441 - 3387 $cm^{-1}$ range indicate aromatic C-H stretching, suggestive of an aromatic ring structure, a trait of phenolic compounds like vanillin. This aligns with the structural analyses performed on bio-based materials incorporating vanillin (Zaidi et al., 2024). A pronounced peak near 1700 $cm^{-1}$ is associated with C=O stretching vibrations, characteristic of the aldehyde group depicted in Figure 3.3 of vanillin (Fatoni et al., 2018). The spectrum also shows peaks in the 1000 - 1300 $cm^{-1}$ region, consistent with stretching and bending vibrations of the aldehyde group, reflecting aldehydic compounds' spectral characteristics (Mohamed et al., 2024). A medium-intensity peak at 1026 $cm^{-1}$, indicative of $O–CH_3$ group stretching, signifies the methoxy functional group in vanillin, distinguishing it from other phenolic compounds. Lastly, absorption peaks between 1427 - 1589 $cm^{-1}$, attributed to C=C-C stretching vibrations, further confirm vanillin's aromatic nature (Sharma and Wazed Ali, 2024).

The spectral analysis of tannic acid reveals a distinct absorption band near 3400 $cm^{-1}$ depicted in Figure 3.2, indicative of phenolic groups which can be seen in Figure 3.3, and a notable band around 3300 $cm^{-1}$, signalling the prevalence of hydroxyl (O-H) groups, thus emphasizing the rich hydroxyl constitution inherent to tannins. A definitive peak observed near 1700 $cm^{-1}$ denotes C=O stretching, signifying the incorporation of carbonyl groups within the ester or carboxylic acid structures of tannic acid. The spectral domain between 1600-1400 $cm^{-1}$, showcasing aromatic C-C stretching vibrations, verifies the aromatic core of tannic acid. These FTIR spectral characteristics resonate with recent research findings, highlighting the complex composition of tannic acid, enriched with phenolic, hydroxyl, carbonyl, and aromatic elements (Wahyono et al., 2019; Xia et al., 2015).

The FTIR analysis of the scaffold, composed of TA, CMC, gelatin, and vanillin, provides significant insights into its molecular structure, with implications for its application in treating burn wounds. The broad absorption peak around 3600 $cm^{-1}$ indicates a prevalent hydrogen bonding, denoting a substantial presence of hydroxyl groups from TA and CMC. This structural characteristic suggests an enhanced mechanical stability and water retention capability of the scaffold, crucial for fostering a moist healing environment (Fahaduddin and Bal, 2024).

A pronounced peak at 1700 $cm^{-1}$ reveals the presence of carbonyl groups, hinting at the aldehyde functions from Vanillin and amide linkages within Gelatin. This peak's position might suggest crosslinking between Vanillin and Gelatin, proposing a chemically integrated structure of the scaffold, potentially influencing its degradation rate and mechanical properties, corroborating research that underlines the importance of scaffold composition in tissue engineering (Huiwen et al., 2024).

The presence of amide linkages in gelatin contributes to the scaffold's biocompatibility and bioactivity substantiated by an N-H bending vibration at 1540 $cm^{-1}$, fostering an environment conducive to cell adhesion and proliferation, aligning with findings on the critical role of amide linkages in scaffold design (Gharahgheshlagh et al., 2024).

The C-O stretching vibrations around 1168 $cm^{-1}$ are attributable to ester or ether bonds in CMC and phenolic ethers in TA, emphasize the scaffold's composite nature, enhancing its structural complexity and functional versatility (Maghsoudi et al., 2024).

The unique peak within the fingerprint region at 1500 $cm^{-1}$ underscores the scaffold's distinctive molecular architecture, essential for understanding its chemical interactions and biological compatibility, supporting its utility in tissue engineering, particularly for burn wound applications where healing facilitation, infection prevention, and tissue regeneration are paramount (Fahaduddin and Bal, 2024).

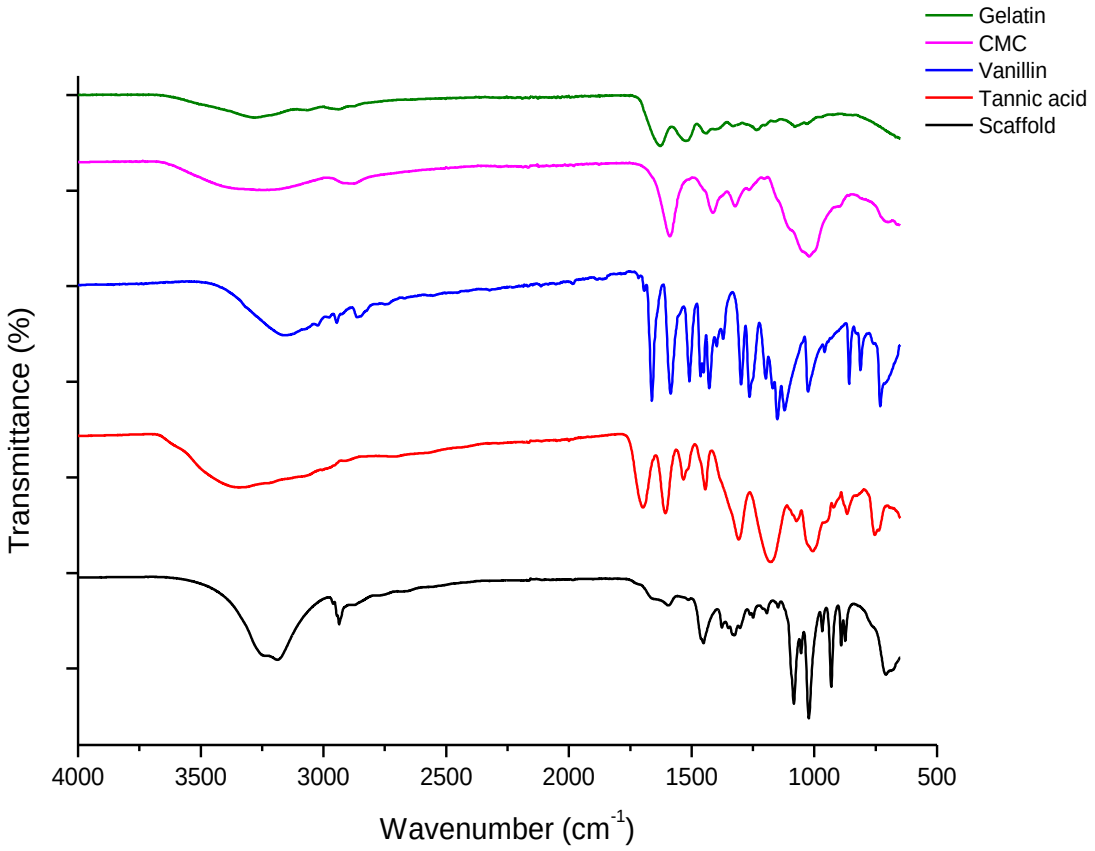


Figure 3-2 The FTIR spectra of gelatin, CMC, TA and the synthesised scaffold.

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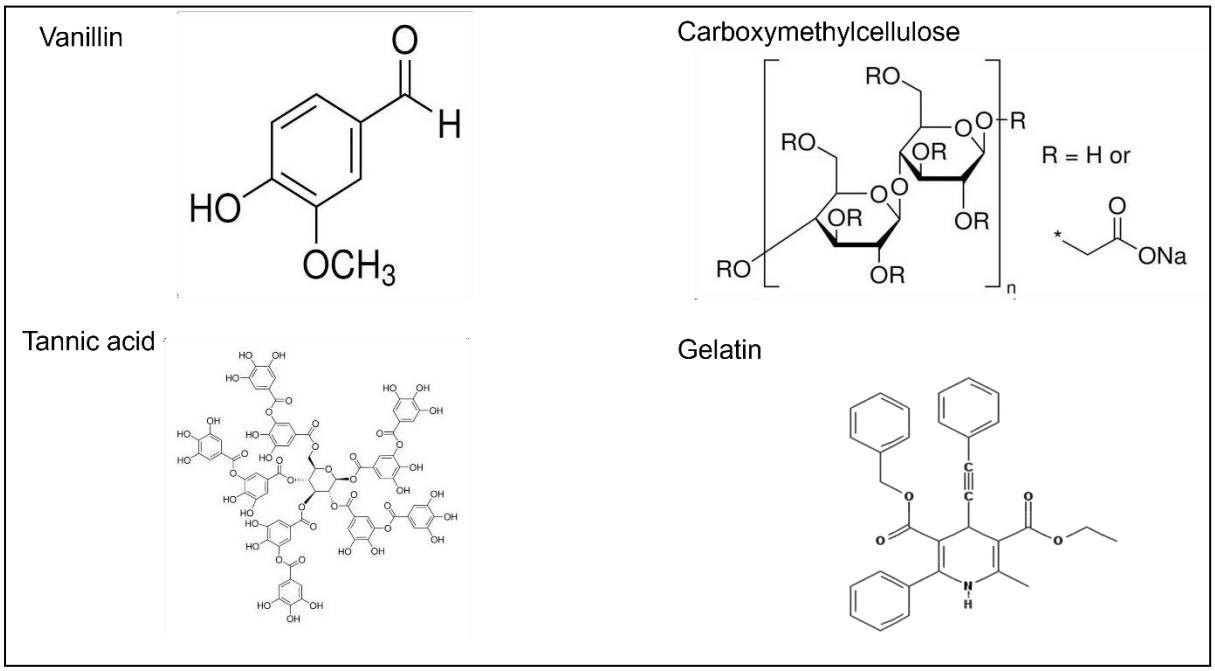


Figure 3-3 The chemical structures of the compounds used to synthesise the scaffold.

### 3.3.3 Scaffold morphology assessment via SEM analysis

Scanning Electron Microscopy (SEM) is a powerful imaging technique widely used across various scientific disciplines to obtain high-resolution images of sample surfaces. By focusing a beam of electrons onto the sample surface, SEM generates detailed images that reveal the topography, composition, and other critical properties of the material under examination (Pallares-Rusiñol et al., 2023). This method is particularly valuable in materials science, biology, and medical research due to its ability to provide insights into the microstructural characteristics of materials at a micro to nanoscale level (Giorgi Filho et al., 2024).

Various microstructural characteristics were observed in the SEM image of the scaffold as seen in Figure 3.4. The textural variations and pores, evident in Figure 3-4 A are indicative of structures that can enhance cell adhesion and nutrient transport, which are critical factors in tissue regeneration. These structures serve as a mesh network of polymeric layers for enhanced bioadhesion and cell generation. These porous structures facilitate the exchange of gases and nutrients, which are vital for cell survival and proliferation within the scaffold (He W et al., 2024).

Heterogeneous structures, rough and irregular surfaces, and layered compositions were also observed in Figure 3-4 B, which are known to support cell infiltration, adhesion, and mechanical interlocking with the wound bed (Cheng et al., 2024; Jalali et al., 2024). These features are essential for integrating the scaffold with the surrounding tissue, ensuring that the scaffold not only acts as a temporary matrix for tissue formation but also actively contributes to the healing process by promoting the infiltration and growth of new tissue cells.

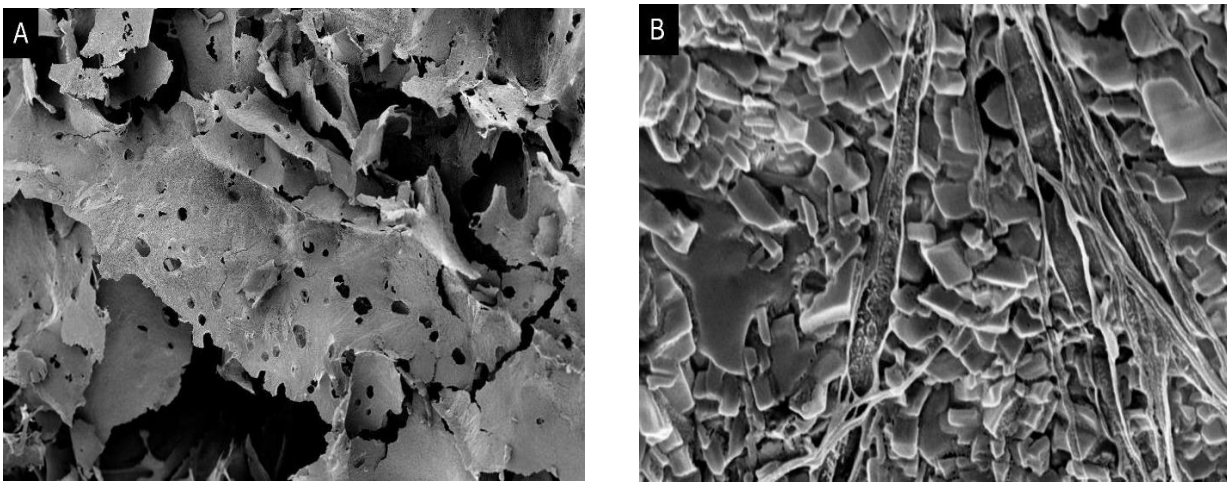


Figure 3-4 Scanning electron microscopy (SEM) images of synthesized scaffold after successful lyophilisation. The scale for image A is 40 nm and for image B is 400 nm.

### 3.3.4 Mechanical analysis of the synthesised scaffold using the Texture Analyser

In the context of acute burn wound healing, where the rapid restoration of skin integrity and function is critical, the mechanical and architectural properties of the scaffold are crucial factors. The scaffold's ability to support cell proliferation and differentiation, combined with its mechanical synergy with innate skin, highlights its potential efficacy in therapeutic applications (Qin et al., 2022; Spicer, 2020; Tseng et al., 2013; Zhao X et al., 2016). The mechanical properties of skin scaffolds, such as tensile and compression strength, are critical for their function during the healing process. The scaffold demonstrated a tensile strength of 0.04248 MPa and a compression strength of 0.063123 MPa. These values are vital as they ensure the scaffold can withstand physical stresses and prevent tearing during wound contraction. Additionally, the stiffness and mechanical features of the scaffold influence cellular behaviours, including adhesion, proliferation, and differentiation, which are essential for effective wound healing (Nokoorani et al., 2021).

Comparing these properties with previous studies on chitosan/gelatin-based scaffolds reveals a range of mechanical attributes. Tseng et al. reported an average tensile modulus of 0.0417 MPa in the wet state, while Pezeshki-Modarres et al. found tensile strengths between 0.072 and 0.097 MPa (Pezeshki-Modaress et al., 2014, Tseng et al., 2013). These comparisons indicate that the current scaffold’s mechanical properties are within a similar range, suggesting it has adequate mechanical robustness to maintain structural integrity in a dynamic healing environment (O’Brien et al., 2004).

The unique combination of CMC and gelatin in the scaffold plays a significant role in its performance. CMC contributes to high tensile strength, ensuring the scaffold remains intact and supports cellular activities, whereas gelatin adds flexibility and moisture retention, crucial for wound healing (Abu Hassan et al., 2024; Singh et al., 2023). This balance between strength and flexibility is essential for the scaffold to function effectively in the healing process.

The scaffold's design features a porous structure with an interconnected pore network, which is essential for nutrient diffusion and cell migration. This design mimics the natural extracellular matrix of the skin, supporting biological requirements while maintaining mechanical compliance. The scaffold’s ability to deform under mechanical loads without concentrating stress preserves its integrity and function in a wound setting, making it a viable option for wound healing applications (Zhang et al., 2024).

In the context of acute burn wound healing, the scaffold's mechanical and architectural properties are crucial for rapid restoration of skin integrity and function. Its support for cell proliferation and differentiation, combined with its mechanical compatibility with the skin, underscores its potential

effectiveness in therapeutic applications. These findings highlight the importance of understanding the mechanical properties of scaffolds to design more effective wound healing materials, ultimately improving patient outcomes (Qin et al., 2022; Spicer, 2020; Tseng et al., 2013; Zhao X et al., 2016).

### 3.3.5 Thermal analysis of the synthesised scaffold and starting materials using DSC

The DSC graph in Figure 3.5 for the scaffold composed of CMC, gelatin, and TA, crosslinked with vanillin, provides critical insights into the thermal behaviour of materials intended for acute burn wound healing applications. The exothermic peak of the scaffold as opposed to the pristine polymers, suggests an energy release, indicative of the formation of a stable crosslinked network, potentially improving the scaffold's mechanical strength and stability and essential for maintaining the integrity of a wound dressing in the dynamic environment of a burn wound (Maiti et al., 2024).

The endothermic transition in gelatin is of particular relevance, as gelatin's melting point corresponds to its sol-gel transition, which can influence the scaffold's ability to conform to the wound bed, providing a protective barrier and supporting moist wound healing, a vital aspect of burn wound treatment (Zhao X. et al., 2016). The inclusion of tannic acid, with its astringent and antimicrobial properties, is also beneficial in burn wound applications, where infection prevention is crucial (Westlake et al., 2023).

The crosslinking with vanillin not only enhances the thermal and mechanical properties of the scaffold but may also contribute to its biocompatibility and bioactivity, making it an excellent candidate for wound healing applications. Vanillin has been shown to improve the physicochemical properties and antibacterial activities of CMC-based composites, which is highly advantageous in the context of burn wound healing, where the risk of infection is high (Ke et al., 2023). The thermal stability imparted by the hydrogen bonded crosslinking suggests that the scaffold can maintain its structural integrity at body temperature and during storage, ensuring the reliability of the dressing during the critical healing process (Alavarse et al., 2022).

Given the scaffold's composite nature and thermal properties, it can be deduced that it has the potential to create an optimal wound healing environment through moisture retention, thermal insulation, and enhanced durability. These properties, alongside the bioactive components that could provide antimicrobial protection and aid in tissue regeneration, are crucial for the effective treatment of acute burn wounds.

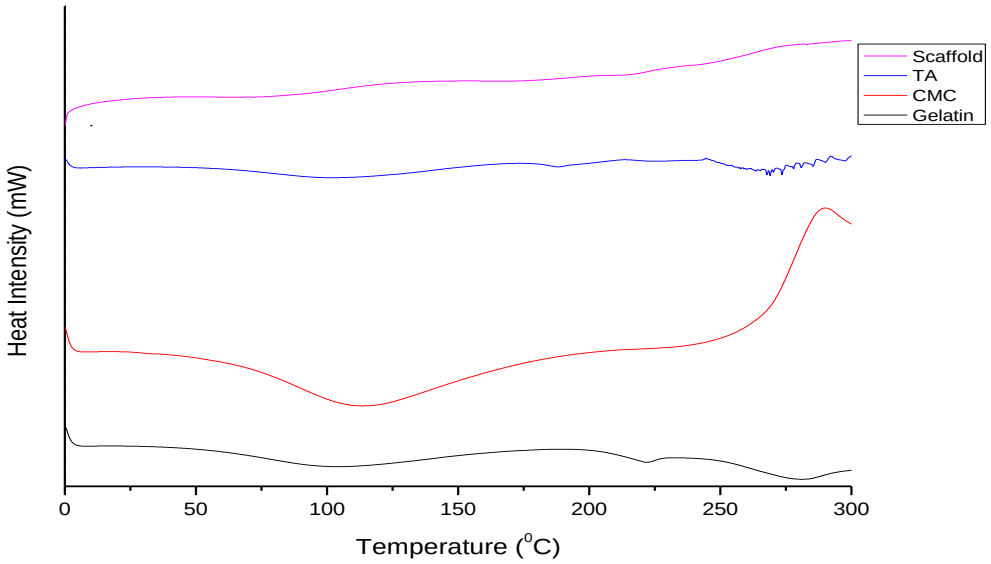


Figure 3-5 Differential Scanning Calorimetry thermograph for the synthesised scaffold, TA, CMC and gelatin.

### 3.3.6 Spectrophotometric analysis undertaken to determine the λ of TA and the calibration curve

In the study, the maximum absorption wavelength ($\lambda_{max}$) for tannic acid was determined to be 280.0 nm. Following the identification of $\lambda_{max}$, a calibration curve was constructed, as shown in Figure 3.6. This curve validates the presence of a linear relationship between the concentration of TA and its absorbance, evidenced by a correlation coefficient ($R^2$) of 0.9977. Therefore, it can be inferred that an increment in the concentration of Tannic Acid leads to a proportional increase in its absorbance, a conclusion supported by literature (Aelenei et al., 2009).

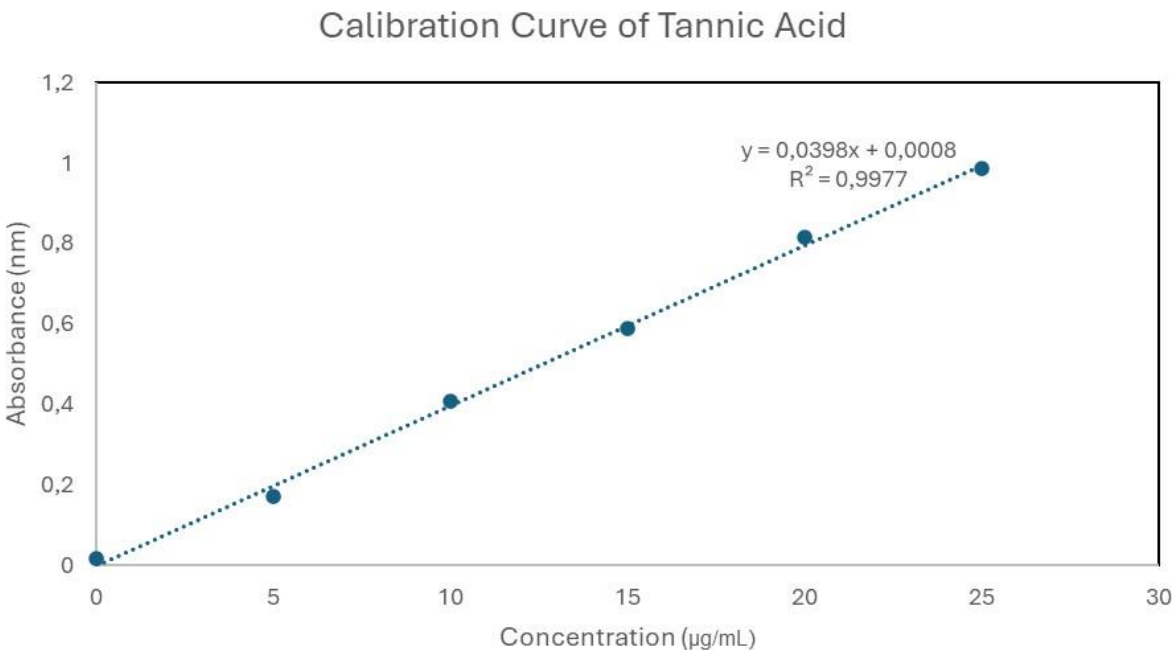


Figure 3-6 Calibration curve of tannic acid utilising maximum wavelength ($\lambda_{max}$) of 280 nm.

### 3.3.7 Tannic acid release studies

The release profile of tannic acid from the scaffold exhibited a biphasic pattern as depicted in Figure 3.7. The release pattern of tannic acid from the scaffold demonstrates a biphasic nature: a crucial initial burst release at $T_0$-$T_{10}$ followed by a sustained release phase at $T_{10}$- $T_{80}$. The early, rapid discharge of tannic acid ensures immediate antimicrobial activity at the wound site, addressing the urgent therapeutic needs of acute burn wounds. This burst release mechanism is aligned with the established need for high local concentrations of therapeutic agents in the early phase of wound healing to combat microbial colonization and effectively manage inflammation (Carvalho et al., 2024; Mekky et al., 2024).

This is supported by the work of Negut et al. who reported that scaffolds designed for burn wound applications should release high initial doses of incorporated drugs to capitalise on the window of opportunity for influencing the wound healing trajectory positively (Negut et al., 2020). The subsequent sustained release maintains therapeutic levels of tannic acid, contributing to the healing process over an extended period and emphasizing the scaffold's multifunctional utility in wound care.

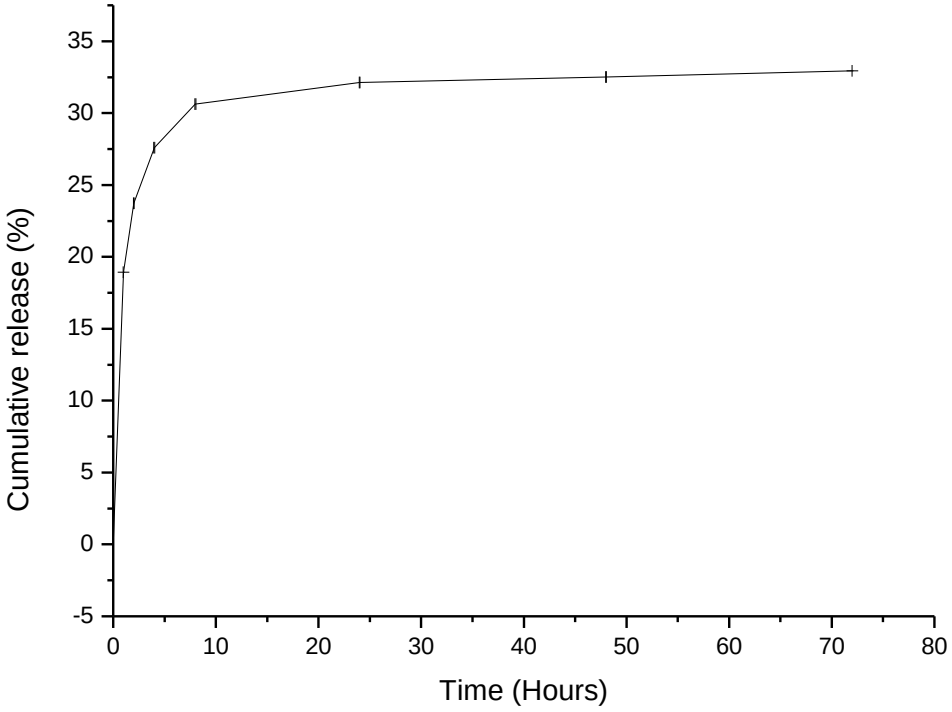


Figure 3-7 Tannic acid release from the scaffold at stimulated conditions of normal physiological pH (pH 7.4) Each point depicts mean ± SD (n=3).

### 3.3.8 Investigation of the biodegradation of the synthesised scaffold and starting materials utilising Zeta Potential

**Rationale for using Zeta Potential as a measure of degradation**

Zeta potential can be a useful indicator of surface stability and potential interactions within biological systems, but it is not typically a direct measure of bulk degradation, especially for complex structures like scaffolds intended for biomedical applications. The usual method of assessing scaffold degradation (such as monitoring weight loss over time or changes in mechanical properties) did not work due to the scaffold dissolving in water.

The composition of scaffolds significantly impacts their degradation rate, as evidenced by research on biofunctionalized 3D-printed scaffolds made from a gelatin/sodium alginate/chitosan tri-polymer complex. These scaffolds displayed rapid degradation when the gelatin content was high, pointing towards the intricate relationship between material composition and scaffold longevity (Singh et al., 2023). This highlights the potential role of zeta potential in measuring scaffold

degradation, offering a window into the electrostatic interactions of the particle charge distribution, in relation to the stability of inflamed pH parameters.

**Degradation results of the synthesised scaffold and starting materials**

The degradation behaviour of a scaffold is crucial due to the need for rapid healing, prevention of infection, and promotion of tissue regeneration. The zeta potential observed for the scaffold and its starting material has been depicted in Figure 3.8.

Gelatin, with its modest decrease in zeta potential as depicted on the graph, suggests a gradual degradation which is beneficial in a burn wound scenario. This is due to gelatin-based scaffolds providing a temporary extracellular matrix for cell migration and new tissue formation while slowly degrading to make space for new tissue (Ndlovu et al., 2021). The controlled degradation profile indicated by the literature and the results in Figure 3.8 aligns with the requirements for acute burn wound healing, where a balance between scaffold stability and degradation is critical (Qin et al., 2022)

The degradation of CMC within scaffolds represents a controlled and significant aspect of scaffold performance for biomedical applications, as highlighted by a series of studies. Saxena et al. highlighted the integration of CMC with other components in nano-biocomposite scaffolds, emphasizing the utility of controlled degradation in applications such as tissue engineering (Saxena et al., 2021). Reeves et al. demonstrated the synthesis of CMC- methacrylate hydrogel cell scaffolds using photopolymerization crosslinking chemistry, which allows for the stabilisation of CMC hydrogels with controllable degradation rate (Reeves et al., 2010). This suggests that the crosslinking chemistry employed significantly influences CMC's stability and degradation within the scaffold. Further exploring the utility of CMC, Hasan et al. tuned the degradation rate of nano-biocomposite scaffolds to promote angiogenesis and vascularization, indicating the versatility of CMC's degradation to meet specific biomedical needs(Hasan et al., 2018). The collective findings from these studies highlight the importance of understanding the degradation of CMC, which is influenced by environmental conditions and the scaffold’s composite materials.

The stability of tannic acid observed in the graph resonates with the study by Natarajan et al., where tannic acid cross-linked collagen scaffolds displayed controlled degradation suitable for wound healing applications (Natarajan et al., 2013). The relative stability of tannic acid in the scaffold suggests its role in providing structural integrity over the studied time frame.

The overall gradual decrease in zeta potential, indicative of scaffold degradation, suggests that the scaffold may maintain its structural integrity long enough to protect the burn wound during the initial critical healing phases. The scaffold's stability in the face of enzymatic and hydrolytic degradation is critical for creating a moist wound environment that can facilitate cell migration, angiogenesis, and extracellular matrix formation, all of which are crucial for healing burns.

Considering the application of acute burn wounds, the scaffold composed of CMC, gelatin, TA and vanillin present a promising composition. It offers a balance between maintaining a barrier to infection and allowing cellular activities necessary for wound repair. The slow and controlled degradation of gelatin and the stabilizing effects of tannic acid could potentially support a conducive healing environment.

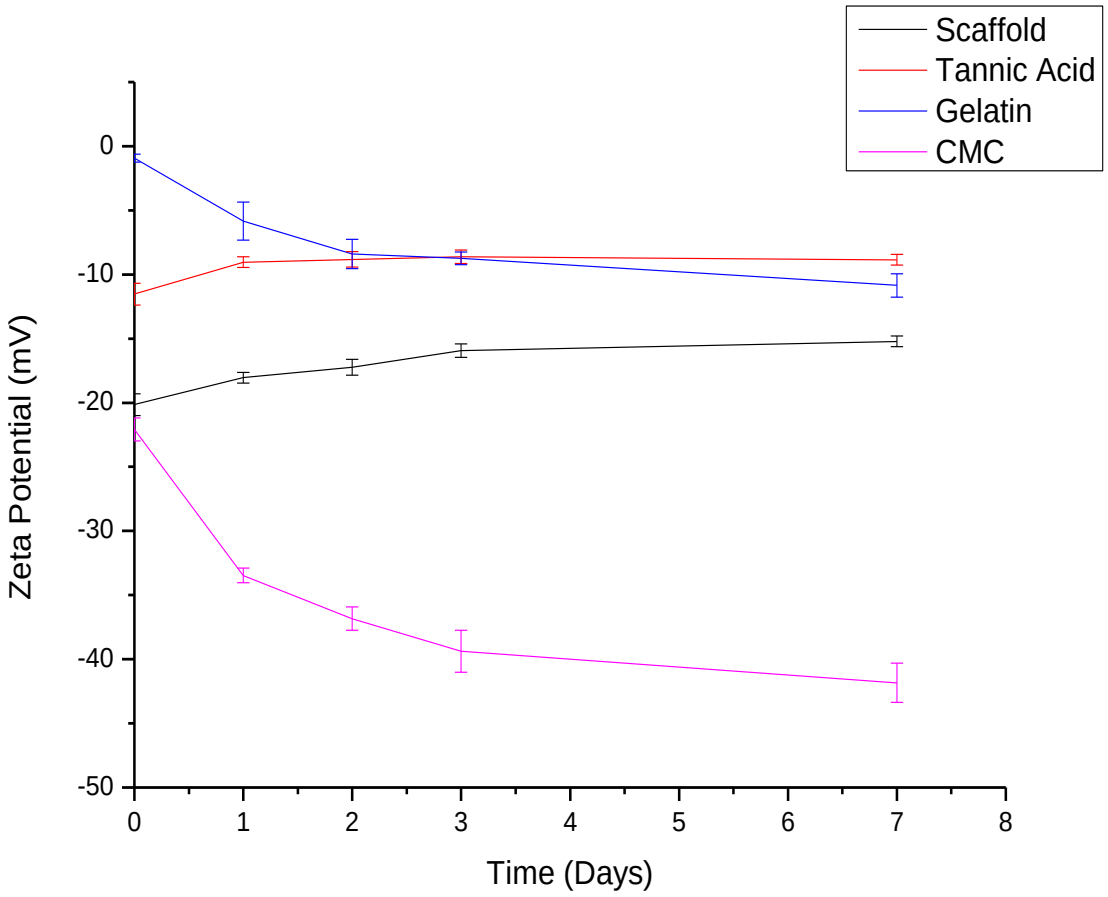


Figure 3-8 The observation of the change in Zeta Potential of the synthesised scaffold and its starting materials over a period of 7 days. Each point depicts mean ± SD (n=3).

### 3.3.9 Evaluation of cytotoxicity of synthesised scaffold and starting materials utilising NIH 3T3 fibroblast cells

In the examination of cell viability in response to scaffold materials, varying trends are evident across different concentrations as seen in Figure 3.9. The cytotoxicity of the scaffold-treated cells were categorised as following by Dahl et al in Table 3-1:

**Table 3-1 Tabulation of rankings of cell viability** (Dahl et al., 2006)**.**

| **Non-cytotoxic** | **Slightly cytotoxic** | **Moderately cytotoxic** | **Severely cytotoxic** |
|---|---|---|---|
| >90 % cell viability, | 60 % – 90 % cell viability | 30 % – 59 % cell viability | <30 % cell viability |

TA's trend remains consistent; cell viability generally rises over time across all examined concentrations. This observation may correlate with the reported antioxidative properties of tannic acid, which can confer cytoprotection against oxidative stress-induced cellular damage. The observable decrease in cell viability at the 48-hour interval at the highest concentration of tannic acid indicates a potential cytotoxic threshold (Ma et al., 2020).

The cell response to CMC appears to vary, with the greatest concentration resulting in a reduction in cell viability that does not resolve by 72 hours. This may be attributable to the altered physicochemical environment induced by higher CMC concentrations which could negatively impact cell viability. Gelatin, a staple in cell culture due to its biocompatibility and facilitation of cell adhesion, consistently maintains high cell viability across all timeframes and concentrations. However, a slight reduction in cell viability at the highest concentration at the 72-hour time point may suggest a limitation in nutrient diffusion because of the gelatin matrix's density.

At subtoxic levels of the synthesised scaffold (0.625 µg/mL and 1.25 µg/mL), an increase in cell viability is noted over the course of the assay, suggestive of an absence of cytotoxicity and a potential promotion of cell proliferation. The maximum concentration tested (2.5 µg/mL), there is an initial reduction in viability at the 48-hour mark, followed by a recovery by 72 hours. This pattern may reflect a delayed adaptive response of the NIH 3T3 cells to higher concentrations of the scaffold materials.

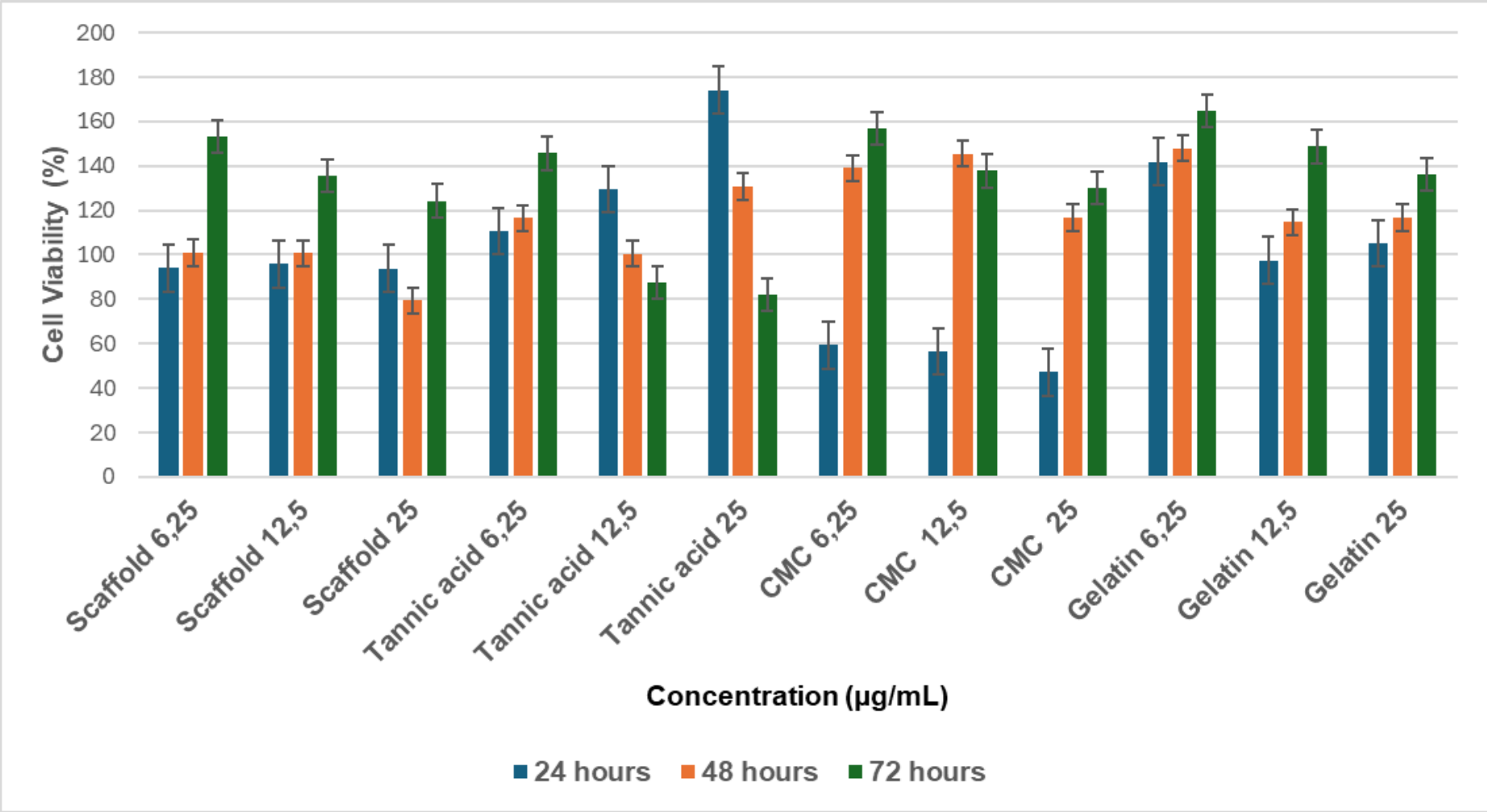


Figure 3-9 MTT assay for evaluation of the effect of the synthesized scaffold, starting materials (CMC, TA and gelatin) on percentage cell viability of NIH 3T3 mouse fibroblast cells. The cells treated with various concentrations of the materials and incubated for 24, 48 and 72 hours. Each percentage cell viability point represents average ± SD (n=3).

# Chapter 4 CONCLUSIONS AND RECOMMENDATIONS

## 4.1 Study Limitations

This study's primary limitations arise from the *in vitro* nature of the experiments, which may not fully replicate the complex biological environment of actual burn wounds. The promising properties of the hydrogel system, such as biocompatibility, biodegradability, and effective drug release profiles, require validation through comprehensive in vivo studies and clinical trials to confirm their efficacy and safety in real-world applications. Additionally, the scalability and cost-effectiveness of producing the hydrogel system at an industrial level were not addressed, which could pose challenges for widespread clinical use. The study focused exclusively on a single type of hydrogel composition, leaving room for the exploration of other formulations that might offer improved or complementary therapeutic benefits. Finally, the long-term stability and storage conditions of the hydrogel were not extensively investigated, and these are critical factors for its practical application in burn wound care.

## 4.2 Conclusion

This study has demonstrated the development and characterization of a novel tannic acid-loaded hydrogel system, tailored for acute burn wound management. FTIR characterization confirmed the integration of tannic acid, CMC, gelatin and vanillin into the scaffold matrix system. DSC further confirmed the crosslinking and chemical reactions formed during the synthesis of the scaffold. The scaffold was revealed to possess several key attributes essential for its application in wound healing, including biocompatibility, favourable mechanical properties, and effective tannic acid release profiles. The *in-vitro* studies on biodegradation and cytotoxicity further demonstrated the safety of the scaffold, while also highlighting its potential in promoting cellular proliferation and wound healing. These findings collectively position the hydrogel system as a leap forward in the treatment of acute burn wounds, offering an alternative to the current paradigms in wound dressing technologies.

## 4.3 Recommendations

Future research should focus on validating the efficacy and safety of the hydrogel system through comprehensive in vivo studies and clinical trials. Advanced characterization is needed to explore the scaffold’s long-term stability, in vivo biodegradation, and interactions with various burn wound types. Exploring a wider array of bioactive agents, such as antimicrobial peptides or growth factors, is recommended to enhance the scaffold’s healing capabilities. Personalizing and customizing the hydrogel properties to align with individual patient needs or specific wound

types could offer more precise and effective treatment options.

Assessing the economic and environmental impact of the hydrogel system is crucial for ensuring its viability and sustainability. Integrating this system into existing burn care protocols and exploring its synergy with other treatments is part of a holistic care strategy.

Research into the hydrogel's thermosensitivity and pH sensitivity presents an opportunity to develop smart, responsive wound care solutions that adapt to the wound's changing microenvironment, potentially enhancing the delivery and release of bioactive components, improving healing efficiency, and mitigating infection risks.

**REFERENCES**


Abu Hassan NA, Fazlina F, Teng LP, et al. (2024) Characterization of hemicellulose-carboxymethyl cellulose blend films: Enhancement of the physicochemical, thermal, and mechanical properties. *Journal of Applied Polymer Science*. John Wiley & Sons, Ltd: e55361.

Aderibigbe BA and Buyana B (2018) Alginate in Wound Dressings. *Pharmaceutics* 10(2). Pharmaceutics.

Aelenei N, Popa MI, Novac O, et al. (2009) Tannic acid incorporation in chitosan-based microparticles and in vitro controlled release. *Journal of Materials Science: Materials in Medicine* 20(5): 1095–1102.

Akhlaq M, Naz S and Uroos M (2024) Facile fabrication of high capacity citric acid cross- linked chitosan and carboxymethyl cellulose-based hydrogel for fast kinetics removal of Cu(II). *Adsorption* 30(3). Springer: 363–375.

Alavarse AC, Frachini ECG, da Silva RLCG, et al. (2022) Crosslinkers for polysaccharides and proteins: Synthesis conditions, mechanisms, and crosslinking efficiency, a review. *International Journal of Biological Macromolecules* 202. Elsevier: 558–596.

Albertyn R, Bickler SW and Rode H (2006) Paediatric burn injuries in Sub Saharan Africa— an overview. *Burns* 32(5). Elsevier: 605–612.

Allorto N, Rencken C and Bishop DG (2023) Burn injuries in KwaZulu-Natal Province, South Africa: Quantifying the healthcare burden. *South African Medical Journal* 113(10). South African Medical Association: 1427–1431.

Allorto NL (2020) Primary management of burn injuries: Balancing best practice with pragmatism. *South African family practice : official journal of the South African Academy of Family Practice/Primary Care* 62(1). S Afr Fam Pract (2004): 1–4.

Allorto NL, Oosthuizen G V., Clarke DL, et al. (2009) The spectrum and outcome of burns at a

regional hospital in South Africa. *Burns* 35(7): 1004–1008.

Anisuzzaman DM, Wang C, Rostami B, et al. (2022) Image-Based Artificial Intelligence in Wound Assessment: A Systematic Review. *https://home.liebertpub.com/wound* 11(12). Mary Ann Liebert, Inc., publishers 140 Huguenot Street, 3rd Floor New Rochelle, NY 10801 USA : 687–709.

Arabpour Z, Abedi F, Salehi M, et al. (2024) Hydrogel-Based Skin Regeneration. *International Journal of Molecular Sciences* 25(4). Multidisciplinary Digital Publishing Institute (MDPI): 1982.

Atiyeh BS, Costagliola M, Hayek SN, et al. (2007) Effect of silver on burn wound infection control and healing: review of the literature. *Burns : journal of the International Society for Burn Injuries* 33(2). Burns: 139–148.

Barakat-Johnson M, Jones A, Burger M, et al. (2022) Reshaping wound care: Evaluation of an artificial intelligence app to improve wound assessment and management amid the COVID-19 pandemic. *International Wound Journal* 19(6). John Wiley & Sons, Ltd: 1561–1577.

Baranovska A and Lutsenko T (2024) PROSPECTS FOR THE USE OF 3D BIOPRINTING TECHNOLOGIES FOR REGENERATIVE THERAPY OF SKIN DAMAGE. *Biomedical Engineering and Technology Issue* 13(1).

Barth A (2007) Infrared spectroscopy of proteins. *Biochimica et biophysica acta* 1767(9). Biochim Biophys Acta: 1073–1101.

Bauer JA, Blocklin AR, Sysel AM, et al. (2024) Safety and efficacy of the Plasma Directed Electron Beam (PDEB$^{TM}$)-implications for enhanced wound healing treatment in military operational medicine and beyond. DOI: 10.1101/2024.02.21.24302399.

Bianchi E, Ruggeri M, Vigani B, et al. (2024) Gas foamed scaffolds as smart 3D structures in skin tissue engineering. *Journal of Drug Delivery Science and Technology* 95. Elsevier: 105541.

Biswas A, Abdullah ASM, Deave T, et al. (2020) Exploration of gaps and challenges in managing

burn injury at district and sub-district government health care facilities in Bangladesh. *Burns Open* 4(1). Elsevier: 16–21.

Biswas A, Bayer IS, Biris AS, et al. (2012) Advances in top–down and bottom–up surface nanofabrication: Techniques, applications & future prospects. *Advances in Colloid and Interface Science* 170(1–2). Elsevier: 2–27

.

Burrows, S, Bowman B, Matzopoulos, et al. (2002). A profile of fatal injuries in South Africa 2000.

Caló E and Khutoryanskiy V V. (2015) Biomedical applications of hydrogels: A review of patents and commercial products. *European Polymer Journal* 65. Pergamon: 252–267.

Cao J, Yuan P, Wu B, et al. (2023) Advances in the Research and Application of Smart-Responsive Hydrogels in Disease Treatment. *Gels 2023, Vol. 9, Page 662* 9(8). Multidisciplinary Digital Publishing Institute: 662.

Carvalho LAN de, Arunachalam K, Pereira N da V, et al. (2024) The genus Cochlospermum Kunth: a comprehensive review of key species with a focus on botanical, pharmacological, and chemical atributes. *DELOS: Desarrollo Local Sostenible* 17(53): e1352–e1352.

Chaganti P, Gordon I, Chao JH, et al. (2019) A systematic review of foam dressings for partial thickness burns. *The American Journal of Emergency Medicine* 37(6). W.B. Saunders: 1184–1190.

Chen Y, Tian L, Yang F, et al. (2019) Tannic Acid Accelerates Cutaneous Wound Healing in Rats Via Activation of the ERK 1/2 Signaling Pathways. Advances in Wound Care 8(7): 341–354

Cheng Y, Li X, Gu P, et al. (2024) Hierarchical Scaffold with Directional Microchannels Promotes Cell Ingrowth for Bone Regeneration. *Advanced Healthcare Materials*. John Wiley & Sons, Ltd: 2303600.

.

Choi JY, Joo YJ, Kang RJ, et al. (2024) Effect of Spray-Type Alginate Hydrogel Dressing on Burn Wounds. *Gels 2024, Vol. 10, Page 152* 10(2). Multidisciplinary Digital Publishing Institute: 152.

Cirillo MD, Mirdell R, Sjöberg F, et al. (2019) Time-Independent Prediction of Burn Depth Using Deep Convolutional Neural Networks. *Journal of Burn Care & Research* 40(6). Oxford Academic: 857–863.

Cubison TCS, Pape SA and Parkhouse N (2006) Evidence for the link between healing time and the development of hypertrophic scars (HTS) in paediatric burns due to scald injury. *Burns* 32(8): 992–999.

Cutting KF (2008) Impact of adhesive surgical tape and wound dressings on the skin, with reference to skin stripping. *Journal of wound care* 17(4). J Wound Care.

Daffue B, Moolman D, Ferreira S, et al. (2018) The causes of burn wounds among adult patients treated at Pelonomi Tertiary Hospital, Bloemfontein. *South African Journal of Surgery* 56(3): 31–36.

Dahl JE, Frangou-Polyzois MJ and Polyzois GL (2006) In vitro biocompatibility of denture relining materials. *Gerodontology* 23(1). John Wiley & Sons, Ltd: 17–22.

Davies P, McCarty S and Hamberg K (2017) Silver-containing foam dressings with Safetac: a review of the scientific and clinical data. *Journal of wound care* 26(Sup6a). J Wound Care: S1–S32.

Deng L, Wang C, Han Z, et al. (2024) A biological antimicrobial agent functionalized bacterial cellulose-based wound dressing. *Cellulose*. Springer Science and Business Media B.V.: 1–13.

Derwin R, Declan Patton |, Avsar P, et al. (2021) The impact of topical agents and dressing on pH and temperature on wound healing: A systematic, narrative review. *International wound journal*, *19*(6), 1397–1408. DOI: 10.1111/iwj.13733.

Derwin R, Patton D, Avsar P, et al. (2022) The impact of topical agents and dressing on pH and temperature on wound healing: A systematic, narrative review. *International wound journal*

19(6). Int Wound J: 1397–1408.

Dubey SK, Parab S, Alexander A, et al. (2022) Cold atmospheric plasma therapy in wound healing. *Process Biochemistry* 112. Elsevier: 112–123.

Fahaduddin and Bal T (2024) Fabrication and evaluation of Dillenia indica-carrageenan blend hybrid superporous hydrogel reinforced with green synthesized MgO nanoparticles as an effective wound dressing material. *International Journal of Biological Macromolecules* 265. Elsevier: 130835.

Fatoni A, Hariani PL, Hermansyah, et al. (2018) Synthesis and Characterization of Chitosan Linked by Methylene Bridge and Schiff Base of 4,4-Diaminodiphenyl Ether-Vanillin. *Indonesian Journal of Chemistry* 18(1). Gadjah Mada University: 92–101.

Fiandeiro D, Govindsamy J and Maharaj RC (2015) Prehospital cooling of severe burns: Experience of the emergency department at edendale hospital, KwaZulu-Natal, South Africa. *South African Medical Journal* 105(6): 457–460.

Gethin G (2007) The significance of surface pH in chronic wounds. *Wounds UK* 3(52).

Gharahgheshlagh SN, Ghadimi T, Latifi N, et al. (2024) Fabricating modified cotton wound dressing via exopolysaccharide-incorporated marine collagen nanofibers. *Materials Today Communications*. Elsevier: 108706.

Giorgi Filho RC, Martinez Junior AM, Calmon MF, et al. (2024) Physicochemical Decellularization of Bovine Pericardium: Effects on DNA Elimination, Extracellular Matrix Preservation, and Biocompatibility. *Waste and Biomass Valorization 2024*. Springer: 1–13.

Gong J, Hou L, Ching YC, et al. (2024) A review of recent advances of cellulose-based intelligent-responsive hydrogels as vehicles for controllable drug delivery system. *International Journal of Biological Macromolecules* 264. Elsevier: 130525.

Goodwin NS, Spinks A and Wasiak J (2015) The efficacy of hydrogel dressings as a first aid measure for burn wound management in the pre-hospital setting: a systematic review of the

literature. *International Wound Journal*. Epub ahead of print 2015. DOI: 10.1111/iwj.12469.

Guo B, Liang Y and Dong R (2023) Physical dynamic double-network hydrogels as dressings to facilitate tissue repair. *Nature protocols* 18(11). Nat Protoc: 3322–3354.

Gustavo Lima W, Cesar Moreira Brito J, Moreira Verly R, et al. (2024) Jelleine, a Family of Peptides Isolated from the Royal Jelly of theHoney Bees (Apis mellifera), as a Promising Prototype for NewMedicines: A Narrative Review. Epub ahead of print 2024. DOI: 10.3390/toxins16010024.

Hasan A, Waibhaw G, Saxena V, et al. (2018) Nano-biocomposite scaffolds of chitosan, carboxymethyl cellulose and silver nanoparticle modified cellulose nanowhiskers for bone tissue engineering applications. *International journal of biological macromolecules* 111. Int J Biol Macromol: 923–934.

Hautmann A, Hedtke T, Sislema-Muñoz S, et al. (2024) Design of a composite wound dressing: Combining an electrospun fleece with a free-standing multilayer film. *Next Materials* 2. Elsevier: 100060.

He H, Yang F, Zhang S, et al. (2024) Bone morphogenetic protein-2 loaded triple helix recombinant collagen-based hydrogels for enhancing bone defect healing. *Biomedical materials (Bristol, England)*. Biomed Mater. Epub ahead of print 22 March 2024. DOI: 10.1088/1748-605X/AD3701.

He W, Li Q, Zhang M, et al. (2024) Leaf-Mimicking Superstructured Porous Membrane with Multiple Bioactive Pro-Healing Functions for Effective Guided Bone Regeneration. *Advanced Functional Materials*. John Wiley & Sons, Ltd: 2316679.

Hu J, Chen Z, Li J, et al. (2024) A novel low-cytotoxicity fluorescent probe applied to monitor pH in vivo and in vitro. *Tetrahedron* 155. Pergamon: 133917.

Huang S, Dang J, Sheckter CC, et al. (2021) A systematic review of machine learning and automation in burn wound evaluation: A promising but developing frontier. *Burns* 47(8). Elsevier: 1691–1704.

Hughes A, Almeland SK, Leclerc T, et al. (2021) Recommendations for burns care in mass casualty incidents: WHO Emergency Medical Teams Technical Working Group on Burns (WHO TWGB) 2017-2020. *Burns* 47(2). Elsevier: 349–370.

Huiwen W, Shuai L, Jia X, et al. (2024) 3D-printed nanohydroxyapatite/methylacrylylated silk fibroin scaffold for repairing rat skull defects. *Journal of biological engineering* 18(1). J Biol Eng: 22.

Ibrahim HM, Taha GM, El-Alfy EA, et al. (2022) Enhancing antibacterial action of gauze by adding gelatin nanoparticles loaded with spectinomycin and chloramphenicol. *Cellulose* 29(10). Springer Science and Business Media B.V.: 5677–5688.

Jalali Sara, Kruppke I, Enghardt Stefan, et al. (2024) Silica Nanofibers with Enhanced Wettability and Mechanical Strength for Bone Tissue Engineering: Electrospinning without Polymer Carrier and Subsequent Heat Treatment. *Macromolecular Materials and Engineering* 309(1). John Wiley & Sons, Ltd: 2300169.

Jia X, Dou Z, Zhang Y, et al. (2023) Smart Responsive and Controlled-Release Hydrogels for Chronic Wound Treatment. *Pharmaceutics 2023, Vol. 15, Page 2735* 15(12). Multidisciplinary Digital Publishing Institute: 2735.

Jin S, Newton MAA, Cheng H, et al. (2023) Progress of Hydrogel Dressings with Wound Monitoring and Treatment Functions. *Gels (Basel, Switzerland)* 9(9). Gels.

Jones EM, Cochrane CA and Percival SL (2015) The Effect of pH on the Extracellular Matrix and Biofilms. *Advances in wound care* 4(7). Adv Wound Care (New Rochelle): 431– 439.

Jones I, Currie L and Martin R (2002) A guide to biological skin substitutes. *British Journal of Plastic Surgery* 55(3). Churchill Livingstone: 185–193.

Jonkheijm A, Hendrijntje Zuidgeest JJ, Van Dijk M, et al. (2013) Childhood unintentional injuries: supervision and first aid provided. *African journal of paediatric surgery : AJPS* 10(4). Afr J Paediatr Surg: 339–344.

Kaal J, Nierop KGJ and Verstraten JM (2005) Retention of tannic acid and condensed tannin by Fe-oxide-coated quartz sand. *Journal of Colloid and Interface Science* 287(1). Academic Press: 72–79.

Kaczmarek B (2020) Tannic Acid with Antiviral and Antibacterial Activity as A Promising Component of Biomaterials—A Minireview. *Materials 2020, Vol. 13, Page 3224* 13(14). Multidisciplinary Digital Publishing Institute: 3224.

Kalmer RR, Karimi A, Dogaheh SG, et al. (2024) Evaluation of the effect of polymer composition on the rheological, mechanical properties and drug-released behavior of novel Eudragit L100-55/gelatin gastro-resistance uncoated capsule. *Polymer Bulletin* 81(11). Springer Science and Business Media Deutschland GmbH: 10369–10392.

Kamenova, K., Prancheva, A., Stoyanova, S., Radeva, L. et al. (2024). Functional Hydrogels for Delivery of the Proteolytic Enzyme Serratiopeptidase. *Gels (Basel, Switzerland)*, *10*(3), 156. https://doi.org/10.3390/gels10030156

Kamoun EA, Kenawy ERS and Chen X (2017) A review on polymeric hydrogel membranes for wound dressing applications: PVA-based hydrogel dressings. *Journal of advanced research* 8(3). J Adv Res: 217–233.

Kanwar A (2018) Skin barrier function. *Indian Journal of Medical Research* 147(1): 117.

Karami P, Zandi M and Ganjloo A (2022) Evaluation of physicochemical, mechanical, and antimicrobial properties of gelatin-sodium alginate-yarrow (Achillea millefolium L.) essential oil film. *Journal of Food Processing and Preservation* 46(7). John Wiley & Sons, Ltd: e16632.

Ke Q, Wang H, Huang X, et al. (2023) Direct addition of vanillin improved the physicochemical properties and antibacterial activities of gelatin/sodium carboxymethyl cellulose composite film. *Industrial Crops and Products* 206. Elsevier: 117653.

Khalid SA, Ghanem AF, Abd-El-Malek A, et al. (2024) Free-standing carboxymethyl cellulose film incorporating nanoformulated pomegranate extract for meat packaging. *Carbohydrate*

*Polymers* 332. Elsevier: 121915.

Koehler J, Brandl FP and Goepferich AM (2018a) Hydrogel wound dressings for bioactive treatment of acute and chronic wounds. *European Polymer Journal* 100 (December 2017). Elsevier: 1–11.

Koehler J, Brandl FP and Goepferich AM (2018b) Hydrogel wound dressings for bioactive treatment of acute and chronic wounds. *European Polymer Journal* 100. Pergamon: 1– 11.

Kojima T, Noguchi Y, Terasaka K, et al. (2024) Engineering pH-Responsive, Self-Healing Vesicle-Type Artificial Tissues with Higher-Order Cooperative Functionalities. *Small*. John Wiley & Sons, Ltd: 2311255.

Kolesov S V, Khutoryanskiy V V, Ciarleglio G, et al. (2024) Fabrication of Alginate/Ozoile Gel Microspheres by Electrospray Process. Epub ahead of print 2024. DOI: 10.3390/gels10010052.

Kondo T and Ishida Y (2010) Molecular pathology of wound healing. *Forensic science international* 203(1–3). Forensic Sci Int: 93–98.

Kong J and Yu S (2007) Fourier transform infrared spectroscopic analysis of protein secondary structures. *Acta biochimica et biophysica Sinica* 39(8). Acta Biochim Biophys Sin (Shanghai): 549–559.

Koohzad F and Asoodeh A (2023) Cross-Linked Electrospun pH-Sensitive Nanofibers Adsorbed with Temporin-Ra for Promoting Wound Healing. *ACS applied materials & interfaces* 15(12). ACS Appl Mater Interfaces: 15172–15184.

Landén NX, Li D and Ståhle M (2016) Transition from inflammation to proliferation: a critical step during wound healing. *Cellular and Molecular Life Sciences 2016 73:20* 73(20). Springer: 3861–3885.

Levy J, Friedman D, Domenico M, et al. (2016) *Safety Data Sheet Dressings*. Available at: moz-extension://4a6c47c4-8649-4af0-acad-5f683385c9f3/enhanced-reader.html?openApp&pdf=https%3A%2F%2Fpluspunt.mediqmedeco.nl%2Ffileupload

er%2Fdownload%2Fdownload%2F%3Fd%3D0%26file%3Dcustom%252Fupload%252 FFile-1569247741.pdf (accessed 25 March 2024).

Li C, Chang F, Gao F, et al. (2024) Chitosan-based composite film dressings with efficient self-diagnosis and synergistically inflammation resolution for accelerating diabetic wound healing. *Applied Surface Science* 642. North-Holland: 158578.

Li Y, Chen Y, Xue Y, et al. (2024) Injectable Hydrogel Delivery System with High Drug Loading for Prolonging Local Anesthesia. *Advanced Science*. John Wiley & Sons, Ltd: 2309482.

Liu L, Li X, Dong G, et al. (2023) Bioinspired Natural Shellac Dressing for Rapid Wound Sealing and Healing. *ACS Applied Materials and Interfaces* 15(37). American Chemical Society: 43294–43308.

Liu X, Sun Y, Wang J, et al. (2024) A tough, antibacterial and antioxidant hydrogel dressing accelerates wound healing and suppresses hypertrophic scar formation in infected wounds. *Bioactive Materials* 34. KeAi Publishing: 269.

Ma M, Zhong Y and Jiang X (2020) Thermosensitive and pH-responsive tannin-containing hydroxypropyl chitin hydrogel with long-lasting antibacterial activity for wound healing. *Carbohydrate Polymers* 236. Elsevier: 116096.

Maghsoudi MAF, Aghdam RM, Asbagh RA, et al. (2024) 3D-printing of alginate/gelatin scaffold loading tannic acid@ZIF-8 for wound healing: In vitro and in vivo studies. *International Journal of Biological Macromolecules* 265. Elsevier: 130744.

Maiti S, Maji B and Yadav H (2024) Progress on green crosslinking of polysaccharide hydrogels for drug delivery and tissue engineering applications. *Carbohydrate Polymers* 326. Elsevier: 121584.

Martin P and Nunan R (2015) Cellular and molecular mechanisms of repair in acute and chronic wound healing. *British Journal of Dermatology* 173: 370–378.

Meas A, Wi E, Chang M, et al. (2024) Carboxylmethyl cellulose produced from wood sawdust for improving properties of sodium alginate hydrogel in dye adsorption. *Separation and Purification*

*Technology* 341. Elsevier: 126906.

Mekky AE, Abdelaziz AEM, Youssef FS, et al. (2024) Unravelling the Antimicrobial, Antibiofilm, Suppressing Fibronectin Binding Protein A (fnba) and cna Virulence Genes, Anti-Inflammatory and Antioxidant Potential of Biosynthesized Solanum lycopersicum Silver Nanoparticles. *Medicina 2024, Vol. 60, Page 515* 60(3). Multidisciplinary Digital Publishing Institute: 515.

Mndlovu H, du Toit LC, Kumar P, et al. (2023) Tannic acid-loaded chitosan-RGD-alginate scaffolds for wound healing and skin regeneration. *Biomedical Materials* 18(4). IOP Publishing: 045009.

Mohamed SK, Ahsin A, Rehman HM, et al. (2024) XRD/DFT, Hirshfeld surface analysis and molecular modelling simulations for unfolding reactivity of newly synthesized vanillin derivatives: excellent optical, NLO and protein binding efficiency. *Journal of Biomolecular Structure and Dynamics*. Taylor & Francis. Epub ahead of print 2024. DOI: 10.1080/07391102.2024.2308774.

Mohammed MA, Syeda JTM, Wasan KM, et al. (2017) An Overview of Chitosan Nanoparticles and Its Application in Non-Parenteral Drug Delivery. *Pharmaceutics* 9(4). Multidisciplinary Digital Publishing Institute (MDPI).

Molan P and Rhodes T (2015) Honey: A Biologic Wound Dressing. *Wounds* 27(6).

Muyonga JH, Cole CGB and Duodu KG (2004) Fourier transform infrared (FTIR) spectroscopic study of acid soluble collagen and gelatin from skins and bones of young and adult Nile perch (Lates niloticus). *Food Chemistry* 86(3). Elsevier: 325–332.

Nabipour H, Aliakbari F, Volkening K, et al. (2024) The development of a bio-based metal-organic framework coated with carboxymethyl cellulose with the ability to deliver curcumin with anticancer properties. *Materials Today Chemistry* 37. Elsevier: 101976.

Natarajan V, Krithica N, Madhan B, et al. (2013) Preparation and properties of tannic acid cross-linked collagen scaffold and its application in wound healing. *Journal of biomedical materials research. Part B, Applied biomaterials* 101(4). J Biomed Mater Res B Appl Biomater: 560–567.

Ndlovu SP, Ngece K, Alven S, et al. (2021) Gelatin-Based Hybrid Scaffolds: Promising Wound Dressings. *Polymers* 13(17). Multidisciplinary Digital Publishing Institute (MDPI).

Negut I, Dorcioman G and Grumezescu V (2020) Scaffolds for Wound Healing Applications. *Polymers* 12(9). Multidisciplinary Digital Publishing Institute (MDPI): 1–19.

Nokoorani YD, Shamloo A, Bahadoran M, et al. (2021) Fabrication and characterization of scaffolds containing different amounts of allantoin for skin tissue engineering. *Scientific Reports* 11(1). Nature Publishing Group: 16164.

Nguyen TX, Nguyen DH, Ho-Man TP, et al. (2022) Cold Plasmamed Beam as a Supporting Treatment of Soft Tissue Injuries in Severe Covid-19 Patients: A Preliminary Report. *Medical Devices (Auckland, N.Z.)* 15. Dove Press: 277

O'Brien FJ, Harley BA, Yannas I V., et al. (2004) Influence of freezing rate on pore structure in freeze-dried collagen-GAG scaffolds. *Biomaterials* 25(6). Elsevier BV: 1077–1086.

Ono S, Imai R, Ida Y, et al. (2015) Increased wound pH as an indicator of local wound infection in second degree burns. *Burns* 41(4). Elsevier: 820–824.

Outwater AH and Van Braekel T (2020) Prehospital care of burn injuries in Africa: A review, 1990–2018. *Burns* 46(8). Elsevier: 1737–1745.

Ozyilmaz ED, Celikkaya R, Comoglu T, et al. (2023) In Vitro and In Vivo Evaluation of Metformin Hydrochloride Hydrogels Developed with Experimental Design in the Treatment of Burns. *AAPS PharmSciTech* 24(8). AAPS PharmSciTech.

Pallares-Rusiñol A, Bernuz M, Moura SL, et al. (2023) Advances in exosome analysis. *Advances in Clinical Chemistry* 112. Academic Press Inc.: 69–117.

Peng L, Zhao J and Tan M (2024) Portable and Wearable Sensing Systems: Techniques, Fabrication, and ... - Google Books. Available at: https://books.google.co.za/books?hl=en&lr=&id=kb_5EAAAQBAJ&oi=fnd&pg=PA179&

dq=development+of+smart+wound+dressings+with+sensors&ots=2gzjsMDxuy&sig=cF WEAo0c-jquNfo0qbqxpkj3bYQ&redir_esc=y#v=onepage&q=development%20of%20smart%20w ound%20dressings%20with%20sensors&f=false (accessed 25 March 2024).

Percival SL, McCarty S, Hunt JA, et al. (2014) The effects of pH on wound healing, biofilms, and antimicrobial efficacy. *Wound repair and regeneration : official publication of the Wound Healing Society [and] the European Tissue Repair Society* 22(2). Wound Repair Regen: 174–186.

Pezeshki-Modaress M, Rajabi-Zeleti S, Zandi M, et al. (2014) Cell-loaded gelatin/chitosan scaffolds fabricated by salt-leaching/lyophilization for skin tissue engineering: in vitro and in vivo study. *Journal of biomedical materials research. Part A* 102(11). J Biomed Mater Res A: 3908–3917.

Pinto e Souza IE, Barrioni BR, Costa MCP, et al. (2024) Development OF pH-sensitive wound dressings using PVA/PAA hydrogels and bioactive glass nanoparticles doped with cerium and cobalt. *Materials Today Communications* 38. Elsevier: 107981.

Qin J, Chen F, Wu P, et al. (2022) Recent Advances in Bioengineered Scaffolds for Cutaneous Wound Healing. *Frontiers in Bioengineering and Biotechnology* 10. Frontiers Media S.A.: 841583.

Qiu Y and Park K (2001) Environment-sensitive hydrogels for drug delivery. *Advanced Drug Delivery Reviews* 53(3). Elsevier: 321–339.

Raepsaet C, Alves P, Cullen B, et al. (2022) Clinical research on the use of bordered foam dressings in the treatment of complex wounds: A systematic review of reported outcomes and applied measurement instruments. *Journal of Tissue Viability* 31(3).
Elsevier: 514–522.

Rahman, M. S., Hasan, M. S., Nitai, A. S., Nam, et al. (2021). Recent Developments of Carboxymethyl Cellulose. *Polymers*, 13(8), 1345. https://doi.org/10.3390/polym13081345

Reeves R, Ribeiro A, Lombardo L, et al. (2010) Synthesis and Characterization of Carboxymethylcellulose-Methacrylate Hydrogel Cell Scaffolds. *Polymers* 2(3). NIH Public Access: 252.

Renani MM, Nazarpak MH, Solati-Hashjin M, et al. (2024) Nanoclay particles as a stem cell adhesion factor in polyvinyl alcohol hydrogel-based wound dressings. *Polymer Engineering & Science*. John Wiley & Sons, Ltd. Epub ahead of print 2024. DOI: 10.1002/PEN.26678.

Ribeiro ARM, Felgueiras HP, Reis S, et al. (2023) Modification of polyvinyl alcohol-based electrospun mats with naturally-derived halochromic molecules for potential applications in wound healing monitoring. MDPI. Epub ahead of print 1 November 2023. DOI: 10.3390/ECMC2023-15658.

Ryu V, Ghoshal M, Chuesiang P, et al. (2024) Mechanisms of microbial photoinactivation by curcumin's micellar delivery. *Frontiers in Food Science and Technology* 4. Frontiers: 1361817.

Sadeghi-Aghbash M, Rahimnejad M, Adeli H, et al. (2024) Catecholamines polymerization crosslinking for alginate-based burn wound dressings developed with ciprofloxacin and zinc oxide interactions. *International Journal of Biological Macromolecules* 260. Elsevier: 129400.

Salih H and Alshrefi SM (2024) pH-Sensitive Swelling Behavior and Drug Release Kinetics of Chitosan-Modified Cs/HEMA Hydrogels. Epub ahead of print 5 March 2024. DOI: 10.21203/RS.3.RS-3978283/V1.

Sarheed O, Ahmed A, Shouqair D, et al. (2016) Antimicrobial Dressings for Improving Wound Healing. *Wound Healing - New insights into Ancient Challenges*. IntechOpen. Epub ahead of print 12 October 2016. DOI: 10.5772/63961.

Saxena V, Hasan A and Pandey LM (2021) Antibacterial nano-biocomposite scaffolds of Chitosan, Carboxymethyl Cellulose and Zn & Fe integrated Hydroxyapatite (Chitosan- CMC-FZO@HAp) for bone tissue engineering. *Cellulose* 28(14). Springer Science and Business Media B.V.: 9207–9226.

Schneider LA, Korber A, Grabbe S, et al. (2007) Influence of pH on wound-healing: A new perspective for wound-therapy? *Archives of Dermatological Research* 298(9). Springer: 413–420.

Schreml S, Szeimies RM, Prantl L, et al. (2010) Oxygen in acute and chronic wound healing. *The British journal of dermatology* 163(2). Br J Dermatol: 257–268.

Shahmohammadi A, Samadian H, Keshel SH, et al. (2024) Burn wound healing using adipose-derived mesenchymal stem cells and manganese nanoparticles in polycaprolactone/gelatin electrospun nanofibers in rats. *BioImpacts* 14(5). Tabriz University of Medical Sciences: 30193–30193.

Shan M, Chen X, Zhang X, et al. (2024) Injectable Conductive Hydrogel with Self-Healing, Motion Monitoring, and Bacteria Theranostics for Bioelectronic Wound Dressing. *Advanced healthcare materials*. DOI: 10.1002/ADHM.202303876.

Sharma V and Wazed Ali S (2024) Durable functionalization of cotton fabric using vanillin as reactive UV protective finish without hampering basic textile properties. *Materials Chemistry and Physics* 315. Elsevier: 128930.

Sharpe JR, Harris KL, Jubin K, et al. (2009) The effect of pH in modulating skin cell behaviour. *British Journal of Dermatology* 161(3): 671–673.

Singh AK, Pramanik K and Biswas A (2023) Constructing a biofunctionalized 3D-printed gelatin/sodium alginate/chitosan tri-polymer complex scaffold with improvised biological and mechanical properties for bone-tissue engineering. *Bio-Design and Manufacturing 2023 7:1* 7(1). Springer: 57–73.

Smolle C, Cambiaso-Daniel J, Forbes AA, et al. (2017) Recent trends in burn epidemiology worldwide: A systematic review. *Burns* 43(2). Elsevier: 249–257.

Somogyi Škoc M, Stevelić N and Rezić I (2024) Development and Characterization of Sustainable Coatings on Cellulose Fabric and Nonwoven for Medical Applications. *Sustainability 2024, Vol. 16, Page 857* 16(2). Multidisciplinary Digital Publishing Institute: 857.

Spicer CD (2020) Hydrogel scaffolds for tissue engineering: the importance of polymer choice. *Polymer Chemistry* 11(2). The Royal Society of Chemistry: 184–219.

Stoica AE, Chircov C and Grumezescu AM (2020) Hydrogel dressings for the treatment of burn wounds: An up-to-date overview. *Materials* 13(12): 1–24.

Stokes MAR and Johnson WD (2017) Burns in the Third World: an unmet need. Annals of *Burns and Fire Disasters 30*(4). Euro-Mediterranean Council for Burns and Fire Disasters (MBC): 243.

Storan ER, Nolan U and Kirby B (2016) Allergic contact dermatitis caused by the tea tree oil-containing hydrogel Burnshield®. *Contact dermatitis* 74(5). Contact Dermatitis: 309– 310.

Sun JY, Zhao X, Illeperuma WRK, et al. (2012) Highly stretchable and tough hydrogels. *Nature* 489(7414). Nature Publishing Group: 133–136.

Supriya H, Tripathi S and Bose S (2023) A review of past promises, present realities and a vibrant future for wound dressing from naturally occurring to sustainable materials. *RSC Sustainability* 1(4). RSC: 763–787.

Szabó A, Pasquariello R, Costa PF, et al. (2023) Light-Based 3D Printing of Gelatin-Based Biomaterial Inks to Create a Physiologically Relevant In Vitro Fish Intestinal Model. *Macromolecular Bioscience* 23(10). John Wiley & Sons, Ltd: 2300016.

Tiwari VK (2012) Burn wound: How it differs from other wounds. *Indian Journal of Plastic Surgery* 45(2): 364–373.

Tomadoni B, Ponce A, Pereda M, et al. (2019) Vanillin as a natural cross-linking agent in chitosan-based films: Optimizing formulation by response surface methodology. *Polymer Testing* 78. Elsevier: 105935.

Tseng HJ, Tsou TL, Wang HJ, et al. (2013) Characterization of chitosan-gelatin scaffolds for dermal tissue engineering. *Journal of tissue engineering and regenerative medicine* 7(1). J Tissue Eng Regen Med: 20–31.

Valcarcel J, Fraguas J, Hermida-Merino C, et al. (2021) Production and physicochemical

characterization of gelatin and collagen hydrolysates from turbot skin waste generated by aquaculture activities. *Marine Drugs* 19(9). MDPI.

Wahyono T, Astuti DA, Komang Gede Wiryawan I, et al. (2019) Fourier Transform Mid- Infrared (FTIR) Spectroscopy to Identify Tannin Compounds in The Panicle of Sorghum Mutant Lines. *IOP Conference Series: Materials Science and Engineering* 546(4). IOP Publishing: 042045.

Wang H, Zhou S, Guo L, et al. (2020) Intelligent Hybrid Hydrogels for Rapid in Situ Detection and Photothermal Therapy of Bacterial Infection. *ACS Applied Materials and Interfaces* 12(35). American Chemical Society: 39685–39694.

Wang K, Ding Q, Qi M, et al. (2024) Integrated Bilayer Microneedle Dressing and Triboelectric Nanogenerator for Intelligent Management of Infected Wounds. *Advanced Functional Materials*. John Wiley & Sons, Ltd: 2316820.

Wang L, Zhou M, Xu T, et al. (2022) Multifunctional hydrogel as wound dressing for intelligent wound monitoring. *Chemical Engineering Journal* 433. Elsevier: 134625.

Wasiak J, Cleland H, Campbell F, et al. (2013) Dressings for superficial and partial thickness burns. *Cochrane Database of Systematic Reviews* 2013(3).

Westlake JR, Laabei M, Jiang Y, et al. (2023) Vanillin Cross-Linked Chitosan Film with Controlled Release of Green Tea Polyphenols for Active Food Packaging. *ACS Food Science and Technology* 3(10). American Chemical Society: 1680–1693.

Wounds International (2014) Effective skin and wound management of non- complex burns. *International Best Practice Guidelines*. Epub ahead of print 2014.

Xia Z, Singh A, Kiratitanavit W, et al. (2015) Unraveling the mechanism of thermal and thermo-oxidative degradation of tannic acid. *Thermochimica Acta* 605. Elsevier: 77–85.

Yu M, Yi C, Lin S, et al. (2024) e-Bandage: Exploiting Smartphone as a Therapeutic Device for Cutaneous Wound Treatment. *Advanced Intelligent Systems*. John Wiley & Sons, Ltd: 2300494.

Zaidi S, Thakur S, Sanchez-Rodriguez D, et al. (2024) Optimal processing conditions of a bio-based epoxy synthesized from vanillyl alcohol. *Polymer Degradation and Stability* 223. Elsevier: 110743.

Zhang W, Liu L, Cheng H, et al. (2024) Hydrogel-based dressings designed to facilitate wound healing. *Mater. Adv* 5: 1364.

Zhang Y ;, Wu B M, Zhang Yulong, et al. (2023) Current Advances in Stimuli-Responsive Hydrogels as Smart Drug Delivery Carriers. *Gels 2023, Vol. 9, Page 838* 9(10). Multidisciplinary Digital Publishing Institute: 838.

Zhao R, Liang H, Clarke E, et al. (2016) Inflammation in Chronic Wounds. *International journal of molecular sciences* 17(12). Int J Mol Sci.

Zhao X, Lang Q, Yildirimer L, et al. (2016) Photocrosslinkable Gelatin Hydrogel for Epidermal Tissue Engineering. *Advanced healthcare materials* 5(1). Adv Healthc Mater: 108–118