# ANTIBACTERIAL ACTIVITY AND BIOCOMPATIBILITY OF POLYPHENOL CROSSLINKED ZNO-CUO NANOCOMPOSITE HYDROGEL AS A WOUND HEALING AGENT

**GADAIME NASRIN K RAMTAN**

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# ANTIBACTERIAL ACTIVITY AND BIOCOMPATIBILITY OF POLYPHENOL CROSSLINKED ZNO-CUO NANOCOMPOSITE HYDROGEL AS A WOUND HEALING AGENT

**by**

**GADAIME NASRIN K RAMTAN**

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## LIST OF SYMBOLS

| | |
|---|---|
| °C | Degree Celsius |
| Ca | Calcium |
| $Cu^{2+}$ | Copper ions |
| CuO | Copper oxide |
| CFU/mL | Colony-forming unit per milliliter |
| g | Gram |
| $H_2O_2$ | Hydrogen peroxide |
| h | Hour |
| Log | Logarithm |
| mL | Milliliter |
| Mg | Milligram |
| mm | Milli meter |
| µg | Micro gram |
| µM | Micromolar |
| µM | Micrometer |
| µL | Microliter |
| % | Percentage |
| rpm | Revolutions per minute |
| sec | Second |
| $O_2 \bullet^-$ | Superoxide |
| OH | Hydroxyl groups |

| | |
|---|---|
| V | Volt |
| $Zn^{2+}$ | Zinc ions |
| ZnO | Zinc oxide |
| w/v | Weight/Volume |
| v/v | Volume/Volume |
| Δ | Delta |

## LIST OF ABBREVIATIONS

| | |
|---|---|
| AB | Alamar Blue™ |
| ATCC | American type culture collection |
| ASTM | American Society for Testing and Materials |
| *C. gigantea* | *Calotropis gigantea* |
| CS | Chitosan |
| $CaCl_2$ | Calcium chloride |
| CPH | Polymeric hydrogel dressing |
| CPZCH | ZnO-CuO nanocomposite embedded in PEG hydrogel dressing |
| CPH-X | Polyphenol embedded in PEG hydrogel dressing |
| CPZCH-X | Polyphenol/ZnO-CuO nanocomposite embedded in PEG hydrogel |
| DMSO | Dimethyl sulfoxide |
| DFUs | Diabetic foot ulcers |
| $DIH_2O$ | Deionized water |
| *E. coli* | *Escherichia coli* |
| EDX | Energy dispersive spectroscopy |
| EDTA | Ethylenediaminetetraacetic acid |
| FTIR | Fourier transform infrared spectroscopy |
| FET | Fish embryo toxicity |
| GAPDH | Glyceraldehyde 3-phosphate dehydrogenase |
| HMOX1 | Heme oxygenase 1 |
| hpf | Hours-post-fertilization |
| Krt-8 | Cytokeratin |
| L929 | Mouse fibroblast |

| | |
|---|---|
| mRNA | Messenger ribonucleic acid |
| *MRSA* | *Methicillin-resistant S. aureus* |
| NF-kβ | Nuclear factor kappa-light-chain-enhancer of activated B cells |
| $NaCl_2$ | Sodium chloride |
| OD | Optical density |
| *P. aeruginosa* | *Pseudomonas aeruginosa* |
| PBS | Phosphate buffer saline |
| PEG | Polyethylene glycol |
| PRP | Platelet rich plasma |
| RIPA | Radioimmunoprecipitation assay |
| ROS | Reactive oxygen species |
| RT-qPCR | Real time quantitative polymerase chain reaction |
| ROS | Reactive oxygen species |
| *S. aureus* | *Staphylococcus aureus* |
| SEM | Scanning electron microscope |
| STIM-1 | Stromal interaction molecule 1 |
| UV | Ultraviolet |
| UV-vis | Ultraviolet visible spectroscopy |
| X | Polyphenol |
| XRD | X-ray diffraction |
| ZOI | Zone of inhibition |

## LIST OF APPENDICES

**AKTIVITI ANTIBAKTERIA DAN BIOSERASI HIDROGEL NANOKOMPOSIT ZNO-CUO POLIFENOL-BERANGKAI SILANG SEBAGAI AGEN PENYEMBUHAN LUKA**

## ABSTRAK

Merawat luka kulit terbuka yang mengeluarkan eksudat memberikan cabaran yang signifikan, terutamanya disebabkan oleh risiko jangkitan bakteria, khususnya strain yang tahan antibiotik, yang boleh menghalang proses penyembuhan. Balutan luka tradisional tidak mencukupi untuk mewujudkan persekitaran penyembuhan yang optimum, kerana ia gagal mengawal mikropersekitaran luka yang kompleks dengan berkesan, yang dicirikan oleh cabaran seperti eksudat berlebihan dan pencemaran bakteria. Kajian ini bertujuan untuk membangunkan dan mencirikan balutan luka pelbagai fungsi yang mengandungi polifenol dan komponen nanokomposit, dengan penilaian merangkumi sifat struktur dan kimia, aktiviti antibakteria, keserasian sel (sitokompatibiliti), keserasian darah (hemokompatibiliti), dan potensi penyembuhan luka. Kajian ini dibahagikan kepada penyediaan polifenol / nanokomposit ZnO-CuO yang terbenam dalam hidrogel PEG (CPZCH-X) bersama analisis sifat struktur dan kimia. Selain itu, analisis fungsi meliputi aktiviti antibakteria, sitokompatibiliti, hemokompatibiliti, dan penyembuhan luka secara in vitro melalui migrasi sel, manakala analisis molekul melibatkan ekspresi protein dan ekspresi gen mRNA. Balutan CPZCH yang mengandungi nanokomposit ZnO-CuO dalam hidrogel PEG menunjukkan sifat pengembangan dan pengecutan yang baik, penting untuk mengekalkan persekitaran luka yang lembap, menyokong pengedaran nutrien, serta menyumbang kepada prestasi balutan yang mesra alam. Balutan CPZCH menunjukkan aktiviti antibakteria terhadap bakteria bukan MDR termasuk

*Staphylococcus aureus*, *Escherichia coli*, serta MDR termasuk *Pseudomonas aeruginosa* dan *methicillin-resistant S. aureus*. Formulasi CPZCH-X (50 μM polifenol) menunjukkan sitokompatibiliti tinggi dengan fibroblas dan hemokompatibiliti cemerlang dengan sel darah manusia. Selain itu, kadar penutupan luka yang dicapai oleh CPZCH-X adalah jauh lebih tinggi berbanding hidrogel perbandingan dalam tempoh 24 jam. Analisis ekspresi molekul menunjukkan penurunan ekspresi *NF-κB*, *HMOX-1* dan *VEGF* disertai peningkatan ekspresi *KRT8*, serta pengekalan tahap STIM-1. Keputusan ini menunjukkan bahawa CPZCH-X memodulasi laluan keradangan dan antioksidan sambil menggalakkan migrasi fibroblas, yang secara keseluruhannya menyumbang kepada potensi penyembuhan luka yang dipertingkatkan. Kesimpulannya, balutan CPZCH-X telah menunjukkan ciri struktur dan kimia yang unggul, bersama sitokompatibiliti dan hemokompatibiliti yang cemerlang, sekali gus menekankan potensi yang besar untuk rawatan luka terbuka yang mengeluarkan eksudat.

# ANTIBACTERIAL ACTIVITY AND BIOCOMPATIBILITY OF POLYPHENOL CROSSLINKED ZNO-CUO NANOCOMPOSITE HYDROGEL AS A WOUND HEALING AGENT

## ABSTRACT

Treating exuding open skin wounds present significant challenges, primarily due to the risk of bacterial infections, particularly those caused by antibiotic-resistant strains, which can impede the healing process. Traditional wound dressings are insufficient for creating an optimal healing environment, as traditional dressings fail to effectively regulate the complex wound microenvironment, which is characterised by challenges such as excessive exudate, and bacterial contamination. The present study aimed to develop and characterize a multifunctional wound dressing containing polyphenol and nanocomposite components, with evaluations encompassing structural and chemical properties, antibacterial activities, cytocompatibility, hemocompatibility, and wound-healing potential. The current study was divided into polyphenol / ZnO-CuO nanocomposite embedded in PEG hydrogel (CPZCH-X) preparation with the structural and chemical properties analyses. In addition, the functional analyses included antibacterial activity, cytocompatibility, hemocompatibility, and *in vitro* wound healing using by cell migration, and molecular analyses involved protein expression and mRNA gene expression. The ZnO-CuO nanocomposite embedded in PEG hydrogel (CPZCH) dressing demonstrated favorable swelling and shrinkage behavior, essential for maintaining a moist wound environment, supporting nutrient distribution, and contributing to eco-friendly dressing performance. CPZCH dressing exhibited antibacterial activity against non-MDR including *Staphylococcus aureus*, *Escherichia coli*, and MDR including

*Pseudomonas aeruginosa*, and *Methicillin-resistant S. aureus*. The CPZCH-X formulation (50 μM polyphenol) displayed high cytocompatibility with fibroblasts and excellent hemocompatibility with human blood cells. Furthermore, the wound closure rate achieved by CPZCH-X was markedly superior to that of comparator hydrogels within 24 hours. Molecular expression analysis demonstrated downregulation of *NF-κB*, *HMOX-1,* and *VEGF* expression accompanied by upregulation of *KRT8*, and maintenance of STIM-1 levels. These outcomes indicate that CPZCH-X modulates inflammatory and antioxidant pathways while promoting fibroblast migration, collectively contributing to enhanced wound-healing potential. In conclusion, the CPZCH-X dressing demonstrated outstanding structural and chemical characteristics, as well as excellent cytocompatibility and hemocompatibility, underscoring substantial potential for Successfultreatment of exuding open wounds.

# CHAPTER 1

# INTRODUCTION

## 1.1 Research background

Dressing open wounds poses a significant therapeutic challenge primarily due to the heightened risk of bacterial infection (Gou et al. 2024; Liu et al. 2023). Traditional wound dressings may not match the specific wound type and exhibit inherent drawbacks including inadequate physical and chemical properties, lack of degradability, incompatibility, and poor barrier function resulting in susceptibility to easily cause bacterial infection (Liang et al. 2022; Pan et al. 2021). Therefore, wound treatment has become a complex repair, a healthcare concern and economic burden worldwide (Lingyu et al. 2021). Malaysia's wound care market is projected to reach approximately US$7.84 million in revenue in 2025. Annual expenditures associated with wound care are enormous, reaching £2.3–3.1 billion in the United‘Kingdom, and $50 billion, in the United”States, and”the global market for wound care product is expected to grow from US$17.0 billion in 2016 to US$18.7 billion by 2027 and US$30.52 billion by the 2030 (Homaeigohar & Boccaccini, 2020; Wang et al. 2024; Yu et al. 2022).

Open skin wounds are often difficult to treat, hence, understanding how to treat the disorders resulting from these wounds can lead to successful healing. Therefore, development of high-performance wound dressings has become a research focus in the medical field, among which hydrogel has the highest standards and management for wound healing. The hydrogel comprises a three-dimensional network of hydrophilic polymers, enabling it to absorb water and swell. Additionally, it possesses the capability to encapsulate bioactive molecules and create a moist environment conducive to promoting the wound healing process (Kibungo et al. 2021).

Tailored hydrogels can be crafted to align with the specific needs of exuding open skin wounds, incorporating essential traits (Huang et al. 2022; Zhong et al. 2020) like biocompatibility, biodegradability, adhesion, antimicrobial, anti-inflammatory, antioxidant properties that foster angiogenesis. Achieving this involves blending synthetic and natural polymers and integrating them with antibacterial and bioactive agents.

## 1.2 Problem statement

Limited therapeutic effectiveness of available wound dressings remains a critical barrier to successful wound healing outcomes. Many conventional dressings lack essential characteristics required for optimal treatment of open, exuding wounds. These limitations include insufficient absorbency to manage wound exudate, inadequate antibacterial protection against opportunistic pathogens, poor mechanical integrity to maintain wound coverage, and minimal bioactivity to support tissue regeneration. Additionally, the widespread emergence of multidrug-resistant bacterial strains, such as *P. aeruginosa* and *MRSA*, has significantly reduced the efficacy of traditional antibiotic therapies in wound care applications. Open wounds colonized by these pathogens often exhibit delayed healing, and persistent inflammation, further emphasizing the need for advanced wound management strategies.

In response to these challenges, the present research focused on the development of a multifunctional four-component hydrogel dressing. This system integrates polymers to provide mechanical reinforcement, structural stability, and moisture retention including chitosan (CS); a natural cationic polysaccharide polymer (Ali et al. 2024), hydrophilic, non-toxic, biodegradable, biocompatible, and hemostatic agent (Khichariya and Verma, 2023; Lu et al. 2022; Piekarska et al. 2023; Yang et al. 2023). Chitosan-based dressing able to stimulate granulation, collagen production,

and infiltration of polymorphonuclear cells during the initial stages of wound healing (Matica et al. 2019), and polyethylene glycol (PEG); a synthetic polymer, soluble in water, hydrophilic, flexible, non-toxic, biocompatible, relatively cheap, and able to absorb and retain water inside dressing (Quadir et al. 2024; Naeimi et al. 2020). In addition, PEG has an important role in mechanical properties improvement of CS wound dressing (Jonidi et al. 2024; Okur et al. 2020; Zhang et al. 2022).

Incorporation of zinc oxide (ZnO) and copper oxide (CuO) nanoparticles contributes potent broad-spectrum antibacterial activity targeting resistant bacterial populations. Each nanoparticles such as metal oxides; zinc oxide (ZnO) and copper oxide (CuO) have distinct physical and chemical properties, especially those synthesized through green synthesis with plant-derived biomolecules that are stable with the cells, providing non-chemical therapeutic alternatives, environmentally friendly, and low toxic (Amir et al. 2024; Ramesh et al. 2024). ZnO and CuO are antibacterial agents, where ZnO has potential to produce reactive oxygen species (ROSs), which play a major role in preventing the bacteria reproduction, and promote the wound healing process, while CuO can penetrate bacterial cells and disrupt the cells function by triggering heightened oxidative stress reactions, ultimately resulting in their elimination (Cheng et al. 2023; Jayanetti et al. 2024; Wang et al. 2024a).

Additionally, polyphenol has been included to deliver antioxidant and anti-inflammatory effect that promote tissue repair, modulate oxidative stress, and accelerate the wound healing process (Cheng et al. 2023). This integrative approach is intended to overcome the fundamental limitations of commercial wound dressings by providing enhanced absorbency, mechanical stability, antibacterial performance, and bioactivity supportive of effective tissue regeneration.

## 1.3 Research objectives

### 1.3.1 General objective

Development of antibacterial and cytocompatible polyphenol-crosslinked ZnO-CuO nanocomposite hydrogel (CPZCH-X) with wound healing potential.

### 1.3.2 Specific objectives

1. To synthesize and characterise the polyphenol / ZnO-CuO nanocomposite embedded in PEG hydrogel (CPZCH-X).
2. To determine the antibacterial potential against non-multidrug resistance (non-MDR) including *Staphylococcus aureus*, *Escherichia coli*, and MDR including *Pseudomonas aeruginosa*, and *Methicillin-resistant S. aureus* strains of CPZCH by agar disk diffusion, time-kill, and bacterial cell adherence analyses.
3. To evaluate the cytocompatibility by Alamar blue assay, determine hemocompatibility by hemolysis, blood clotting, platelet adhesion analyses, and assess toxicity by fish embryo toxicity (FET) analysis of CPZCH-X.
4. To elucidate wound healing potential of CPZCH-X by the cell migration and molecular analyses, including RT-PCR of mRNA expression for *NF-κB*, *HMOX-1*, *KRT-8*, and *VEGF*, and Western blot assessment of protein levels for NF-κB, KRT-8, and STIM-1.

# CHAPTER 2

# LITERATURE REVIEW

## 2.1 Open skin wounds

An open wound occurs due to the disruption of the integrity of the skin and underlying subcutaneous layers, including the mucous membrane, which may cause visible bleeding and exposure of underlying tissues to the external environment (Hamil et al. 2020; Mamun et al. 2024). In addition, interference with the physiological organization of skin cells and the disruption of their normal functions. This facilitates the spread of microorganisms to the deeper layers of the skin and entering the lymph nodes. Therefore, all scars must be cleaned, properly covered. An effective wound covering aids in controlling bleeding prevents contamination by absorbing blood and exudates, moreover, accelerating of the wound healing process (Patenall et al. 2024). Open wounds can be classified into two types based on their healing duration: acute wounds and chronic wounds.

### 2.1.1 Acute skin wounds

Acute skin wounds as shown in Figure 2.1 are unintentional injuries to skin often precipitated by trauma to the skin due to injuries such as lacerations, abrasions or surgery (Dai et al. 2020; Yu et al. 2022). These wounds can heal within a short period without leading to further medical complications and typically follow a natural, orderly healing process even without external assistance (Yu et al. 2022). Acute wounds are easily healed completely in less than 3 months (Nguyen et al. 2023). In 2014, acute wounds accounted for 17.2 million hospital visits in the United States (Sen et al. 2021). The treatment of these wounds must ensure normal physiological healing (Dai et al. 2020), as the wounds must have an adequate blood supply. Inflammation, infection, or residual debris in wounds may delay or prevent proper healing.

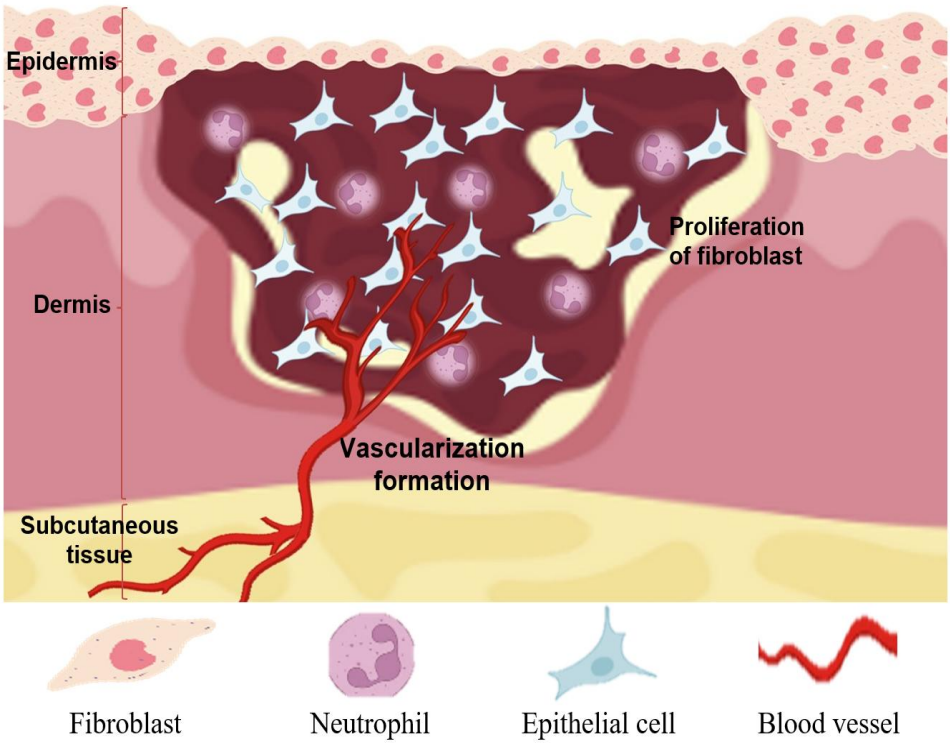


**Figure 2.1 Schematic illustration of acute skin wounds.** In acute wounds, blood supply to the wound site persists, along with re-epithelialization and fibroblast proliferation. The figure was created using BioRender.

### 2.1.2 Chronic skin wounds

Chronic skin wounds as shown in Figure 2.2 are those that result from the delayed healing of acute wounds (Vivcharenko et al. 2021). These wounds are difficult to control and typically take between 8 to 12 weeks to treat and heal (Mathew-Steiner et al. 2021; Sen. 2021). When left untreated or improperly managed, chronic wounds can lead to severe medical complications such as sepsis, tissue removal, limb amputation, and potentially death (Sen. 2021). These wounds, which remain open for more than a month, often do not progress through the normal healing process. Additionally, an imbalance between regulatory molecules that contribute to healing processes causes the tissue repair process to halt at one of the stages of wound healing

(Grubbs et al. 2023; Vivcharenko et al. 2021). The prolonged inflammatory stage is the primary factor responsible for chronic wounds formation.

This type of open skin wound is characterised by the presence of exudate, emit odors, intense pain, and is difficult to treat with antibiotics when infected with MDR skin pathogens (Eriksson et al. 2022). Chronic skin wounds are categorized into diabetic foot ulcers, pressure ulcers, and vascular leg ulcers (venous and arterial ulcers) (Pasek et al. 2023; Tavakoli et al. 2020) as shown in Figure 2.2. These wounds are often complicated by conditions such as diabetes and obesity, leading to prolonged open wounds that require specialized care (Sen et al. 2023). In 2022, chronic wounds made up 77.6% of all open wound cases reported in Malaysia (Morat and Ajemi, 2024). Treatment of open wounds poses a major therapeutic challenge due to the ease of bacterial infection (Eriksson et al. 2022; Liu et al. 2023) leading to sepsis.

Diabetic foot ulcers (DFUs) are known as a disorder that occurs in the skin of the foot of a diabetic patient, as the risk of developing diabetic foot ulcers constitutes 15% of the life of the diabetic patients (Al-Basra et al. 2022), with financial spending amounting to approximately 1.38 billion dollars annually to care for the healing of these wounds (Urso et al. 2021). DFUs include three types: neuropathic, neurological deficiency, and ischemic. Neuropathy is the predominant type among these types, especially sensory neuropathy (Baker et al. 2023), where this type is a sensory or autonomic impairment, sensory impairment equals 80% of patients suffer from DFUs, which lead to muscle wasting and foot deformity, making the patient vulnerable to many traumas, subsequently to ulceration, in addition, autonomic dysfunction that leads to dryness and fragility of the skin (Basra et al. 2022) as a result of poor sweating in the lower extremities.

Pressure ulcers ($PU_S$) are wounds that often develop in areas with bony prominence on the feet and require proper drainage to alleviate pressure for healing. Pressure ulcers typically affect elderly individuals who are bedridden for extended periods or immobilized patients, leading to a lack of movement and increased vulnerability to ulcers. These ulcers can severely impact a patient's quality of life (Fukaya et al. 2022). This type of ulcer is characterised by localized injury to the skin and deeper tissues due to prolonged pressure, can manifest as either an open wound or intact skin, with tissue damage resulting from intense or prolonged mechanical loading, such as compression, tension, and shear (Sardo et al. 2023). Approximately 3 million people worldwide suffer from pressure ulcers (Parvizi et al. 2023), making $PU_S$ the third most costly disorder after cardiovascular disease and cancer.

Venous and arterial diseases are among the main causes of chronic ulcers in the lower extremities. The primary cause of venous ulcer formation is venous hypertension, which occurs due to venous reflux (incompetence) or blockage (Milan et al. 2019). Arterial leg ulcers result from insufficient access of oxygen to the tissues due to clogged arteries. Peripheral arterial occlusive disease (PAOD) and atherosclerosis are common diseases that lead to blocked arteries in the legs, and 10%-20% of PAOD patients also suffer from venous leg ulcers (Alagha et al. 2024; Pannier and Rabe, 2013). Approximately 1% of the world's population is at risk of developing venous ulcers, with 0.3% of venous ulcers remaining active and non-reversible. The incidence of the disease rises with age, impacting over 4% of individuals aged 65 and older. When these wounds are not treated properly and effectively, ~30% of healed venous ulcers recur in the first year (Aguiar et al. 2023), with the rate rising to 78% after 2 years.

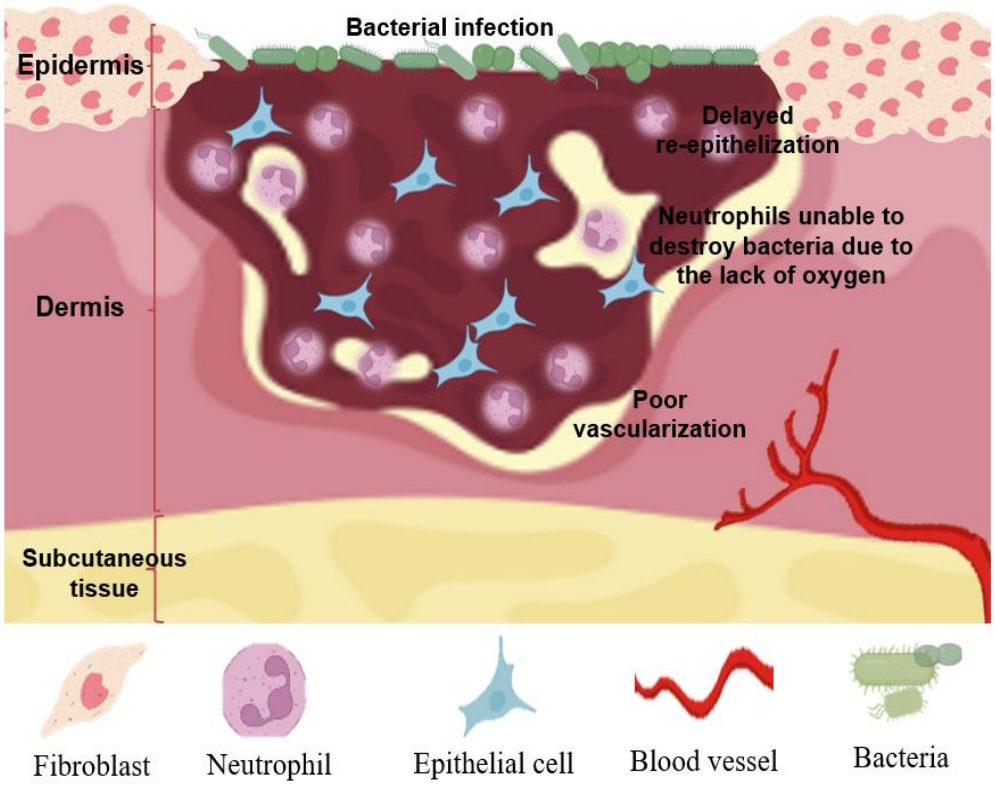


**Figure 2.2 Schematic illustration of chronic skin wound.** In chronic wounds, bacterial infections impair angiogenesis, hinder fibroblast proliferation and reepithelization. The figure was created uisng BioRender.

## 2.2 Challenges in the wound care

Wound care has become a complex issue, presenting a major healthcare challenge and economic pressure globally. Frequent and painful dressing changes, especially over extended periods, can significantly complicate the overall management of the wound. Moreover, this repeated intervention may lead to increased treatment expenses. (Lingyu Sun et al. 2021; Wilkinson et al. 2020). Policymakers and healthcare leaders gain valuable insights from understanding the economic implications of wound care, where the costs associated with annual expenditures on wound care are substantial (Queen et al. 2024) (Figure 2.3), and projections indicate that the global market for wound care products will increase from US$17.0 billion in 2016 to US$18.7 billion by 2027. Meanwhile, in Malaysia,

the wound care market is forecasted to grow at a steady annual rate of 0.72% from 2025 to 2029 (Homaeigohar & Boccaccini, 2020; Yu et al. 2022). Despite the challenging nature of treating these wounds, a comprehensive understanding of the wounds management can lead to successful healing, improve treatment strategies, accelerate healing, and facilitate the development of new, more effective therapies.

### 2.2.1 Wound healing stages

Wound healing is a multifaceted and dynamic biological process aimed at restoring the structure and function of injured tissue. It progresses through a series of interconnected and overlapping stages: hemostasis, inflammation, proliferation, and tissue remodelling. Hemostasis (coagulation); the first phase which begins with injury to the microvascular bed, leading to fibrin clot formation, degranulation, along with blood coagulation and platelet aggregation. Inflammatory phase, the second phase is characterised by the infiltration of the wound and the surrounding tissue by inflammatory cells including neutrophils and monocytes infiltrate within 24 to 72 hours post-injury. Then lymphocytes migrate to the wound area, where the cell debris and pathogens are removed (Brumberg et al. 2021; Hong et al. 2023).

The proliferation phase, which is the third stage of wound healing, occurs between days 3 and 14 after injury. During this period, the initial fibrin matrix is replaced by newly formed granulation tissue. The final phase, known as maturation or remodeling, is governed by a balance between the proteolytic activity of matrix metalloproteinases (MMPs) and the regulatory effects of their tissue inhibitors (TIMPs). (Alven et al. 2022; Choudhary et al. 2024; Erlyn et al. 2024). This leads to the development of thick scar tissue, which is marked by the absence of cells and blood vessels.

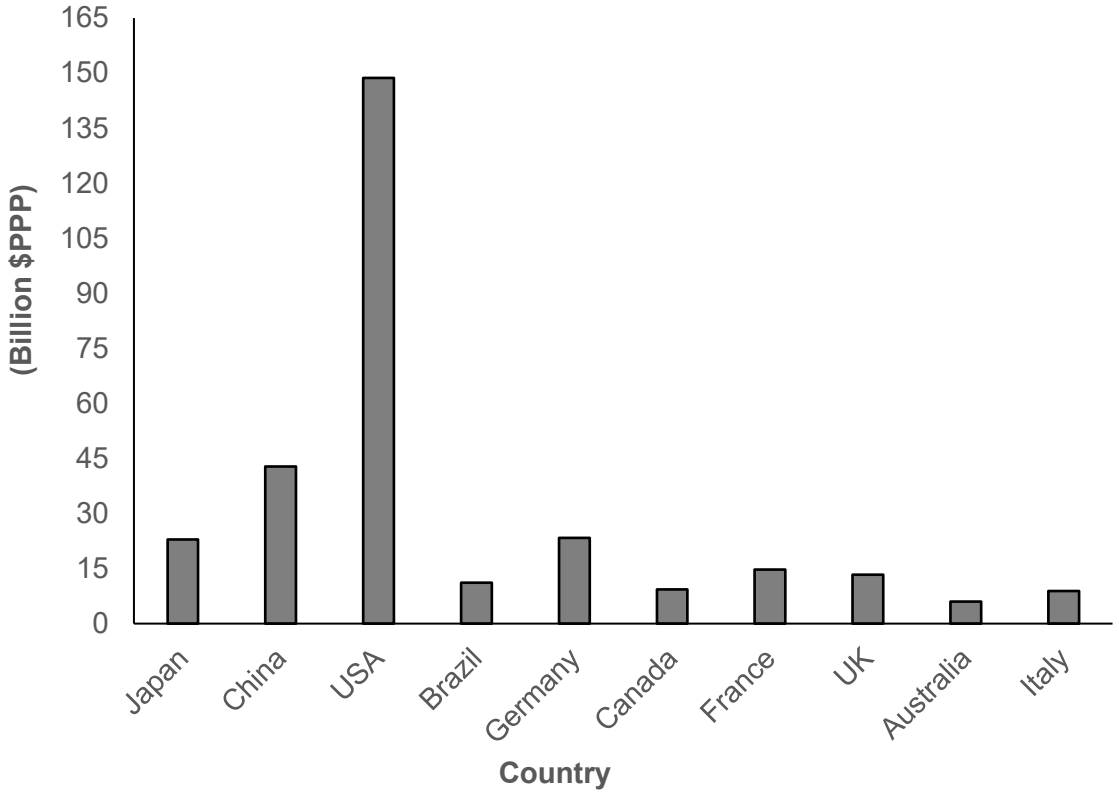


**Figure 2.3 Global top 10 spenders on wound care provision.** The USA leads in expenditure, followed by China, measured in billion PPP dollars (Queen et al. 2024).

Acute wounds can be repaired by the natural wound healing process over a period of 8 to 12 weeks as shown in Figure (2.4 a), depending on the depth and size of the damaged skin tissue. In contrast, chronic wounds may resulting in considerably more severe outcomes due to impaired normal wound healing caused by a complex array of pathogenic factors. Unlike acute wounds, the healing process in chronic wounds typically stops during the early inflammatory phase. This delay can persist for up to 12 weeks after the injury, preventing progression to the proliferation and healing stages as shown in Figure (2.4b). The key characteristics of chronic wounds include a prolonged inflammatory phase and excessive accumulation of neutrophils within the wound site (Brumberg et al. 2021; Falanga et al. 2022; Raziyeva et al. 2021). The presence of ongoing infections indicates a dominance of MMP levels over the respective tissue inhibitors.

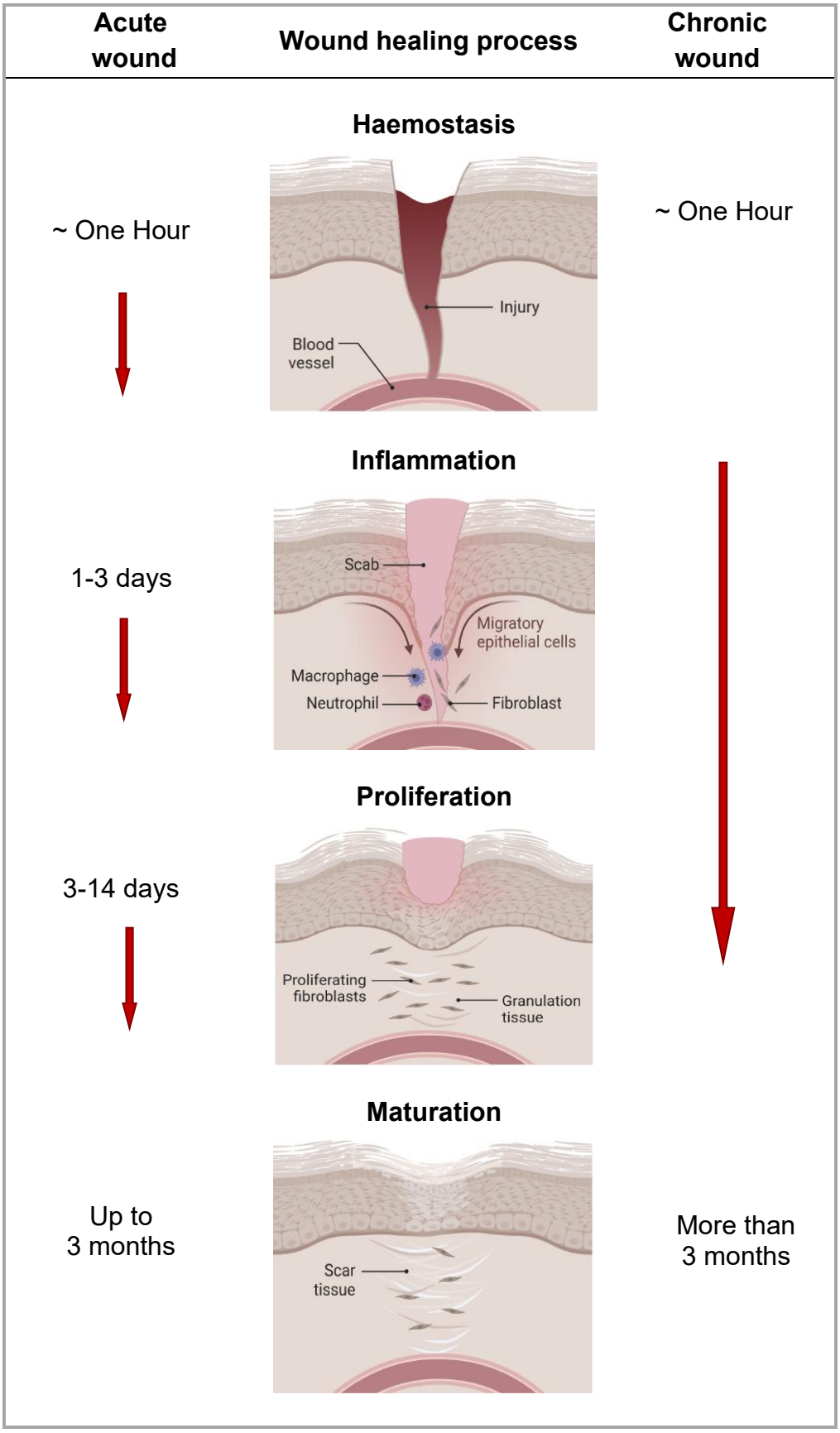


**Figure 2.4 llustration of wound healing process.** Prolonged inflammation delays the healing of chronic wounds. Figures obtained from BioRender.

Bacteria are among the primary contributors to wound complications and delayed healing in chronic wounds (Awuor et al. 2023). Bacteria interfere with the formation of new blood vessels, resulting in chronic hypoxia and reduced access to essential micronutrients. Moreover, in approximately 60% of these wounds, bacterial contamination can lead to the formation of biofilms (Bagheri et al. 2022). Biofilms are a very fine layer rich in glycoprotein, which contributes to spread bacteria by adhering to the wound layer (Wakade & Shende, 2021). The most isolated bacterium in these wounds is *Staphylococcus aureus* (39%), followed by *Escherichia coli* (19%), *Pseudomonas aeruginosa* (12%), and methicillin-resistant *S. aureus* (9%). Other less prevalent bacterial species account for the remaining 21% of chronic wound infections (Awuor et al. 2023), as illustrated in Figure 2.5.

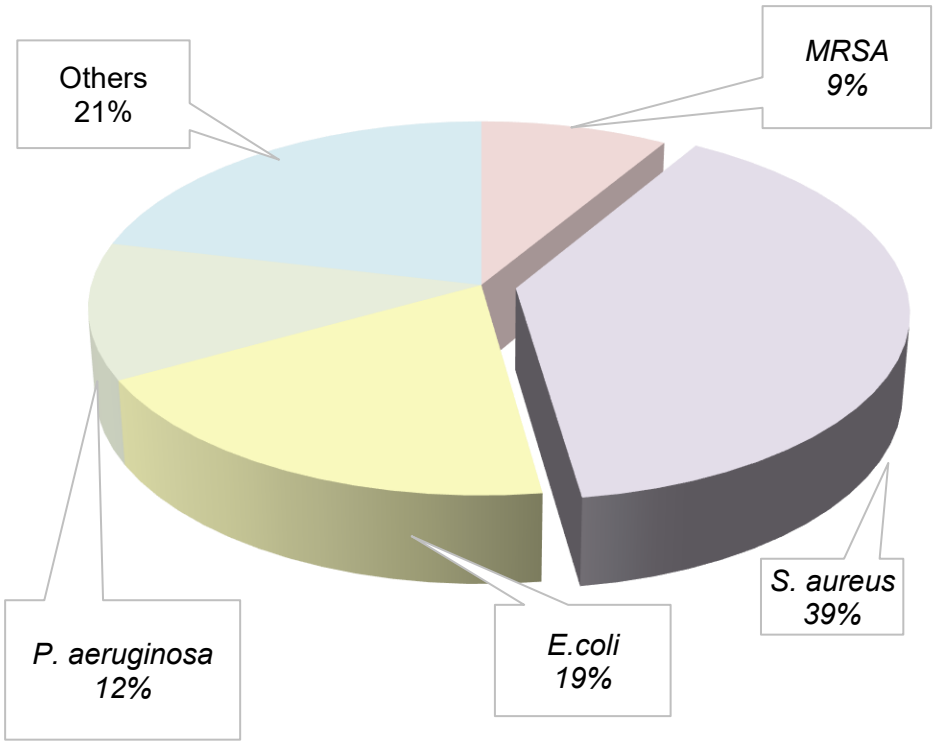


**Figure 2.5 Microorganisms commonly associated with chronic wounds.** *S. aureus* represents the most common pathogen in chronic wounds, followed by *E. coli*, *P. aeruginosa*, and *MRSA*.

Antibiotic wound dressings demonstrate effectiveness in reducing bacterial colonization and promoting healing; however, several significant limitations persist. A primary concern is the development of antibiotic resistance. Overuse or improper application of antibiotic-embedded dressings can encourage bacterial adaptation, leading to resistance that complicates the treatment of subsequent infections (Liang et al. 2022). Antibiotics incorporated in wound dressings may lack efficacy against all bacterial species present in wounds, particularly multidrug-resistant strains (MDR) (Ouyang et al. 2022). Furthermore, antibiotics often exhibit limited activity against biofilms, which contribute to persistent infections (Liang et al. 2022).. These challenges have stimulated research into alternative antibacterial agents, such as nanoparticles, aiming to reduce the emergence of resistance (Zhong et al.2020). Overall, although antibiotic-based wound dressings remain valuable, their inherent limitations demand careful application and continuous innovation in wound care strategies.

## 2.3 Diverse wound dressings and applications for optimal healing

Applying wound dressings to the injured area is the primary therapeutic intervention recommended, this practice protects the affected areas, reduces the inflammatory process, and accelerates healing (Teodoro et al. 2024). The most commonly used wound dressings consist of conventional materials such as cotton, bandages, and gauze. These dressings are affordable and provide excellent absorbency (Zhang et al. 2024). However, traditional wound dressings, despite the long history of use, are not customized to individual needs or specific wound conditions, because of mainly passive role in wound healing, where these dressings cause the exhibit poor barrier function, which may aggravate the wound condition (Chen et al. 2020; Sadeghianmaryan et al. 2024).

In addition, these dressings can lead to dehydration and may cause adhesions when in prolonged contact with the wound (Zhang et al. 2024). It will then potentially result in secondary harm and delay the healing process. These constraints have stimulated the development of innovative wound dressing types.

Traditional wound dressings have been replaced by modern dressings due to the inability to provide adequate drainage, requiring secondary dressing, random debridement, which may remove newly formed granulation tissue during changes, lack of moisture retention, and exposure to bacterial contamination (Brito et al. 2024). Modern dressings contain a highly absorbent layer, which promotes the rapid formation of granulation tissue and supports the movement of cells from the wound periphery towards the center (Brumberg et al. 2021). Due to the varying nature of wounds, particularly those with different causes and healing mechanisms, it is essential to select appropriate dressings tailored to the specific type and healing stage of the wound (Brumberg et al., 2021), as illustrated in Table 2.1.

**Table 2.1** Types of open wounds and the characteristics of wound dressings applied for each type.

| Wound type | Applicable dressing (example) | References |
|---|---|---|
| Surgical wound | • Dressing is made from calcium alginate fibers which form a non-adherent gel upon contact with blood or wound exudate.<br>• Example: Algisite M | Pan et al. 2021 |
| Abrasions | • Film dressings are transparent semipermeable polymer membranes and coated with an adhesive.<br>• Examples: Tegaderm and Opsite dressings. | Shin et al. 2023 |
| lacerations | • Film dressings are transparent semipermeable polymer membranes and coated with an adhesive.<br>• Examples: Tegaderm and Opsite dressings. | Shin et al. 2023 |
| Diabetic foot ulcers | • Semipermeable non-adhesive and adhesive seals presented by foams and hydrocolloids Examples: UrgoStart contact dressing Allevyn, Biatain | Brumberg et al . 2021 |
| Pressure ulcers | • Semipermeable non-adhesive foam dressings and hydrocolloids.<br>Examples: Mepilex Ag, Signal$^{TM}$, and DuoDerm ExtraThin | Brumberg et al. 2021 |
| Chronic venous ulcers | • Semipermeable foam dressings Examples: Mepilex, Allevyn, Contreet Ag, Coloplast | Shi et al. 2020 |

Modern wound dressings are typically semi-occlusive or occlusive and are primarily made from polymers (Table 2.2). These dressings are categorised into interactive (films and foams), and advanced interactive (hydrocolloids and hydrogels) (Abruzzo et al. 2021; Assis et al. 202; Przekora et al. 2020). Film dressings are transparent, adhesive, and porous materials that serve as bacterial barriers. Due to limited fluid absorption capacity, film dressings are not appropriate for wounds with heavy exudate. Film dressings are commonly used as secondary coverings placed over primary dressings. Examples such as Tegaderm™ and Hydrofilm™ are suitable for managing superficial wounds and abrasions (Eskens et al. 2020; Michelin et al. 2021; Savina et al. 2021). Foams are semi-permeable dressings consisting of a hydrophobic layer and a hydrophilic foam layer that faces the wound such as Askina Foam™.

These dressings are typically used to treat ulcers in the lower extremities (Vivcharenko et al. 2021; Wang et al. 2021a). The main drawback of these dressings is the need for frequent replacement.

Hydrocolloids are an elastic matrix containing hydrophilic polymers, can absorb large amounts of fluid, maintain a moist environment, and protect the wound from bacteria. Hydrocolloids have an outer layer that acts as an effective barrier without the need for secondary dressings, and accelerate healing by promoting autolysis, which helps remove dead tissue Hydrogels are highly polymeric materials, capable of maintaining a moist wound environment that promotes self-debridement (Lauranoa et al. 2022). Hydrogel-based dressings are widely used on exuding wounds such as ulcers, can reduce the wound temperature by up to $5C^0$, easy to remove without causing patient pain, and promote tissue granulation, and facilitate autolysis (Brumberg et al. 2021; Hawthorne et al. 2021). However, a secondary dressing is often required to ensure adherence to the wound.

The concept of an ideal dressing varies based on the wound's type, location, size, depth, secretions, inflammation, and adhesion. According to the European Wound Management Association (EWMA), ideal dressings should balance moisture, protect from infection, facilitate debridement, and promote epithelialization (Ansari et al. 2024; Ousey et al. 2018; Rippon et al. 2021), durable, act as a microbial barrier, absorb fluids, eliminate excess secretions, and prevent leakage. Dressings should alleviate pain, be comfortable, easy to apply, and biodegradable to prevent painful removal and tissue damage, biocompatible, non-toxic, cost-effective, and keep cells viable while reducing necrosis (Firlar et al. 2022; Nguyen et al. 2023; Seçil & Derman, 2023; Vivcharenko & Przekora, 2021). Ideal dressings have a porous structure, antioxidant properties, and haemostatic capabilities (Ji et al. 2024).

Finally, ideal dressings must exhibit mechanical properties, anti-inflammatory activity, and promote angiogenesis and granulation tissue regeneration (Shang et al. 2024) (Figure 2.6).

**Table 2.2** Advantages and disadvantages of types of wound dressings materials.

| Wound dressing | Advantages | Disadvantages | References |
|---|---|---|---|
| Film | • Semi-permeable, facilitating gas exchange while preventing external bacterial entry.<br>• Flexible and moisture retentive.<br>• Self-adhering.<br>• Enables visual wound monitoring. | • Non-absorbent and impermeable to fluid; thus, they can cause maceration. | Britto et al. 2024 |
| Foam | • Highly absorbent.<br>• promotes autolytic debridement.<br>• permeable to gases and water vapors. | • Requires additional dressing.<br>• Unsuitabe for dry wounds and dry scars. | Aduba et al. 2016; Kirwan et al. 2016 |
| Hydro-colloids | • Maintain moist environment inside the wound. | • Formation of a thick, yellow, malodorous gel. Gel may be mistaken for signs of wound infection.<br>• Can cause maceration of the surrounding skin. | Sood et al. 2014 |
| Hydrogel dressing | • Capable of absorbing fluids and retaining optimal wound moisture, facilitating cell proliferation and migration.<br>• Resemblance to natural tissue, adaptability to shape, tissue adherence, intelligent medication release, and self-repair capabilities. | • Inadequate encapsulation.<br>• Insufficient control. | Zhu et al. 2023; Bartkowiakband Li al. 2023 |

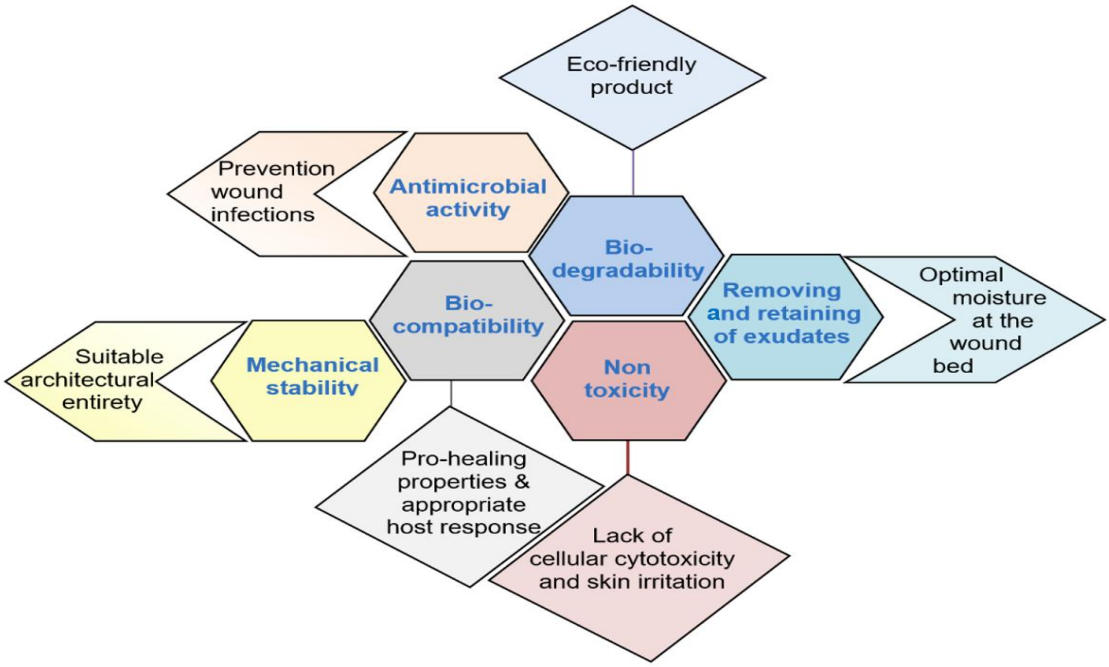


**Figure 2.6 Ideal dressing properties.** A schematic representation of the most important requirements that wounds need to heal and the effective role of each requirement in accelerating the healing of skin wounds.

## 2.4 Hydrogel technology in wound dressing

Due to the porous three-dimensional cross-linked structure and high-water content, hydrogels offer significant advantages in wound dressing (Op't Veld et al. 2020; Yu et al. 2022). Hydrogel dressings are formulated to maintain a moist environment that enhances wound healing. A cooling effect is achieved by reducing the wound surface temperature by up to 5 °C, which helps alleviate pain. Easy removal from the wound bed minimizes tissue trauma. Hydrogel dressings serve as a barrier against microbial penetration, prevent tissue dehydration, and promote autolytic debridement through rehydration of necrotic tissue. This makes the dressings suitable for acute and chronic wounds and promotes re-epithelialization without leaving residue (Tavakoli et al. 2020; Stoica et al. 2020; Vasile et al. 2020; Xu et al. 2020). The soft and flexible nature of these dressings makes dressings compatible with human skin during movement (Liang et al. 2021). This combination of characteristics makes hydrogels highly effective and versatile in wound care applications.

### 2.4.1 Cross-linking polymeric networks for hydrogel formation

Cross-linked polymer networks consist of polymer chains that are interconnected to create a stable three-dimensional structure, which improves the material's mechanical strength, elasticity, and overall structural stability. Polymeric networks of hydrogels are created through chemical cross-linking or physical cross-linking (Figure 2.7). The method of cross-linking determines the chemical and physical characteristics, and functions of the hydrogel dressing (Alven et al. 2022). Chemical cross-linking involves covalent bonding within the components, enhancing mechanical integrity and degradation resistance, such as conjugation reaction, free radical polymerization, and enzymatic. Conjugation reaction cross-linking can be achieved through mild conditions such as the Schiff's base reaction, Michael addition reaction, and Diels–Alder addition reaction (Lu et al. 2021). Free radical polymerisation; involves the generation of free radicals via heating, electrolysis, high-energy radiation ultraviolet radiation, and plasma initiation (Su et al. 2021). Enzymatic cross-linking occurs with natural polymers promoted by enzymes like urease, tyrosinase, transglutaminase, and horseradish peroxidase (HRP) (Liu et al. 2020; Pham et al. 2020; Zhong et al. 2020a). This process preserves biological activity and enables fast gelation without generating harmful by substances.

Physical crosslinking is formed through interactions between molecules and can easily transition from liquid to gel when exposed to various ambient conditions such as crystallisation, ionic interaction, hydrogen bonding, and hydrophobic interaction. Crystallisation through freezing and thawing; a common physical bonding method. During freezing cycle, ice crystals arrange polymer chains around themselves, and upon melting, a microporous structure forms. The temperature, polymer component content, time, and number of cycles,

can be adjusted to achieve different pore sizes, morphologies, mechanical strengths, and other characteristics (Xiao et al. 2021).

Meanwhile, Ionic interaction involves reversible bonding between oppositely charged functional groups. This dynamic process is an efficient approach for forming stable yet adaptable connections within polymer networks or between bioactive compounds (Su et al. 2021; Wang et al. 2020c). Hydrogen bonding: a dynamic interaction, allows hydrogels to spontaneously rebuild after being destroyed (Bi et al. 2020; Su et al. 2021;). Hydrophobic interaction refers to the cross-linking of hydrogels within water-soluble polymers that possess hydrophobic terminal groups, side chains, or monomer units.

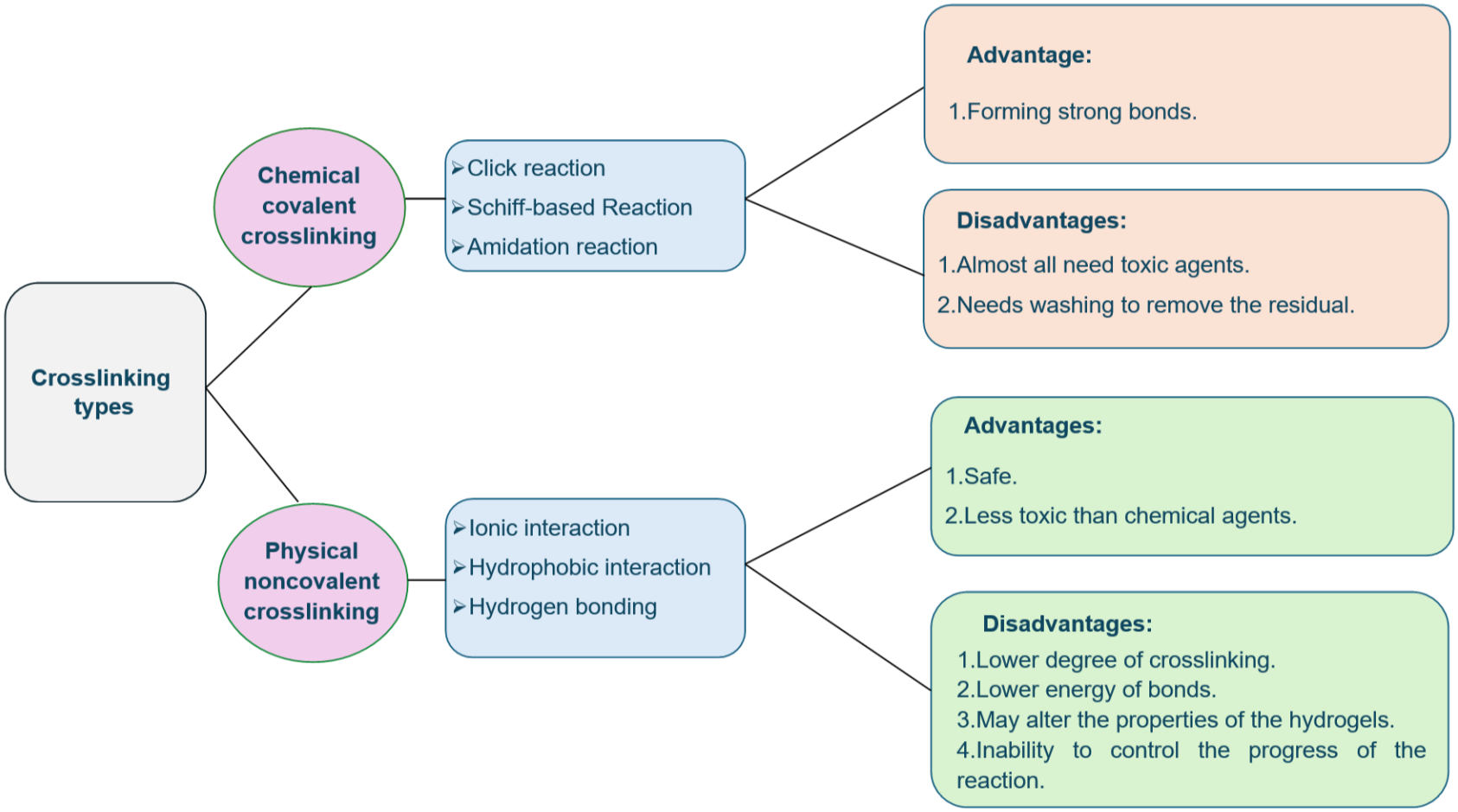


**Figure 2.7** **Types of cross-linking in polymer networks for wound dressings.** Advantages and disadvantages of each cross-linking method.

### 2.4.2 Types of hydrogel polymers

The most commonly utilized hydrogels as biomaterials are swellable polymers, derived from both natural and synthetic polymers (Figure 2.8). Natural polymers are known as biopolymers which are large molecules derived from plant, animal and microbial sources. Although biopolymers can roughly mimic native extracellular matrix (ECM) and cellular milieu, these polymers are importantly heterogeneous and may undergo significant biodegradation that can alter the material's physicochemical properties as well as their behavior (Alven et al. 2022). Synthetic polymers are chemically manufactured polymers that exhibit properties that can be controlled, and predictable, such as low production costs, easy shape control, and regulated degradation, along with consistent chemical and physical characteristics, as well as reliable mechanical strength. In addition, synthetic polymer is purer and does not contain impurities (Alven et al. 2022; Negut et al. 2020). Therefore, synthetic polymers have gained increasing attention (Zhu et al. 2023). These polymers are characterised by good structural controllability, which provides new opportunities in wound dressing application.

Natural polymers such as chitosan, alginate, collagen, gelatin, hyaluronic acid, fibrin, cellulose, and DNA are characterised by providing innate biodegradability, biocompatibility, and presence of bioactive adhesion sites. In addition, the design of hydrogels can be diversified through modification of these polymers via cross-linking or self-assembly. Chitosan (CS), a natural cationic polysaccharide derived from chitin, possesses unique properties including high liquid absorption due to hydrophilic nature, wetting properties, biocompatibility, biodegradability, antibacterial activity against most bacteria, non-toxicity, and cost-effectiveness (Jia et al. 2023). These properties enable chitosan-containing hydrogels to exhibit swelling behavior,

absorb secretions and prevent secretion accumulation by promoting drainage (Okur et al. 2020; Zeng et al. 2022). However, a common drawback of these dressings is poor mechanical properties (Alven et al. 2022), such as limited elasticity and stability in physiological media (Okur et al. 2020; Wang et al. 2023). To address this issue, chitosan is often combined with synthetic polymer to improve the performance of dressings by enhancing elasticity and ductility.

Synthetic polymers include polyethylene glycol, polyethylene oxide, poly(ε-caprolactone), and polyglycolic acid. Polyethylene glycol (PEG), a synthetic viscous amphiphilic polymer (Oliveira et al. 2023; Prete et al. 2023), plays a crucial role in hydrogel dressings. PEG endows the hydrogel with a high modified mechanical property, and easy control over structure and chemical composition (Zhu. 2022; Mir et al. 2018). The advantages of PEG include biocompatibility, biodegradability, water solubility, transparency, non-toxicity, low cost, high flexibility, heat resistance, which result in high swelling capacity of the hydrogel, PEG prevents scar formation, by facilitating protein absorption, cell adhesion and proliferation (Jonidi et al. 2024; Ribeiro et al. 2024; Su et al. 2021; Zhang et al. 2022). The high hydrophilicity of PEG enhances exudate absorption and the swelling of hydrogels (Vakil et al. 2022). Thus, the dressing maintains a moist environment that may reduce pain.