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REVIEW

Deep Eutectic Solvents in Chronic Wound Management: Current Developments and Future Prospects

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Abstract: Chronic wounds pose a significant and growing global health challenge, affecting millions of individuals and often leading to prolonged suffering and increased healthcare costs. A major barrier to effective healing is wound infection, which disrupts the natural repair process and contributes to the chronicity. Therefore, innovative strategies for infection control are urgently required. Deep Eutectic Solvents (DESs) have recently gained attention as promising drug delivery systems owing to their multifunctional properties. In addition to serving as penetration enhancers that improve drug permeation, DESs exhibit intrinsic antimicrobial and antibiofilm activities, making them attractive candidates for managing infected wounds. This review highlights the fundamentals of DESs in the context of chronic wound management. It provides an overview of the wound healing process, pathophysiology of chronic wounds, and the role of biofilms in persistent infections. It further explores the dual role of DESs as penetration enhancers and antibiofilm agents, summarizing the recent DES-based formulations under investigation. Finally, this review discusses the current challenges and future prospects of integrating DESs into clinical practice. Collectively, DESs represent novel and versatile therapeutic platforms that have the potential to transform the treatment landscape of chronic wound healing.

Keywords: antibiofilm agent, chronic wound, deep eutectic solvent, penetration enhancer, wound healing

Introduction

Injuries caused by surgery, pathological conditions (eg, diabetes and vascular diseases), or extrinsic factors (eg, cuts, burns, and pressure) that compromise the structure and integrity of the skin are defined as acute and/or chronic wounds.[1,2] Acute wounds typically heal within 4–6 weeks with complete restoration of functional and anatomical skin integrity.[3] However, chronic wounds take longer to heal, typically requiring more than 6 weeks,[3] due to pathological conditions such as venous stasis, autoimmune diseases and diabetes.[2] Prior infections, tumors, and inflammation may also result in chronic wounds. Delays in the healing process provide opportunities for bacterial infection and growth, thereby hindering optimal and functional restoration of skin integrity.[2] Diabetic foot ulcers are one of the most common chronic wounds, and have been estimated to affect 9.1–26.1 million people annually.[4] Basic treatment strategies for chronic wounds include sharp debridement, surgical drainage of abscesses, debridement of osteomyelitis, and antimicrobial therapy.[5] Wound dressings can be used to create and maintain a moist environment to enhance wound healing. A moist wound environment reduces pain and scarring, facilitates autolytic debridement and activates collagen synthesis.[6] It also boosts the migration of keratinocytes to the surface of the wound and supports the function of growth factors, nutrients, and other soluble mediators in the wound microenvironment. Moist wound dressings include foams, films, hydrogels, hydrocolloids and alginates.[6]

The stratum corneum (SC) is the outermost layer of skin that is non-living in nature. This renders the skin impermeable and hinders the drug permeation.[7,8] Only a few drug molecules can permeate intact skin. Specifically, molecules with low molecular weight (<500 Da) and optimal lipophilicity (log$P$ = 1–3) can penetrate the skin.[8]

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Therefore, chemical penetration enhancers (CPEs) such as azone, terpenoids, ethanol, sulfoxide, and glycosides are used to enhance drug permeation.[9] CPEs enable drug molecules to permeate and accumulate in deeper skin regions,[10] resulting in an increased drug concentration at the site of injury including the biofilms, thereby enhancing wound healing.

Biofilm formation can occur in wounds. A biofilm contains microorganisms entrenched in extracellular polymeric substances (EPS). It is comprised of bacteria, fungi, viruses, proteins, extracellular DNA, and other biogenic factors.[11] Biofilms are more common in chronic wounds than in acute wounds, and are attributed to delayed wound healing.[12] This hinders effective treatment of skin and soft tissue infections.[13] Antibiofilm agents disrupt and prevent biofilm formation,[14] thereby enhancing chronic wound healing. Antibiofilm agents include antibiotics, surfactants, antimicrobial peptides, bacteriophages, quorum sensing inhibitors, nanoparticles and hydrogels.[15]

Deep eutectic solvents (DESs) are eutectic mixtures of two or three compounds associated with hydrogen bond interactions.[16] The melting point of DES is lower than that of each individual component. Usually, DES exists in the liquid state at room temperature.[16] DES can act as penetration enhancer[17] and antibiofilm agent[15] for chronic wound healing. However, few studies have been conducted to explore the properties of DES as penetration enhancers and antibiofilm agents.

This review provides a brief overview of DES, the physiology of wound healing, the features of chronic wounds, and biofilm formation. Here, we examine the role of DES as both a penetration enhancer and antibiofilm agent for chronic wound healing. Additionally, this review provides an insight into current DES-based formulations, and discusses the obstacles and future perspectives associated with the use of DES for chronic wound healing.

Deep Eutectic Solvents

A DES contains two or three components that interact with each other through hydrogen bonding to form a eutectic mixture.[16] The DES exhibits a melting point that falls below that of each constituent compound. The characteristic feature of DES is a very large depression of the freezing point, resulting in a liquid state of the mixture at temperatures below 150°C.[16] Typically, DESs are prepared by mixing a quaternary ammonium salt with metal salts or a hydrogen bond donor that has the potential to form a complex by interacting with the halide ion present in the quaternary ammonium salt.[16,18]

DESs are considered a new class of ionic liquids (IL) because of their many characteristic similarities. DESs and ILs are two different types of solvents; however, they have been used interchangeably in literature. ILs are composed of one type of discrete anion and cation, whereas DESs are formed from a eutectic mixture of Lewis or Brønsted acids and bases containing a variety of cationic and/or anionic species.[19] High thermal stability, low volatility, low vapor pressure, and tunable polarity are some of the general characteristics of both DESs and ILs. DESs are biodegradable, inexpensive, non-toxic, and easy to prepare; however, ILs are non-biodegradable, expensive, and highly toxic.[20]

As a pioneer, Abbott et al illustrated significantly deep melting point depression of eutectic mixture of hydrogen bond donors (HBDs) and acceptors (HBAs).[21] A 1:2 mole fraction combination of choline chloride (melting point = 302°C) and urea (melting point = 133°C) resulted in a eutectic composition (melting point = 12°C), which is a liquid at ambient temperature.[21] Urea, glycerol, l-lactic acid, and d-fructose are commonly used HBDs to prepare DES, whereas examples of HBAs include choline chloride, nicotinic acid, l-alanine, and methyltriphenylphosphonium bromide.[22]

Classification of DESs

DESs are prepared by mixing HBD and HBA at a certain molar ratio, which results in depression of the melting point of the solvent.[23] The DESs were classified into four categories, as listed in Table 1.[19,24] DESs can be represented by general formula[19] as follows:

$$Cat^{+}X^{-}zY$$

where Cat^{+} is generally a phosphonium, ammonium, or sulfonium cation; X is a Lewis base, which is usually a halide anion; Y is a Lewis or Brønsted acid; and z represents the number of Y molecules interacting with the anion.

Type I DESs consist of quaternary ammonium salts and metal chlorides, whereas Type II DESs contain quaternary ammonium salts and hydrated metal chlorides.[25] Type I DESs have a high melting point owing to the presence of non-

Table 1 Classification of DESs into Four categories[19,24] Cat^+ is Generally a Phosphonium, Ammonium, or Sulfonium Cation; X is a Lewis Base, That is Usually a Halide Anion; and z Represents the Number of Molecules Interacting with Anion That Can Be Metal Salt, Hydrated Metal Salt or Hydrogen Bond Donor

TYPE I $Cat^+X^-zMCl_x$ Metal (M) = Zn, Sn, Fe, Al, Ga, In MCl_x is metal salt.	**TYPE II** $Cat^+X^-zMCl_x\cdot yH_2O$ Metal (M) = Cr, Co, Cu, Ni, Fe $MCl_x\cdot yH_2O$ is hydrated metal salt.
TYPE III Cat^+X^-zRZ Z = $CONH_2$, COOH, OH RZ is hydrogen bond donor.	**TYPE IV** $MCl_x + RZ = MCl_{x-1}^+ \cdot RZ + MCl_{x+1}^-$ Metal (M) = Al, Zn and Z = $CONH_2$, OH MCl_x is metal salt and RZ is hydrogen bond donor.

hydrated metal halides. Type III DESs consist of quaternary ammonium salts and HBDs, whereas Type IV DESs are composed of metal chlorides and HBDs (eg, ethylene glycol and urea).[25] Recently, new types of DESs have been proposed as Type V, which are composed of non-ionic HBAs and HBDs.[26]

Among the different categories, Type III DESs have been extensively studied and are usually based on choline chloride and various hydrogen-bond donors.[23,24] Choline chloride is inexpensive, biodegradable, and non-toxic, making it a natural additive for several animal species.[24]

Based on their aqueous solubility, Type III DESs can be further classified as hydrophilic and hydrophobic DESs.[27] Hydrophilic DESs are prepared from small quaternary ammonium salts (eg, choline chloride) and HBDs (eg, urea, ethylene glycol, glycerol, and small organic acids). They are highly hygroscopic, have a relatively low viscosity and reasonable conductivity.[27] Hydrophobic DESs are composed of hydrophobic compounds, such as thymol, menthol, tetrabutylammonium bromide, and fatty acids as HBAs, along with carboxylic acids and long-alkyl-chain alcohols as HBDs.[24] These solvents are promising for the extraction of natural compounds and metals from aqueous solutions. Compared to hydrophilic DESs, hydrophobic DESs have higher viscosities and low conductivity.[27]

Active pharmaceutical ingredients (APIs) such as lidocaine, ibuprofen, and phenylacetic acid can be used to prepare DES; in such cases, DESs are referred to as therapeutic DESs (THEDES).[24] A DES, which consists of a polymerizable monomer and conductive ion salt along with other components, and possesses the capability to undergo polymerization reactions, is known as a polymerizable DES (PDES). In general, PDESs are used to prepare gel-based formulations.[28]

Type III DES are sometimes referred to as natural DES (NADES).[24] NADESs mostly contain natural primary metabolites such as sugars, sugar alcohols, amines, organic acids, and amino acids, along with water at specific molar ratios.[29] Depending on the nature of the components, NADESs have been categorized into five major groups:[24,30]

- Ionic liquids (prepared from an acid and a base)
- Neutral liquids (composed of sugars only, or sugars with other polyalcohols)
- Neutral liquids with acids (composed of sugars/polyalcohols and organic acids)
- Neutral liquids with bases (composed of sugars/polyalcohols and organic bases)
- Amino acid-containing NADES (composed of amino acids and organic acids/sugars)

Preparation and Characterization of DESs

Common methods used for the preparation of DES include heating and stirring, grinding, vacuum evaporation, and freeze-drying.[31] In the heating and stirring method, the compounds are mixed and heated under constant stirring until a homogenous liquid is obtained. The temperature ranges between 50–100°C because at high temperatures, degradation of the DES may occur due to esterification reaction.[24] A mortar and pestle is used for the grinding method, where the compounds are mixed and crushed at room temperature to form a clear liquid.[24] In the evaporation method, the components are dissolved in water, then most of the water is evaporated under vacuum at 50°C, and finally dried in a desiccator until a constant weight is achieved.[31] In the freeze-drying method, an aqueous solution of DES or the individual compounds used for DES preparation are freeze-dried to sublimate water to obtain a pure form of solvent.[32]

Methods assisted by microwaves and ultrasound have been used to prepare DES. The microwave-assisted method is ultra-fast because, in this method, individual components (ingredients) are mixed in glass bottles and exposed to microwaves for a few seconds (< 30s).[32] In the ultrasound (US)-assisted method, various concentrations (10%, 30%, 75%) of components are prepared in distilled water, then kept in glass vials fitted with screw cap and finally exposed to US waves (37 KHz, 30 W) at 50°C until homogenous liquids are obtained.[32]

Various tools and techniques have been employed to characterize DESs. Intermolecular interactions between DES components (or between drug and DES components in THEDES) can be identified using Fourier transform infrared spectroscopy (FTIR).[33] Moreover, proton and carbon nuclear magnetic resonance (NMR) spectroscopy can confirm the chemical structure and purity of the DESs and drug-DES systems.[33] Differential scanning calorimetry (DSC)[33] and thermogravimetric analysis (TGA)[34] can be employed to identify the phase transformation and determine the thermal decomposition behavior of the DESs respectively.

UV-Vis spectroscopy[25,33] and high-performance liquid chromatography (HPLC)[25] are commonly employed to identify the concentration of API (drug) dissolved in the DESs, which in turn helps to determine the drug solubility and release profile. Likewise, a pycnometer can be used to determine the density and a viscometer to determine the viscosity of the DESs.[25] A conductivity meter,[35] pH meter,[35] Du Noüy ring method,[36] and refractometer[36] can be employed to determine the electrical conductivity, pH, surface tension, and refractive index of the DESs, respectively.

Chronic Wound

Physiology of Wound Healing

Wound healing is achieved through four overlapping and highly coordinated phases: hemostasis, inflammation, proliferation and remodeling.[37] Table 2 shows the phases of wound healing and the key events.

Hemostasis

Hemostasis is initiated when the integrity of the skin is compromised. Vasoconstriction occurs to prevent blood loss and clot formation seals the vessel. Platelet aggregates result in the formation of an initial hemostatic plug, and initiate coagulation and complement cascades. Prothrombin is activated to form thrombin within the tissue, which, in turn, cleaves fibrinogen to generate fibrin, forming a clot with platelets and plasma fibronectin. In addition, various mediators are released to attract macrophages and fibroblasts to the injury site.[37,38]

Table 2 Phases of Wound Healing with Key Events

Phases	Key Events	References
Hemostasis	• Vasoconstriction prevents blood loss. • Platelets aggregation forms initial hemostatic plug.	[37,38]
Inflammation	• Release of cytokines and growth factors. • Migration of neutrophils, monocytes, lymphocytes and plasma cells to the site of injury. • Release of reactive oxygen species and proteases by neutrophils. • Vasodilation and phagocytosis	[39,40]
Proliferation	• Re-epithelization covers the wound surface with new skin. • Neovascularization (angiogenesis) restores vascular integrity. • Granulation fills defective tissue with new connective tissue.	[39]
Remodeling	• Wound contraction closes full-thickness wound. • Maturation involves the conversion of the extracellular matrix from granulation to scar tissue.	[41]

Inflammatory Phase

The inflammatory phase is triggered by the release of mediators from injured capillaries and tissue cells, activated platelets (along with their cytokines), and other by-products of hemostasis. Neutrophils migrate to the injury site and initiate wound repair by activating the fibroblasts and epithelial cells. In addition, monocytes, lymphocytes, and plasma cells arrive at the site of injury.[39] Local vessels dilate as a result of coagulation and complement cascades during the hemostasis phase. Bradykinin (generated by the coagulation cascade) and anaphylatoxins, such as C3a and C5a (generated by the complement cascade), increase the permeability of blood vessels and facilitate the migration of inflammatory cells at the wound site.[37] Monocytes differentiate into macrophages, which results in the formation of phagocytic cells. They ingest surviving microorganisms, fibrin clots, dead neutrophils, and other cellular debris. In addition, they synthesize NO (nitric oxide) and secrete cytokines that initiate wound repair.[39]

Proliferative Phase

Degradation of the initial fibrin-platelet matrix and invasion of endothelial cells and fibroblasts occur at the beginning of the proliferative phase. This phase overlaps with the inflammatory phase. Cellular migration through the fibrin clot and provisional matrix is facilitated by the secretion of proteases belonging to serine, cystine, and matrix metalloproteinase (MMP) families.[37] The major events in this phase include covering the wound surface with new skin (re-epithelialization), restoration of vascular integrity (neovascularization), and filling of defective tissue with new connective tissue (granulation).[39]

Keratinocytes completely cover the defective skin surface and initiate re-epithelialization. Locally released growth factors stimulate keratinocyte proliferation. New keratinocytes migrate to the repair site, which requires a fluid environment. Migration involves complex steps that are directed by a chemotactic gradient created by various growth factors. Keratinocytes secrete proteolytic enzymes in the absence of a fluid surface, which enable them to find the moisture necessary for migration.[38,39]

Neovascularization, or angiogenesis, is the process of re-establishing vascular network.[42] This event is stimulated by tissue hypoxia and growth factors. A hypoxic wound environment is created by closure of the wound surface. The fibrin clot formed in the hemostasis phase temporarily covers the wound surface, thereby creating a closed system for angiogenesis to proceed.[39] Hypoxia induces secretion of angiogenic growth factors by macrophages. Additionally, angiogenesis is stimulated by lactic acidosis.[39] Furthermore, fibroblasts and vascular endothelial growth factors stimulate angiogenesis along the edges of wounds.[37,39]

Replacement of the fibrin clot scaffold with new tissue rich in hyaluronic acid, fibronectin, and other extracellular matrices (ECM) results in granulation tissue.[37,39] Such tissue is metabolically active, highly vascular, and supports the proliferation of a variety of cells and proteins, thereby resulting in a pinkish-red appearance.[39] Fibroblasts (dermal cells) are the predominant cell types found in the granulation tissue. Fibroblasts produce collagen, along with other substances that comprise the ECM.[39,43] ECM consists of fibronectin (promotes adhesion and migration), hyaluronan (promotes tissue hydration), collagens/elastin (provides tissue resiliency and strength), and chondroitin sulfate (involves in the regulation, storage, migration, and expression of a variety of substances, including enzymes, growth factors, and coagulation proteins).[39]

Remodeling Phase

During the remodeling phase, wound contraction contributes to the successful closure of full-thickness wounds enhancing the cosmetic appearance.[37,41] It also strengthens the scar because the newly formed epithelium that lacks glandular, follicular, nervous, and vascular components, has to cover a smaller area of the wound. Therefore, a high degree of contraction is desirable for wound healing.[41]

At the end of wound contraction, myofibroblasts disappear by apoptosis or revert to a quiescent fibroblastic phenotype because of reduced tension within the ECM. The components of the ECM change to ascertain the integrity, strength, and function of the replacement tissue during the remodeling phase. The final phase of wound repair involves the conversion of the ECM from granulation to scar tissue, which is referred to as maturation. Proteoglycans replace hyaluronan, supporting the aggregation and deposition of collagen fibers, which in turn provides tensile strength to the wound.[41] ECM remodeling depends on the presence of proteolytic enzymes produced by mesenchymal and inflammatory cells,[41] such as matrix metalloproteinases (MMPs),[44] cathepsins, and serine.[41] Wound remodeling may continue for up to two years, and during this period, collagen content does not increase; however, under the influence of local mechanical factors, collagen fibers rearrange themselves into a more organized lattice-like structure, thereby increasing the tensile strength of scar tissue.[41]

Features of Chronic Wounds

Disruption of the normal wound healing process results in the development of chronic wounds, which pose a significant challenge to patients, healthcare professionals, and the overall healthcare system.[45] Chronic wounds do not follow the predictable stages. The three major etiologies include venous, diabetic, and pressure ulcers.[46] Venous ulcers occur in the legs and are primarily caused by dysfunctionalization of valves. Diabetic ulcers start as small scratches that usually go unnoticed due to impaired nerves in the lower extremities. However, they may become seriously infected due to poor circulation, compromised immune systems, and damaged capillaries. Pressure ulcers can affect bedridden patients who have limited mobility.[46] Although different chronic wounds have different etiologies at the molecular level, they express some common features, such as persistent infections, and deficiency of stem cells that are often dysfunctional, along with elevated levels of proinflammatory cytokines, reactive oxygen species (ROS), senescent cells, and proteases.[5]

Microorganisms, repeated tissue injury, and platelet-derived factors such as transforming growth factor-β (TGF-β) or ECM fragment molecules result in a constant influx of immune cells, which in turn amplifies the proinflammatory cytokine cascade, leading to higher levels of proteases.[5] Proteases are regulated by their inhibitors in acute wounds; however, in chronic wounds, protease levels increase in comparison with the levels of their respective inhibitors, which subsequently results in the destruction of ECM and degradation of growth factors.[5,47] The proteolytic destruction of the ECM attracts inflammatory cells and prevents the proliferative phase of wound healing, thereby magnifying the inflammation cycle.[5]

ROS produced by immune cells at low concentrations can protect microorganisms. However, in chronic wounds, hypoxic and inflammatory environments lead to uncontrolled ROS production, which damages ECM proteins and cause cell damage.[5,48] Moreover, the presence of senescent cells with impaired proliferative and secretory capacities is a characteristic feature of chronic wounds, which makes them unresponsive to typical wound-healing signals. Oxidative stress in senescent cells leads to DNA damage-related cell cycle arrest and unusual metabolic changes in patients with diabetes, resulting in defects in intracellular biochemical pathways.[5]

The environment of chronic wounds is alkaline, with pH ranging from 7.2 to 8.9.[46,49] However, acidic environments are essential for fibroblast proliferation, collagen formation, oxygenation, angiogenesis, and macrophage activity. An alkaline environment affects these key processes and prevents healing by further colonization by pathogenic bacteria. Therefore, the occurrence of biofilms is higher in chronic wounds, making them more resistant to biocides.[46]

Biofilm Formation

A bacterial biofilm consists of microorganisms embedded in extracellular polymeric substances (EPS) that adhere to living or inert surface.[11] Biofilms contain not only bacterial cells, but also fungi, viruses, extracellular DNA, proteins, and other biogenic factors. They help bacterial growth by providing a protective environment. Biofilm-producing infections are highly polymicrobial, which further increase virulence and complicate the treatment.[11]

Biofilm formation occurs in five stages, as illustrated in Figure 1.[50] In the first stage, there is reversible attachment of microbes to the surface via flagella, pili, or other surface appendages. The second stage includes irreversible attachment facilitated by EPS secretion. The third stage is characterized by cell proliferation and microcolony formation. Growth and differentiation occur in the fourth stage, resulting in a mature biofilm with features, such as water channels and towering clusters of cells. In the fifth (final) stage, active and/or passive detachment results in the dispersion of biofilm cells.[11,51]

DESs as Penetration Enhancers for Chronic Wound Treatment

Transdermal drug delivery offers advantages such as improved patient compliance owing to its non-invasive nature, avoidance of first pass metabolism, sustained and controlled drug delivery, reduced side effects, and direct access to target sites.[52] Low permeability of the skin is a major challenge in the development of topical and transdermal drug delivery systems.[8] Skin barriers protect the organs from foreign toxins and prevent water loss. The outermost non-living layer of the skin, that is, the stratum corneum (SC), hinders permeation of drugs.[52] Drug molecules with properties such

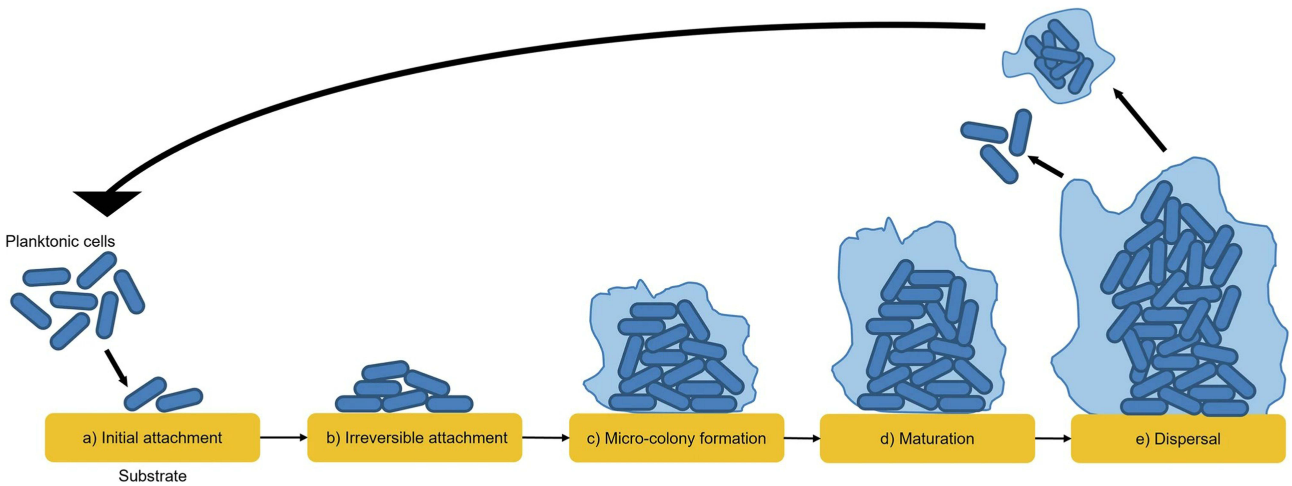

Figure 1 Stages of biofilm formation. Reproduced with permission from *Liu* et al.[50]

as low molecular weight (<500 Da) and optimum lipophilicity (log$P$= 1–3) can easily penetrate the skin. Therefore, only a limited number of molecules can permeate an intact skin.[8]

The "bricks and mortar" model is generally used to depict the SC, which is the main barrier to transdermal permeation of molecules.[53] Figure 2 shows the brick-and-mortar model of the SC.[54] In this model, SC morphologically and functionally represent a two-compartment system consisting of corneocytes (bricks) and intercellular matrix (mortar). Corneocytes are primarily composed of fibrous protein networks, whereas intercellular matrix is mainly composed of neutral lipid.[55] While penetrating the SC, molecules are transported via either the transcellular pathway (corneocytes) or intercellular pathway (tortuous lipid).[53]

The low solubility and permeability of oral drugs render them unsuitable for topical and transdermal delivery systems.[9] Various physical, pharmaceutical, and chemical methods have been developed to address these issues. The most common method employed to enhance skin permeability is the use of chemical penetration enhancers (CPEs) such as ethanol, terpenoids, glycosides, sulfoxides, and azones. CPEs act by modifying SC fluidity, increasing the drug's skin disruption coefficient, and generating hydrophilic pores in the SC to establish drug reservoir.[9] Indeed, CPEs enable drug molecules such as antibiotics, analgesics, and anti-inflammatory agents to penetrate tissues and accumulate in deeper skin regions,[10] thereby increasing the local drug concentration at the wound site and promoting wound healing.

Understanding the mechanisms of action of DES will aid in designing DES-based formulations for the treatment of chronic wounds. DESs penetrate SC primarily through mechanisms, such as liquid modification, protein modification, and lipid exchange.[9] DESs play a crucial role in liquid modification of SC. Hydrophilic DESs act by opening tight junctions and facilitating intercellular transport, whereas hydrophobic DESs act by increasing the transport channels and

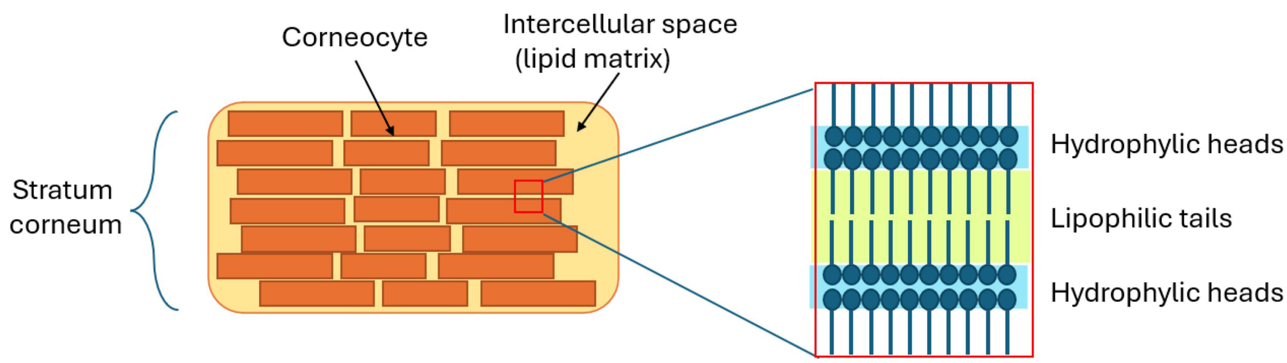

Figure 2 Bricks and mortar model of the SC. Corneocytes are considered bricks and intercellular matrix as mortar. Reproduced with permission from *Banas* et al.[54]

facilitating transcellular transport.[9] In contrast, amphiphilic DESs can pass through the lipid bilayer and destroy the lipid membrane, resulting in increased skin permeability.[9,56] DESs can interact with keratin and disrupt its ordered arrangement in the SC, inducing conformational changes in the protein molecules.[57,58] In addition, DESs can denature keratin to form vacuoles, thereby enhancing drug penetration.[9] When used as drug solvents, DES enhances the penetration of macromolecular drugs through a mechanism known as "lipid exchange." DESs selectively dissolve and replace lipids and facilitate the diffusion of drug molecules into the deeper layers of the skin.[9]

Sakuragi et al reported that DES facilitates lysozyme penetration by interacting with lipids in the SC, thereby exhibiting the property of DES as a skin penetration enhancer for proteins.[59] Additionally, Boscariol et al demonstrated that DES can promote the transdermal permeation of bioactive macromolecules by disrupting the skin.[60] In this study, DES promoted the passage of molecules through the intercellular (composed of lamellar lipids) and transcellular (composed of corneocyte lipid envelopes) routes. Molecules slipped around the lamellar lipids in the intercellular route; however, in the transcellular route, small transient openings were created to allow molecules to pass through.[60] Similarly, in a study by Li et al, DESs enhanced the skin penetration of molecules such as oxymatrine and quercetin.[61] This study showed that DESs are safe for promoting skin permeability.[61] Song et al demonstrated that DES enhanced the skin permeability of L-ascorbic acid.[62] The increased solubility of DES in skin lipids (SC) facilitated the penetration of L-ascorbic acid.[62] Furthermore, in a recent study, Czyrski et al showed that DES enhanced permeation mainly by loosening lipid packing and increasing lipid and protein mobility.[57] In this study, a blend of propylene glycol and DES enhanced the transition of keratin's secondary structure from α-helix to β-sheet conformation.[57]

Owing to the lack of understanding regarding toxicity and safety, only a small number of CPEs have been used for commercial purposes. Therefore, there is an urgent need for novel biodegradable and biocompatible skin-penetration enhancers. DESs can be used as CPE alternatives to harmful organic solvents in the treatment of chronic wounds. In addition, DESs can be used as solubilizing agents, drug reservoirs, stabilizing agents and surfactants.[9]

Chronic wounds are open and lack an intact SC. However, when penetration enhancers such as DESs are used to treat open wounds, they increase the permeation of drugs through the partially intact surrounding skin for localized effect and promote wound healing.[63] Additionally, DESs can penetrate the biofilms in the wounds which is essential to disrupt them and control wound infections.[13]

DESs as Antibiofilm Agents for Chronic Wound Healing

Biofilm formation hinders the treatment of skin and soft tissue infections. A biofilm is a three-dimensional matrix consisting of microbial cells entrenched in an EPS.[13] EPS is primarily made of proteins, polysaccharides, and/or extracellular DNA. The biofilm matrix enhances microorganism survival when exposed to antibiotics, resulting in antimicrobial tolerance and treatment failure.[13] Biofilm formation, which is common in chronic wounds, prevents wound contracture and epithelialization, induces chronic inflammation, prevents normal epidermal differentiation following healing, and disrupts host immune response.[12]

An agent that prevents and disturbs biofilm formation in wounds is commonly known as an antibiofilm agent.[64] Antibiofilm agents target various stages of biofilm formation, such as initial attachment, irreversible attachment, microcolony formation, maturation, and dispersal, to enhance wound healing.[15] Such agents act by targeting cell surface-associated adhesins such as proteins, appendages, and EPS, thereby interrupting the attachment of microorganisms to the wound surface. EPS production and cellular division are also targeted to inhibit the early stages of biofilm development.[14] Disruption of biofilm formation can be achieved through various means, such as physical removal of the biofilm, elimination of dormant cells, targeting social interactions (particularly in polymicrobial biofilms), and degradation of the EPS matrix.[14] Additionally, antibiofilm agents can achieve biofilm dispersion by inducing EPS matrix remodeling and activating dispersal mechanisms.[14] Various approaches are available for managing chronic wound biofilms (Table 3).

DES disrupts biofilm structure and enhances biofilm removal. DES can solubilize proteins and polysaccharides that are part of the biofilm and other components present in the biofilm matrix.[73] DES can restrain microbial adhesion and proliferation and promote biofilm detachment and removal. Moreover, it can control inflammatory cascade associated with wounds and decrease cell stress by inhibiting the production of reactive oxygen species.[74]

Table 3 Approaches for the Management of Chronic Wound Biofilms

Strategies	Significance to Chronic Wound Healing	References
Sharp debridement	• Removes biofilm, dead tissue and niduses. • Promotes healing by exposing viable underlying tissues. • Controls inflammation. • Reduces bacterial burden within the wound. • Promotes granulation tissue formation.	[12,15]
Antibiotics/ antiseptics	• Prevent biofilm spreading and reforming after debridement. • Less effective due to resistance to microorganisms.	[12]
pH modulation	• Lowering pH inhibits bacterial proliferation and decreases toxicity created by bacterial end-product. • Acid treatment with citric acid, boric acid and acetic acid lowers wound pH.	[15]
Surfactants	• Lower surface tension of liquid used for cleaning wound. • Interfere with the adhesion of microbes to the wound surface. • Enhance liquid infiltration and assist in elimination of debrided cells and microbes.	[15,65]
Antimicrobial peptides	• Form pores on bacterial cell membrane that results in leakage of cellular content and causes death. • Exhibit immunomodulatory effect and reduce inflammatory components. • Induce epithelial cell migration and angiogenesis.	[15,66,67]
Photodynamic substances	• Produce cytotoxic reactive oxygen species (ROS) when exposed to light of particular wavelength in the presence of oxygen. • ROS damages microbial proteins, DNA and membranes and in turn results in cell death.	[15,68]
Bacteriophages	• Disrupt biofilm matrix by producing phage-coded enzymes such as alginase and polysaccharide depolymerase. • Lyse bacterial cell by multiplying within the host cell and producing new virus particles.	[69]
Quorum sensing inhibitors	• Inhibit cell-to-cell communication in bacterial colonies and production of virulence factor. • Inhibit autoinducer synthesis and signal transduction cascades. • Degrade signaling molecules and dysregulate biofilm signaling.	[15,69]
Nanoparticles	• Act as carrier for delivery of antimicrobial agents. • Metal/metal oxide-based nanoparticles possess intrinsic antimicrobial properties. • Nanoparticles loaded with EPS degrading moieties penetrate into biofilm matrix. • Stimuli-responsive nanoparticles cause thermal damage of biofilm.	[70]
Hydrogels	• Act as carrier for delivery of biofilm-killing agents. • Act as debriding agent. • Enhance re-epithelialization of chronic wound.	[71,72]
DESs	• Disrupt biofilm by solubilizing proteins and polysaccharides. • Restrain microbial adhesion and proliferation. • Control inflammation. • Exhibit intrinsic antibacterial and antifungal properties.	[73–75]

Wikene et al illustrated that the hydrophobic photosensitizer-solubilizing property of DES coupled with photodynamic therapy presented potential for antimicrobial activity.[76] A DES containing a hydrophobic compound (curcumin) photo inactivated *Escherichia coli* at a relatively low concentration of curcumin, ie 1.25 µM.[76] Nystedt et al have demonstrated the antibiofilm properties of neutral DES. In a pre-established bacterial biofilm, neutral DES demonstrated bactericidal activity against *P. aeruginosa* and *S. aureus*.[13] Moreover, biofilm formation by both bacterial species was inhibited by DES at or below 0.5 times the minimum inhibitory concentration (MIC) (sub-MIC levels).[13] In a recent study, Swebocki et al showed that DESs eradicated methicillin-resistant *Staphylococcus aureus* (MRSA) and *Escherichia coli* biofilms at concentrations below 1% v/v, thereby exhibiting antibiofilm activity.[75] DES induced significant morphological changes in both strains. Moreover, in the study, DES exhibited antifungal activity against *Candida albicans* and *Candida auris*.[75]

The hygroscopic and chaotropic effects of DES can modify proteins and disorganize slough, thereby enhancing wound healing of wounds.[77] The slough is devitalized or dead proteinaceous host tissue attached to viable tissue. In contrast, biofilms are viable and made of bacteria-derived tissues, where bacteria are embedded in an EPS matrix primarily composed of polysaccharides.[78]

DESs possess cytotoxic properties and play a significant role in the extraction and purification of virus-like particles.[79] Hydrophilic DES can pass through the cell membrane and exhibit toxic effects by disrupting both the pH and the anion pool of the cytoplasm. An increase in internal acidity (lowering pH) adversely affects the integrity of purines, resulting in enzyme denaturation and oxidative stress, thereby affecting microbial cell viability.[80,81] In a recent study, Shaw et al demonstrated the antifungal properties of DES against the pathogenic fungal species *Candida albicans*, suggesting that DES acts as an antibiofilm agent.[82] DES interacted with cell wall components, primarily β-glucans, chitin, and glycoproteins, and blocked the uptake of nutrients and other compounds, thereby resulting in cell death due to starvation.[82] However, this study did not include data on the effects of DESs on fibroblasts. Other studies, nevertheless, have reported that DESs exhibit good biocompatibility with murine fibroblasts,[83] along with excellent viability and proliferation of both fibroblasts and keratinocytes.[84]

DES-Based Formulations for Chronic Wound Treatment

Different DESs- and DES-based formulations have been used to treat chronic wounds. DESs are primarily used as penetration enhancers and antibiofilm agents in the treatment of chronic wounds. They are also used for therapeutic purposes, wound dressing, and debridement. Examples of DES used for chronic wound healing are presented in Table 4.

Table 4 DES-Based Formulations for Wound Healing

Formulations	Type of DES	Role of DES	Effect on Wound Healing	References
Hydrophobic DES based on menthol and stearic acid	Therapeutic DES (THEDES)	Therapeutic application	Stronger hydrogen bond interactions between menthol and stearic acid, no relevant cytotoxicity in HaCaT cells, potentiated wound healing, and presented antibacterial properties against *Staphylococcus epidermidis* and *Staphylococcus aureus* strains, some of which were methicillin resistant	[85]
Citric acid and xylitol mixture for collagen	Natural DES (NADES)	Excipient for wound dressing	Potential topical preparation containing collagen for wound healing and antibacterial properties; inclusion of a photosensitizer allowed for antimicrobial photodynamic therapy	[86]
Mannose and choline chloride-based DES; Hydroxyethyl methacrylate (HEMA) and 2-(dimethylamino)- ethyl methacrylate) DMAEMA based DES containing dopamine	NADES	Wound dressing hydrogel	Outstanding cell compatibility and antibacterial properties (*Staphylococcus aureus, Escherichia coli*) and promoted wound healing (nearly 100% skin recovery in 9 days)	[87]

(*Continued*)

Table 4 (Continued).

Formulations	Type of DES	Role of DES	Effect on Wound Healing	References
Mannose and choline chloride-based DES; Polyvinlyl alcohol, DES and honey based fast dissolving nanofibers	NADES	Wound dressing	In vitro cell viability in NIH/3T3 and HepG2 cells was >90%, total bacterial reduction of 37.0% and 37.9% against *Escherichia coli* and *Staphylococcus aureus*, respectively, following 6 hours incubation with nanofibers, accelerated wound healing process and improved the wound healing rate on rat skin to 85.2% at day 6	[88]
DES composed of choline chloride and glycerol, choline chloride and 1,4-butanediol	NADES	Eco-friendly green solvent to extract bioactive oil palm leaves falvonoids	Extracted flavonoids using NADES exhibited antioxidant and wound healing properties in vitro, NADES extracts exhibited non-cytotoxicity effects on 3T3 fibroblast cells at ≤1000 μg/mL	[89]
Hydrogel composed of dopamine coated sodium hyaluronate and the natural DES with in situ reduction of silver nanoparticles (Ag NPs)	NADES	Wound dressing	Hydrogel presented good cytocompatibility against NIH-3T3 fibroblast cells, exhibited prominent antibacterial effects against wound bacteria, and facilitated regeneration of mouse skin tissue in the wound area	[90]
Choline geranate integration into electrospun protein scaffolds	NADES	Wound dressing	A compatible platform for proliferation of human dermal neonatal fibroblasts, a concentration-dependent inactivation of exogenously applied solution of both gram-positive (*Enterococcus* spp.) and gram-negative pathogens (*Pseudomonas aeruginosa*) was observed	[91]
Nitrogen and chloride co-doped carbon dots (N/Cl-CDs) based on choline chloride-urea-glycine ternary DES and Ag NPs	NADES	Doping material of carbon dots	N/Cl-CDs exhibited oxidase-like activity and excellent antibacterial activity against *Escherichia coli, Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* (MRSA), addition of Ag NPs to N/Cl-CDs significantly enhanced the oxidase and antibacterial activities (1.8 mg/mL nanocomposite completely inactivated 10[5] cfu/mL of MRSA in 90 mins)	[92]
Novel ternary PDES based on natural tannic acid, choline chloride and hydroxyethyl methacrylate	Polymerizable DES (PDES)	Wound dressing	Excellent self-healing and antibacterial properties, can be used in LCD 3D printing without any UV-photoblocker	[93]

(*Continued*)

Table 4 (Continued).

Formulations	Type of DES	Role of DES	Effect on Wound Healing	References
Menthol based NADES with free fatty acids (myristic acid, lauric acid and stearic acid)	NADES	Antibiofilm agent	Effective antimicrobial properties towards Gram-positive bacterial and fungal strains, a 4:1 molar ratio of menthol and lauric acid led to effective biofilm removal/ dispersion of not only MRSA and *Candida albicans* but also *Escherichia coli*, without need of any additional physical force or antibiotic while not compromising normal keratinocyte proliferation and migration in wounds	[74]
Tadalafil and lidocaine loaded DES	Choline chloride-based DES	Topical vehicle	Enhanced retraction of the cut wound area, reduced scarring of the burn wounds, and antimicrobial activity against fungi (*Candida albicans*) and bacterial strains (*Enterococcus faecalis, Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa*)	[94]
DES composed of choline chloride and malonic acid	NADES	Solvent for extraction of β-chitin from Indo-West Arabian Sea squid pens (*Uroteuthis duvaucelli*)	Extracted β-chitin based gels have potential in wound healing applications due to their immunological, antibacterial, and biocompatibility properties	[95]
Allantoin-choline chloride/zinc chloride binary mixtures	DES	Wound dressing	In rabbit wounds, the allantoin-choline chloride (15% v/v) exhibited better wound healing as compared to allantoin-zinc chloride	[96]
Composite hydrogel prepared with choline chloride, acrylamide and acrylic acid-based DES and β-cyclodextrin as a filler	DES	Wound dressing	Potential wound healing property due to self-healing ability of the composite hydrogel with excellent mechanical properties and pH responsiveness along with the existence of a hydrophobic cavity of β-cyclodextrin for drug delivery	[97]
Chitosan-based supramolecular biofilm material for wound dressing based on DES	NADES	Wound dressing	Potent antibacterial properties against *Staphylococcus aureus* (98.86%±1.90%) and *Escherichia coli* (99.69%±0.53%) with excellent biocompatibility and biodegradability	[98]
Chitosan-based supramolecular aerogel with "skeletal structure" constructed in NADES	NADES	Wound dressing	Exhibited inhibitory effect on *Staphylococcus aureus* and *Escherichia coli* with excellent biocompatibility and effectively promoted wound healing	[99]

(*Continued*)

Table 4 (Continued).

Formulations	**Type of DES**	**Role of DES**	**Effect on Wound Healing**	**References**
Sodium hyaluronate hydrogel for wound healing based on DES	DES	Wound dressing	Treatment with combination of hydrogel and electrical stimulation resulted in a stronger therapeutic effect on wound healing compared to commercial DuoDERM dressing	[100]
Eutectogel based on DES and poly (vinyl alcohol)	NADES	Wound dressing	Enhanced transdermal delivery vehicle for drug delivery to wounds	[101]
DES for essential-oil delivery and bacterial-infected wound healing	DES	Wound dressing	Clove essential oil loaded DES nanoformulations effectively promoted MRSA-infected wound healing in vivo with good biocompatibility	[102]
Conductive hyaluronic acid/DES (acrylamide/cholinechloride/ glycerol) composite hydrogel for skin burn healing under electrical stimulation	PDES	Wound dressing	Combined with exogenous electrical stimulation, the conductive eutectogel exhibited the ability to reduce inflammation, stimulated cell proliferation and migration, promoted collagen deposition and angiogenesis, and facilitated skin tissue remodeling with great potential as a dressing for burn wounds	[103]
Multifunctional eutectogel based on a PDES prepared by the incorporation of diallyldimethylammonium chloride and glycerol in the presence of polycyclodextrin/dopamine-grafted gelatin/oxidized sodium alginate	PDES	Wound dressing	Eutectogels achieved 90% antibacterial effect, showed excellent biocompatibility and improved wound healing process in vivo	[104]
DES composed of betaine and urea	NADES	Debridement and antibiofilm agent for chronic wounds	DES possessed a high debriding capacity, strong antibiofilm capability, reduced bacterial load evaluated in artificial slough and ex vivo wounds; significant effects on debriding and antibiofilm capacity was observed in a murine model of diabetic wound; superiority in reduction of bacterial load and wound area with no adverse effects in leg ulcers	[77]
3D printed wound dressing composed of a hydrophobic DES (acetic acid or octanoic acid combined with menthol)-formulated emulgel incorporating curcuminoids	Hydrophobic DES	Wound dressing	Emulgel components worked synergistically with curcuminoids to significantly enhance anti-biofilm activity against *Staphylococcus aureus*, offering an effective strategy to prevent wound infections	[105]

(*Continued*)

Table 4 (Continued).

Formulations	Type of DES	Role of DES	Effect on Wound Healing	References
Xylan-chitosan based films with DES	DES	Wound dressing (DES served as the solubilization medium for the biopolymers and as a plasticizer and compatibilizer of xylans and chitosan)	Films possessed non-cytotoxicity towards human keratinocytes (HaCaT cell line, cell viability ≥80%), provided UV-light protection (transmittance ≤38%, 200–400 nm), exhibited good antibacterial activity against MRSA (≥4-log cfu/mL reduction, in vitro assay; 2-log cfu/mL reduction, ex vivo assay), showed in vitro wound healing in 2D scratch assays with 76% wound area closure after 30 h and 17% of relative cell viability in the simulated wound area after 72 h achieved with a 3D hydrogel model	[83]
Poly (DES) electroactive chitosan eutectogel	PDES	Wound dressing	PDES hydrogel exhibited remarkable antibacterial performance (up to 99%) and blood compatibility, achieving rapid hemostasis (within 15s) and reducing blood loss (36 mg); additionally, the hydrogel prevented skin wound infections, enhanced collagen deposition, and facilitated microvascular reconstruction at the wound site, significantly improving infected wound healing (in 12 days)	[106]
Polyvinyl alcohol/chitosan hydrogel based on DES	NADES	Wound dressing	In vitro studies showed excellent hemostatic properties, good biocompatibility and broad-spectrum antibacterial properties of the hydrogel; in MRSA-infected skin wound model, the hydrogel accelerated healing of infected wounds via inhibiting the intensification of inflammation, increasing collagen deposition, accelerating re-epithelialization, and promoting the formation of hair follicles and new blood vessels	[107]

(*Continued*)

Table 4 (Continued).

Formulations	Type of DES	Role of DES	Effect on Wound Healing	References
All-natural kelp decellularized scaffold prepared using DES	DES	Decellularizing bioactive kelp scaffold (Im-Gly2)	Scaffold demonstrated intrinsic antioxidant and immunomodulatory effects and reduced ROS production in RAW264.7 macrophages; accelerated healing of in vivo diabetic wounds by alleviating inflammation, angiogenesis, granulation tissue formation, collagen deposition, and re-epithelialization	[108]
NADES-based chitosan hydrogel	NADES	NADES as gelation solvent	Following photoimmunomodulation treatment, significant wound healing effects, including increased cell migration, improved mitochondrial membrane potential, reduced intracellular ROS and differential protein expression in AGS and HT29 cells were observed	[109]

Challenges and Future Perspectives

DESs are increasingly being recognized as promising drug delivery systems, particularly for the treatment of chronic wounds. Various classes of DES, such as THEDES, NADES, PDES, and conventional DES, have shown potential for enhancing wound healing. Some formulations primarily function as permeation enhancers, facilitating drug penetration into the skin layers and thereby accelerating the repair process. Others exhibit intrinsic biological activities, including antimicrobial and antibiofilm properties, which play critical roles in the reduction of wound-associated infections. In addition, several DES formulations have demonstrated synergistic effects such as stimulating cell migration, promoting angiogenesis, and supporting overall tissue regeneration.

As wound healing is a highly complex and dynamic process, a thorough evaluation of DES toxicity is essential to avoid harmful effects on damaged tissues. Rigorous in vitro and in vivo investigations are required to confirm the safety and therapeutic efficacy of each newly developed DES in human applications. To date, most studies have focused on topical or transdermal applications, with a limited exploration of alternative routes of administration for internal wound management. Although the preliminary findings are encouraging, comprehensive preclinical and clinical studies are necessary to establish DES-based therapies as viable clinical options. Furthermore, such studies will require adherence to strict regulatory standards, including FDA evaluations, before any formulation reaches the market.

Combination approaches represent a promising direction, in addition to stand-alone DES formulations. DES may be incorporated into advanced delivery systems, such as spray-dried or freeze-dried formulations, into lipid nanocarriers or can be used to form microemulsions, creams, or gels to enhance stability and therapeutic performance. Similarly, combining DES with established excipients or delivery platforms can generate synergistic systems that maximize healing outcomes.

Future research should prioritize the design of novel DES using biocompatible and safe excipients with the goal of identifying alternatives that minimize toxicity while maximizing therapeutic benefits. Mechanistic studies are also essential to elucidate the pathways through which DES exerts antimicrobial, antibiofilm, and wound-healing effects. Finally, attention to formulation stability, scalability, and shelf life is critical for translating DES-based systems from laboratory research into market-ready products capable of addressing the global burden of chronic wounds.

Conclusion

DESs have emerged as innovative alternatives for the management of chronic wounds. Their intrinsic antimicrobial, antibiofilm, and wound healing properties highlight their potential as therapeutic candidates in this field. Although chronic open wounds lack an intact SC, the penetration enhancing properties of DES-based formulations facilitate deep drug permeation through the surrounding partially intact skin. This, in turn, promotes wound healing by enabling effective biofilm penetration, disruption, and infection control. Moreover, the incorporation of DES in advanced delivery systems or combination formulations can further enhance their safety, stability, and efficacy while providing synergistic benefits for tissue repair and regeneration. Despite encouraging preliminary findings, extensive preclinical and clinical investigations are essential to validate their safety, elucidate their mechanisms of action, and establish regulatory approval pathways. Overall, DES represent a promising platform for drug delivery and hold significant potential for translation into market-ready therapies aimed at improving the outcomes of chronic wound care.

Disclosure

The authors report no conflicts of interest in this work.

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