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Development strategy of novel drug formulations for the delivery of doxycycline in the treatment of wounds of various etiologies

Olena Saliy [a,1], Mariia Popova [a,2,*], Hanna Tarasenko [a,3], Olga Getalo [b,4]

[a] Department of Industrial Pharmacy, Kyiv National University of Technologies and Design, Mala Shyianovska (Nemyrovycha-Danchenka) Street, 2, Kyiv 01011, Ukraine
[b] Department of Industrial, Clinical pharmacy and Clinical pharmacology, Shupyk National Healthcare University of Ukraine, Dorohozhytska Street 9, Kyiv 04112 Ukraine

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ABSTRACT

Doxycycline hyclate (DOXH) is a broad-spectrum antibiotic derived synthetically from tetracycline. Despite its use in clinical practice for more than 40 years, DOXH remains an effective antibiotic with retained activity. The potential advantages of DOXH for wound healing therapy include its mechanisms of action, such as anti-inflammatory effects, antioxidant properties, modulation of cellular processes, stimulation of collagen synthesis, and antimicrobial activity. As current standards of care aim to improve wound healing by promoting rapid closure, a relevant direction is the development of novel DOXH formulations for parenteral delivery that enhance both skin regeneration and control of infectious conditions.

Oral delivery is the most common and commercially available route for administering DOXH therapeutic agents. However, parenteral delivery of DOXH, where the antibiotic substance is not in a solid state (as in powdered or compressed solid form) but rather dissolved in any carrier, presents challenges regarding DOX solubility and the stability of DOXH solutions, which are major factors complicating the development of new formulations for parenteral administration.

This review discusses the achievements in research strategies and the development of new pharmaceutical formulations for the delivery of doxycycline in the treatment of wounds of various etiologies.

1. Introduction

Wounds of various etiologies, such as second-degree burns, chronic wounds, and ulcers affect millions of people and remain a significant health problem worldwide. Treatment of wounds is a challenge to wound care professionals and is often associated with high costs and ineffective or limited treatment outcomes (Frykberg et al., 2015). The epidemiology of injuries includes road traffic accidents, sports, war conflicts, and assault/interpersonal violence (Prevaldi et al., 2016). The military conflict between Ukraine and Russia, which led to the imposition of martial law and hostilities, has resulted in a large number of patients with open gunshot and infected wounds who need immediate and effective treatment. The normal wound healing process occurs in a well-synchronized sequence of controlled stages: hemostasis, inflammation, proliferation, and remodeling (Velnar et al., 2009). Any deviations from the normal wound-healing process lead to abnormal scar formation and a chronic condition that is more susceptible to infections (Díaz-García et al., 2021). Chronic wounds, such as venous ulcers, exhibit dysfunctions in the extracellular matrix that hinders tissue healing, thus large skin injuries and chronic wounds are healing slowly to their susceptibility towards infections and fluid losses (Kolimi et al., 2022). Thus, wound healing is impaired by multiple factors, such as the presence of co-morbidities, diabetes, neuropathy, immunosuppression, vascular or venous insufficiency, or any condition that results in a weakened immune system (Ackermann et al., 2013).

Current Standards of care aim to enhance wound healing and

* Corresponding author.
E-mail address: popova.me@knutd.edu.ua (M. Popova).
[1] ResearchGate: https://www.researchgate.net/profile/Olena-Saliy-2 ID Scopus: 57219560195 ResearcherID: AAC-5721-2019
[2] ResearchGate: https://www.researchgate.net/profile/Mariia-Popova-9
[3] Scopus Author ID: 58032095000ResearcherID: JHT-0528-2023
[4] ResearchGate: https://www.researchgate.net/profile/Olga-Getalo-2

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promote rapid closure; moreover, the development of therapeutic interventions is an important direction that improves skin regeneration while simultaneously controlling infectious complications (Kolimi et al., 2022). Novel therapeutic strategies for wound healing are based on biological processes and natural wound-healing mechanisms. Facilitating drug delivery to the affected site, synthesizing tissue- and skin-compatible scaffolds, and creating protective coverings on wounds are among the principles considered in the development of new drug formulations (Saghazadeh et al., 2018).

Currently, the increasing global rise in pathogen resistance to widely used antibiotics necessitates a return to the practice of using certain classic drugs with preserved activity. There is a need to explore new avenues and delivery methods for antibiotics to target sites of disease-causing microorganisms and to utilize antimicrobial agents that have not yet developed widespread resistance (Reygaert, 2018).

DOXH is a broad-spectrum antibiotic derived synthetically from tetracycline. Despite being used in clinical practice for over 40 years, DOXH remains an effective antibiotic with preserved antibacterial activity (Rusu and Buta, 2021). DOXH formulations are well-tolerated by the patients and are typically administered orally at a dose of 100 mg once or twice daily (Holmes et al., 2009). However, the range of DOXH-based medicinal products available on the market is very limited, most of which are solid oral dosage forms such as immediate-release tablets and capsules, oral suspension powders, syrups, dispersible tablets, and delayed-release solid dosage forms. (Saliy et al., 2022). The oral route of administration is the most common and desired method for delivering DOXH. However, despite the variety of pharmaceutical formulations available for oral drug delivery, there is often a need for parenteral administration in the treatment of wounds to prevent common side effects such as gastrointestinal disorders or esophagitis (Sloan et al., 2008), abdominal pain, oral ulceration, dysuria, gastrointestinal irritation, and more (Holmes et al., 2009).

The antibacterial activity of doxycycline is mediated by a large number of mechanisms. The anti-inflammatory effects of doxycycline have been studied, including the inhibition of neutrophil activation and migration, T-lymphocytes activation and proliferation, inhibition of phospholipase, angiogenesis, nitric oxide synthesis, and granuloma formation, as well as the suppression of inflammatory cytokines release (TNFα, IL-1β, IL-6, IL-8) and decrease of reactive oxygen species. (Rusu and Buta, 2021) Doxycycline has been described in scientific literature as an inhibitor of matrix metalloproteinase (MMP) activity, where it inhibits MMPs at sub-antimicrobial doses and is the only MMP inhibitor approved by the Food and Drug Administration (Liu et al., 2017). Therefore, the combination of DOXH mechanisms of action, which promote skin regeneration while simultaneously inhibiting infectious processes, makes it an attractive potential treatment option for wounds of various etiologies.

Parenteral delivery of DOXH is currently available on the pharmaceutical market only in the form of powders or lyophilisates for solution for injection, which are used in limited indications. However, there is a need for parenteral drug delivery, such as local skin application, in the treatment of wound infections due to several proposed advantages, including ease of administration, painless and non-invasive delivery, patient compliance, and self-application without the need for intervention by experienced medical professionals, thereby reducing the burden on healthcare services. Local application has an advantage in targeted wound treatment compared to systemic administration of drugs into the circulatory system, which is not site-specific and is ineffective for wounds with compromised blood supply, such as severe burns and chronic wounds (Xue et al., 2018). Therefore, research and investigation in this direction hold great interest and are crucial areas for pharmaceutical sciences.

Current scientific research on effective drug delivery systems aims to enhance the efficacy of existing antibacterial, anti-inflammatory, and antiviral effects of DOXH, as well as to reduce resistance (Skwarczynski et al., 2022). However, parenteral delivery of DOXH, where the antibiotic substance is not in solid form (such as in powder or compressed solid form) but dissolved in any carrier, presents challenges in terms of DOXH solubility and stability of solutions with DOXH, which are significant factors complicating the development of the new formulation for parenteral forms. The doxycycline salt, known as hyclate, is classified as slightly soluble in water (European Pharmacopoeia, 2017). Different forms of doxycycline salts, such as hyclate and monohydrate, are therapeutically equivalent. Hyclate has greater solubility than monohydrate, making it preferable for inclusion in formulations of parenteral forms. However, water-based solutions of DOXH prepared by conventional methods face stability issues during long-term storage, including changes in color, pH shift, formation of impurities, and doxycycline degradation products, which can lead to toxic side effects and pose hidden risks in clinical practice (Jutglar et al., 2018; Saliy et al., 2021). DOXH is a highly photosensitive drug, a feature that limits the stability of the corresponding dosage forms (Kogawa et al., 2014), and is prone to degradation through oxidation and epimerization, by the identified impurities such as 4-epidoxycycline (4-EDOX), 4,6-epidoxycycline (4,6-EDOX) and 4-epimetacycline (4-EMTC), that forms in aqueous doxycycline solutions, causing changes in solution color (Yekkala et al., 2003).

Therefore, there is an increased need to research and develop new stable pharmaceutical formulations with doxycycline for effective treatment methods of wound infections. However, to achieve the best results in terms of health benefits and treatment, the simplicity of formulation design plays a crucial role in providing optimal, patient-friendly, and economically viable production, storage, and transportation.

The main aim of this research is to analyze new data and summarize the results regarding pharmaceutical forms as DOXH delivery systems in the treatment of wounds of various etiologies.

2. Mechanisms of wound healing action of doxycycline hyclate

The molecular environment of chronic wounds contains abnormally high levels of pro-inflammatory cytokines (tumor necrosis factor [TNF]-alpha and interleukin [IL]−1beta) and matrix metalloproteinases (MMPs), which impair normal wound healing. The potential advantages of using DOXH for wound healing therapy lie in its mechanisms of action, including anti-inflammatory effects, antioxidant properties, modulation of cellular processes, stimulation of collagen synthesis, and antimicrobial activity (Stechmiller et al., 2010).

2.1. Inhibition of matrix metalloproteinases (MMPs)

MMPs play a crucial role in tissue remodeling during wound healing. Excessive MMP activity can lead to excessive degradation of extracellular matrix (ECM) components, impairing the wound healing process. DOXH suppresses the activity of MMPs, specifically MMP-1, MMP-2, and MMP-9, which are involved in the degradation of collagen and other ECM components. Doxycycline helps to maintain the integrity of the extracellular matrix by inhibiting MMP activity, thus promoting proper wound healing (Samartzis et al., 2019). Doxycycline's MMPs inhibition prevents excessive degradation of collagen and promotes the deposition of new collagen, leading to improved wound healing and reduced scar formation (Palasuk et al., 2018). The mechanism of doxycycline's MMP inhibition relies on chelating zinc ions found within the active region of MMPs, preventing MMPs from destroying ECM components (Karna et al., 2001).

Depending on the dosage, DOXH reduces the proteolytic degradation of azocoll, a substrate for MMPs, in the fluid of chronic wounds, achieving an 89 % reduction at a concentration of 2.3 mg/ml (Chin et al., 2003). Research findings indicate that doxycycline regulates MMP activity and prevents excessive tissue degradation during wound healing. Doxycycline's mechanism of MMP inhibition has a positive impact on wound healing, particularly in chronic wounds.

2.2. Modulation of fibroblast activity

Fibroblasts are key cells involved in wound healing and scar formation (Moore et al., 2020).

Doxycycline regulates the activity of fibroblasts and the deposition of collagen in the wound bed, leading to healing with reduced scar formation. Prolidase is an enzyme involved in collagen degradation, particularly in the processing of proline, an essential amino acid for collagen synthesis. It has been proven that doxycycline suppresses the activity of prolidase and the biosynthesis of collagen in human skin fibroblasts. Doxycycline induces coordinated inhibition of prolidase activity and collagen biosynthesis (with an IC50 of approximately 150 μg/ml) and gelatinolytic activity in cultured human skin fibroblasts. The inhibition of prolidase activity by doxycycline suggests that it may interfere with the recycling process, leading to a reduction in collagen biosynthesis (Karna et al., 2001).

Furthermore, the decrease in collagen biosynthesis observed in the study is explained by the suppression of prolidase activity, rather than changes in the quantity of prolidase receptor proteins or integrin (Karna et al., 2001). This indicates that the effect of doxycycline on collagen biosynthesis is mainly mediated through its impact on prolidase activity.

The inhibitory effect of doxycycline on prolidase activity and collagen biosynthesis cannot be solely attributed to its direct action on these processes. Studies also suggest that the anti-inflammatory properties of doxycycline and its ability to form complexes with metal ions, such as Mn^{2+}, which are necessary for prolidase activity, may contribute to its inhibitory effects (Karna et al., 2001).

Thus, doxycycline suppresses prolidase activity and regulates collagen biosynthesis in human skin fibroblasts. It also exhibits antioxidant potential. Doxycycline increases the quantity of type I collagen and elastic fibers and accelerates the closure of skin wounds.

2.3. Anti-inflammatory action

Chronic inflammation can impair wound healing by prolonging the inflammatory phase and delaying the transition to the proliferation and remodeling phases (Moore et al., 2020). Doxycycline has anti-inflammatory properties and can modulate immune responses. It has been shown to reduce the recruitment of inflammatory cells, such as neutrophils and macrophages, to the site of the wound. By reducing inflammation, doxycycline creates a more favorable environment for wound healing and minimizes tissue damage, ultimately leading to reduced scar formation (Samartzis et al., 2019).

Studies have shown that doxycycline inhibits the production of pro-inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), and interleukin-6 (IL-6). These cytokines play a crucial role in the initiation and maintenance of the inflammatory response. Tumor necrosis factor-alpha (TNF-α) is a pro-inflammatory cytokine that plays a pivotal role in immune response and inflammation. TNF-α exerts its action by binding to two different cell surface receptors, TNF receptor 1 (TNFR1) and TNF receptor 2 (TNFR2). After binding to TNFR1, TNF-α initiates a signaling cascade that leads to the activation of nuclear factor kappa B (NF-κB), a transcription factor involved in the regulation of many genes related to inflammation and immune reactions. Activation of NF-κB induces the expression of various pro-inflammatory mediators, including cytokines, chemokines, adhesion molecules, and enzymes such as cyclooxygenase-2 (COX-2). These mediators promote the recruitment and activation of immune cells, enhancing the inflammatory response (Dos Santos et al., 2022).

2.4. Reduction of oxidative stress

Oxidative stress contributes to inflammation and impairs wound healing. Doxycycline possesses antioxidant properties and can scavenge reactive oxygen species (ROS) (Clemens et al., 2018). By reducing oxidative stress, doxycycline helps mitigate inflammation and creates a more favorable environment for wound healing. Oxidative stress refers to an imbalance between the production of ROS and the ability of the body's antioxidant defense systems to neutralize them.

Under normal physiological conditions, the human antioxidative defense system including enzymes, such as superoxide dismutase (SOD), catalase, and glutathione peroxidase, helps neutralize ROS and maintain balance. However, when there is excessive ROS production or a decrease in antioxidant capacity, oxidative stress can occur (Clemens et al., 2018). In vitro analysis demonstrated that local application (topical administration) of doxycycline (10 mg/kg/day, 30 mg/kg/day) improves oxidative balance by reducing protein carbonyls, malondialdehyde, nitric oxide, and hydrogen peroxide (H_2O_2). Furthermore, after 21 days of local applications of doxycycline, there was an increase in antioxidant enzymes (catalase and superoxide dismutase), demonstrating its antioxidant potential (Altoé et al., 2021).

2.5. Angiogenesis

Angiogenesis is a crucial process in wound healing as forms new blood vessels to supply oxygen and nutrients to the healing tissue.

Studies have demonstrated the anti-angiogenic properties of doxycycline. It is believed that the anti-angiogenic effect of doxycycline is mediated through various mechanisms. One mechanism involves the inhibition of matrix metalloproteinases (MMPs), which are enzymes involved in the breakdown of extracellular matrix components (Merentie et al., 2018). MMPs play a critical role in angiogenesis by degrading the extracellular matrix to facilitate the formation of new blood vessels. In addition to MMP inhibition, doxycycline has been found to impact other signaling pathways involved in angiogenesis. It has been shown to suppress the expression of vascular endothelial growth factor (VEGF), a key factor promoting angiogenesis. By reducing VEGF expression, doxycycline can inhibit signaling pathways that contribute to blood vessel formation (Merentie et al., 2018).

Furthermore, it has been found that doxycycline inhibits the proliferation and migration of endothelial cells, which are the building blocks of blood vessels. This can further contribute to its anti-angiogenic effects by preventing the formation of new blood vessels in the treatment of chronic wounds (Merentie et al., 2018). Overall, doxycycline demonstrates anti-angiogenic activity through its ability to inhibit MMP activity, reduce VEGF expression, and suppress the proliferation and migration of endothelial cells. These mechanisms contribute to its ability to regulate angiogenesis and prevent excessive or aberrant blood vessel formation.

2.6. Antibacterial action

Wound infection is one of the major factors that delay healing, as microorganisms compete with the host immune system.

The causative agents of wound infection include opportunistic aerobic or anaerobic microorganisms that coexist with the human body, such as *Staphylococcus, Streptococcus, Escherichia coli, Proteus, Xanthomonas, Synergistes, Clostridia, Bacteroides, Fusobacteria* and others. Doxycycline exhibits a broad spectrum of activity and is effective against gram-positive and gram-negative, aerobic and anaerobic bacteria spirochetes and mycoplasma. Doxycycline is sensitive to pathogens found in purulent wounds, including *Staphylococcus, Streptococcus* (including *S.pneumoniae), E. coli*; pathogens of septic wounds such as *Clostridium tetani, Bacillus anthracis, Klebsiella, Corynebacterium diphtheriae* and some fungi (Xu et al., 2017).

Periodontal pathogens involved in the progression of periodontitis ulcers, such as *Porphyromonas gingivalis, Bacteroides forsythus, Actinobacillus actinomycetemcomitans, Prevotella intermedia, Campylobacter rectus, Fusobacterium nucleatum*, are particularly sensitive to doxycycline (Voronkina et al., 2022).

Doxycycline suppresses strains producing beta-lactamases, which are predominantly encountered in deep periodontal pockets. The

bacteriostatic action of DOXH aims to inhibit bacterial growth by allosterically binding to the 30S ribosomal subunit of prokaryotes during protein synthesis and possibly to the 50S ribosomal subunits, thereby blocking the binding of aminoacyl-tRNA to mRNA and inhibiting bacterial protein synthesis. Doxycycline inhibits collagenase activity in vitro (Connell et al., 2003).

DOXH exhibits systemic action in various tissues.

Its high lipophilicity allows it to penetrate numerous membranes to reach target molecules. Tetracyclines act as cationic coordination complexes to cross OmpF and OmpC porin channels in gram-negative bacteria. Similarly, in Gram-positive bacteria, the neutral, lipophilic form penetrates the cytoplasmic membrane. Passage through the cytoplasmic membrane is energy-dependent and proton-motive force-driven (Chopra et al., 2001).

The bacteriostatic action of DOXH aims to inhibit bacterial growth by allosterically binding to the 30S prokaryotic ribosomal unit during protein synthesis. DOXH prevents the binding of charged aminoacyl-tRNA (aa-tRNA) to the A-site of the ribosome, halting the elongation phase and leading to an unproductive cycle of protein synthesis. Doxycycline affects the binding rate of triple complex formation (comprised of elongation factor Tu [EF-Tu], GTP, and aa-tRNA) with the ribosome.

The development of microbial resistance to antibiotics poses a potential risk. However, the local application of 1 % doxycycline has a significantly higher concentration (i.e., 10,000 mg/ml) than the minimum inhibitory concentration required for a 50 % reduction in pathogenic growth, thereby minimizing the likelihood of developing doxycycline-resistant bacteria (Level IV) (Fluit et al., 2019).

3. Opportunities and challenges in the development of parenteral drug delivery systems for the treatment of wounds of various etiologies

3.1. Extemporaneous solution

DOXH in the form of an extemporaneous solution was investigated for the treatment of non-healing wounds in patients with diabetes. Impaired wound healing, such as diabetic foot ulcers and acute skin wounds, is associated with complex physiological mechanisms, including constantly high concentrations of cytokines in the wound, which sustain the inflammatory response and induce high concentrations of proteases that degrade growth factors, receptors, and matrix proteins necessary for proper wound healing. DOXH exhibits inhibitory effects on MMP-9 through the chelation of catalytic zinc.

Local application of DOXH has been shown to reduce proteolytic activity in the wound fluid of chronic venous leg ulcers and clinical use of 1 % DOXH solution for local treatment has demonstrated favorable effects on wound healing, while oral administration of DOXH, investigated for the treatment of chronic leg ulcers, showed less effective action over time due to insufficient levels of DOXH in the wound to inhibit MMP activity (Serra et al., 2015).

The use of a 2 % DOXH solution on chronic foot ulcers provides a sufficient level of the drug to inhibit MMP in the wound itself. Clinical evaluation of the 2 % DOXH solution was conducted in a study of wound healing that not only did not close during the first 55 days of Standard of care, but also increased in size during this period. The addition of 2 % DOXH solution locally to the Standard of care regimen had a positive effect, particularly in reducing the size of the wound during the first two weeks of use and promoting healing until complete closure (Gabriele et al., 2019). The impact of local DOXH application on skin scarring was investigated in vivo using a model of skin wound injury. A single dose of 3.90 mM doxycycline (2 mg/mL) administered within 12 h of injury was found to significantly reduce scar thickness by 24.8 % ($^*P < 0.0001$) without compromising tensile strength. The same effect could not be achieved with the oral administration of doxycycline. In doxycycline-treated scar matrices, collagen I content was significantly reduced ($^*P = 0.0317$) and fibers were favorably arranged with significantly increased fiber randomness ($^*P = 0.0115$), which is a promising direction in treating traumatic wounds. (Moore et al., 2020).

Topical application of doxycycline solution (2 mg/ml in phosphate-buffered saline, four times daily) was used in different phases of treatment of corneal erosion after sulfur mustard (SM) exposure, where high MMP-9 activity and low MMP-2 activity were detected in all exposed eyes and corneas of the experimental group. During the acute treatment phase, doxycycline reduced MMP-9 activity. Prolonged topical treatment with doxycycline reduced MMP-9 activity in tear fluid, cornea, and severity of damage. Doxycycline was clinically effective only if treatment was started before neovascularization occurred (Horwitz et al., 2014).

Thus, long-term local treatment with low doses of DOXH is capable of significantly reducing the expression of MMP-9 and NGAL in wound tissues. However, limitations in creating a parenteral form have been found in the inability to assess the impact of low DOXH doses on the development of bacterial resistance. DOXH solutions were prepared only before application, and the stability of DOXH solutions over time, i.e., the delivery of DOXH in the form of solutions for topical application, is only possible under stationary conditions.

3.2. Doxycycline hyclate gels for local delivery

One of the areas of growing interest in the delivery of DOXH is the development of soft dosage forms based on polymers that can be applied topically, mucosally, or at the site of the wound. Examples of such forms include simple classical gels, in situ gels, gel emulsions, hydrogels, etc.

3.2.1. In situ forming gel

In situ, gel systems are polymeric compositions that are in the sol state before application but change to gel forms under physiological conditions. Various natural and synthetic polymers can undergo in situ gelation and have the potential for use in parenteral drug delivery: buccal, rectal, vaginal, ocular, intraperitoneal forms, etc. (Kouchak et al., 2014). Some drug formulations are commercially available, such as Atridox (Atrix Laboratories), which contains DOXH in an in situ gel system (Javali et al., 2012).

As an example of the effective use of DOXH in the form of an in situ gel, we can mention gels for the treatment of periodontitis, in case of inflammatory diseases affecting the tissues supporting the teeth, as well as in open periodontium and mucoskeletal wounds, by direct intrapocket administration (Phaechamud et al., 2015; Raval et al., 2018).

Various influences of polymers, such as ethyl cellulose, eudragit RS, shellac, bleached shellac, cholesterol, benzyl benzoate, and N-methyl-2-pyrrolidone in gel formulations were studied during model drug development (Puyathorn et al., 2023). Doxycycline-loaded in situ gels were investigated for their physicochemical and microbiological properties, including viscosity, rheology, injectability, water diffusion rate in the gel, gel formation, doxycycline release profile, degradation of the gel in vitro, and antimicrobial activity. Such a system can be easily introduced to the target site with minimal injection force. A Doxycycline-loaded cholesterol-based in situ gel system containing 10 % benzyl benzoate was found to be the most suitable for sustained release over 10 days, exhibited antimicrobial activity against *Staphylococcus aureus, Streptococcus mutans* ATCC 27,175, and *P. gingivalis* ATCC 33,277 and was recommended as a dosage form for the treatment of inflammatory and wound processes in periodontitis (Phaechamud et al., 2017). A thermo-reversible in situ gel of doxycycline hydrochloride can transmute from sol-gel transition with a change in replication to environmental temperature vicissitudes made up of gallic acid (GA) and tamarind seed polysaccharide (TSP). The optimized gel composition (DT8) was evaluated for stability and compatibility, appearance, gelation temperature, and gravitational flow simulation, in vitro over 12 h. Faster wound epithelialization and higher wound contraction rate in rats compared to the control group were demonstrated (Gupta et al., 2021).

Therefore, suitable criteria for doxycycline-loaded in situ gel systems include a good safety profile, consumer properties, and effective

antimicrobial activity against wound infection pathogens such as *Staphylococcus aureus, E. coli, Candida albicans, Streptococcus mutans*, and *Porphyromonas gingivalis.*

3.2.2. Gel emulsions

An important area of wound care is the provision of lipids to the tissues.

Certain lipids, such as polyunsaturated fatty acids (PUFAs), play a key role in the structure of cellular membranes and are essential for anabolic events during tissue regeneration. PUFAs cannot be synthesized in sufficient quantities and therefore must be supplied through an appropriate delivery route.

Expected, that the coexistence of dispersed bioavailable oil and aqueous phases in a single pharmaceutical form may be crucial for effective wound healing (Shingel et al., 2008). To provide a localized antibiotic delivery system to inaccessible wound sites, in situ forming microspheres (ISM) have been developed through solvent exchange-induced self-assembly. Overall, in situ forming microspheres is a non-aqueous emulsion consisting of polymeric non-aqueous droplets filled with an antibiotic dispersed in an external oily phase. After injection and contact with the aqueous environment of physiological fluids, the internal phase of the emulsion solidifies based on precipitation properties through the solvent exchange mechanism and subsequently encapsulates the active substance within the obtained microspheres (Voigt et al., 2012). Doxycycline was studied as a model drug against periodontal pathogens, and a pharmaceutical formulation was prepared where Eudragit® RS PO (ERS) was included in the internal phase of in situ forming microspheres as a matrix-forming agent using dimethyl sulfoxide (DMSO) or 2-pyrrolidone (PYR) as a solvent. Olive oil and camellia oil were used as external oily phases. The Doxycycline-loaded ISM formulation has advantages in terms of injectability over a gel formed in situ (ISG) due to the lubricating effect of the external oily phase and lower viscosity compared to ERS-based ISG. ISM containing these two oils showed high injectability, phase transformation behavior, and sustained release of doxycycline for 7 days. The obtained microspheres exhibited a spherical shape with a porous structure and demonstrated effectiveness against pathogens (Chuenbarn et al., 2022). Chitosan microspheres loaded with doxycycline were developed using a novel water-in-oil emulsion technique, involving oil phase ionic gelation. Microspheres were prepared by using 6 % v/v of chitosan (3% w/v in acetic acid), soybean oil–n-octanol oil mixture (1:2 v/v) as the continuous phase, and 5 % span 80 as an emulsifier. Doxycycline was entrapped by the equilibrium swelling method with 8.4 % total entrapment. The doxycycline-loaded microspheres exhibited a minimum inhibitory concentration (MIC) of 16.5, 17.4, 11.2, and 98.3 μg against *Klebsiella pneumoniae* (ATCC 15,380), *E. coli* (ATCC 25,922), *Staphylococcus aureus* (ATCC 9144), and *Pseudomonas aeruginosa* (ATCC 25,619), respectively (Shanmuganathan et al., 2008). A stable, controlled-release gel composition containing 10 % doxycycline based on glyceryl monooleate with the addition of 5 % sesame oil was developed. The gel was injected and converted to a semi-solid form in the periodontal pocket and adhered to the mucosal membrane (Singh et al., 2021). The use of aqueous-insoluble hydrophobic ibuprofen as a matrix-forming agent for doxycycline loading with subsequent in situ gel formation by removal of the solvents dimethylsulfoxide and N-methylpyrrolidone was described (Puyathorn et al., 2023). Thus, non-aqueous solutions and carriers have been used in the development of new gel-emulsion forms for the protection and stabilization of doxycycline, and a wide range of plant oils containing essential PUFAs such as sesame oil, olive oil, camellia oil, and soybean oil have been incorporated into the formulation.

3.2.3. Hydrogels

Hydrogels, which are crosslinked networks of hydrophilic polymers, have been widely studied for the treatment of dermal wounds and ocular wounds in the past decade. Hydrogels are hydrophilic polymers composed of three-dimensional viscoelastic networks that absorb and retain water, exceeding their dry weight several times, and swell in physiological environments while maintaining their three-dimensional structure (Tavakoli et al., 2020). They have advantages such as fluid absorption, moistening of the wound bed, and cooling of the wound surface, which can alleviate symptoms such as erythema, burning, itching, pain, and skin damage, making them suitable for use in parenteral drug delivery systems, wound dressings, hygiene products, and regenerative medicine (Firlar et al., 2022). Depending on the amount of water in the tissues, hydrogels can absorb or release water into the wound environment. Maintaining a moist wound bed is widely recognized as being the most ideal environment for effective wound healing (Jacob et al., 2021).

Doxycycline hydrogels based on polyethylene glycol (PEG) were investigated and evaluated for their effectiveness in wound healing of skin and eye injuries after exposure to sulfur mustard chemical warfare agent. An eight-armed PEG polymer containing multiple thiol (-SH) groups was crosslinked using hydrogen peroxide (H_2O_2) hydrogel) or 8-armed S-thiopyridyl (S-TP hydrogel) to form the hydrogel. It was found that the addition of glycerol, polyvinylpyrrolidone (PVP), and PEG 600 to the formulation contributed to the retention of the hydrogel on the skin for up to 24 h. Histological studies of the skin showed that doxycycline-loaded hydrogels (0.25% w/w) provided an improved degree of wound healing on the skin after sulfur mustard exposure compared to untreated skin and placebo hydrogels (Anumolu et al., 2011). The penetration of doxycycline through blistered damaged cornea was 2.5–3.4 times higher than through intact cornea. Histology and immunofluorescence studies showed a significant decrease in matrix metalloproteinase-9 (MMP-9) and improved corneal healing after vesicant injury with doxycycline hydrogels compared to an equivalent dose of doxycycline delivered in phosphate-buffered saline (PBS, pH 7.4) (Anumolu et al., 2010). A collagen-based hydrogel was developed for the prevention and/or treatment of infections resulting from skin wounds (superficial burns, surgical wounds). Glutaraldehyde at a concentration of 0.30 % was used as a crosslinking agent. Crosslinking with glutaraldehyde reduced the release of doxycycline from the parenteral system, but the collagen/ doxycycline gel remained stable against enzymatic degradation (Ghica et al., 2012).

Doxycycline hydrogels exhibit non-Newtonian pseudoplastic flow behavior and are biocompatible with human skin fibroblasts and endothelial cells, which is important for their further use in wound healing.

3.2.4. Aerogels

Aerogels are obtained through supercritical drying of gels, which is the only drying method capable of preserving the nanostructure of a dry nanoporous material with high porosity. Aerogels and their combinations are considered interesting compositions for wound healing due to their high water absorption capacity (Yahya et al., 2020). Alginate and pectin aerogels in a multilayer structure serve as carriers for pharmaceutical agents (Del Gaudio et al., 2013). A new composition of aerogel in the form of polymer microcapsules containing an alginate-based core-shell and amidated pectin loaded with doxycycline was obtained using coaxial spraying technology.

Alginate with a high mannuronic acid content, capable of stimulating cytokine production by human monocytes, was used as a component of the shell and as a wound healing enhancer. The microcapsule core was based on an amidated pectin matrix containing doxycycline. Both polymers allowed the formation of a multilayer structure with controlled release of doxycycline at the site of wound injury. The degree of cross-linking between alginate and pectin in the microcapsules resulted in a highly varied nanoporous texture of the polymer matrix. The ability of the particles (core-shell) to swell and gel upon contact with wound fluids is directly related to the concentration of alginate. However, all compositions transformed into a gel state between 40 and 50 min (De Cicco et al., 2016).

However, the widespread application of aerogels is limited due to their multi-step technological process (sequential coating application, polymer cross-linking, etc.), multi-step processes, or prolonged preparation times of the medicinal product.

3.3. Nanofibers

The electrospinning process is a commonly used technique in biomedicine with promising applications, including drug delivery carriers, tissue engineering, and wound dressings (Bhattarai et al., 2019). Electrospun nanofibers are considered promising candidates for biomedical applications due to their flexibility in providing unique medical properties, controlled release system of active substances, and the potential for industrial production using biopolymers and their blends, including polylactic acid (PLA), poly(lactide-co-glycolide) (PLGA), poly(ethylene-vinyl acetate) (PEVA), and polycaprolactone (PCL). Encapsulation of antibiotics in electrospun PLA fibers plays a key role in the treatment of local wounds and tissue reconstruction as it does not affect the bioactivity of the active substance (Chun Xu et al., 2020).

The fabrication of nanofibers using the electrospinning technique in the system of polylactic acid-hydroxyapatite-doxycycline (PLA-HAP-Doxy) as a drug delivery carrier is described. Two different approaches were used to prepare Doxycycline-loaded nanofibers: immobilization on the electrospun mat's surface and encapsulation in the fiber structure. PLA-HAP nanofibers containing Doxycycline were fabricated through physical adsorption with low Doxycycline content (3 and 7 wt.%), providing a suitable membrane for sustained release rather than immediate release of the drug in the treatment of local wounds and tissue regeneration (Farkas et al., 2022).

Poly(lactic acid) nanofibers containing doxycycline/polylactic acid (DCH/PLA) with different loading contents of doxycycline in the range of 5–30 % were developed using a simple electrospinning method. The mechanical properties, vapor permeability, and absorbency of the nanofibers meet the requirements for wound dressings. By adjusting the loading content of doxycycline, the wettability of the nanofibers can be shifted from hydrophobic to hydrophilic, and the release rate of doxycycline can be controlled for an extended period ranging from three days to two weeks. The DCH/PLA nanofibers demonstrated positive antibacterial activity against *E. coli*, indicating their ability to treat/prevent infectious wounds (Cui et al., 2019).

Kefiran, a microbial biopolymer, is capable of forming nanofibers through the electroforming method. The developed kefiran nanofibers are potential carriers for DCH delivery. Eight different samples with variations in kefiran concentration and process parameters were made. The inhibition zone for doxycycline-loaded nanofibers was 22 mm and 20 mm for *S. aureus* and *E. coli*, respectively. Additionally, kefiran nanofibers were evaluated for cytotoxicity against L929 cell lines. Doxycycline at a concentration of 1200 μg/mL showed 33.2 % cell viability after 72 h. Kefiran nanofibers in combination with doxycycline successfully reduce the side effects of doxycycline, including cytotoxicity, and show promise in wound healing (Dadashi et al., 2019).

Doxycycline-loaded nanofibers have great potential as wound dressings for the treatment of chronic wounds. They demonstrate accelerated wound healing to a greater extent than local doxycycline treatment due to the sustained release of doxycycline with fewer side effects.

3.4. Buccal films

Buccal drug delivery aims to administer drugs to the mucosal membrane lining the cheeks, located on the inner side of the oral cavity, and is capable of facilitating both local and systemic drug delivery, thereby avoiding first-pass metabolism and enzymatic degradation of drugs. Buccal film formulations are currently scarce in the commercial market; however, the scientific literature describes numerous therapeutic and clinical possibilities where mucoadhesive buccal films can be utilized to provide high-quality, effective, and safe treatment in the wound healing field. (Shipp et al., 2022). One of the main advantages of film application is that it increases the contact between the drug and the lesion so that the film acts as a dressing that separates the injured mucosal surface from the oral environment. Buccal films can protect the wound surface, reducing pain and improving the efficacy of oral cavity disease treatment (Stembirek et al., 2013).

The investigated mucoadhesive films, which were prepared by casting, consisted of a blend of hydroxypropyl methylcellulose (HPMC) E3, K4, and carbopol 940, and were loaded with doxycycline for application in the oral cavity. The formulation consisting of HPMC K4 in combination with C940 at a ratio of 5:3 was found to be the most effective in vitro and was evaluated in vivo for experimentally induced periodontitis, demonstrating its effectiveness in improving clinical parameters and reducing salivary MMP-8 levels. The prepared mucoadhesive buccal film with doxycycline, due to its adhesive properties, ensures close contact with the soft tissues of the gums and facilitates local antibiotic release (Dinte et al., 2023). The optimization of mucoadhesive buccal film compositions aims to achieve effective subtherapeutic (antibiotic) concentrations of DOXH that can act as MMP inhibitors and remain stable at 25 °C. Studied film composition, which contains microspheres in combination with DOXH, β-cyclodextrin, $MgCl_2$, sodium thiosulfate, high-quality hydroxypropyl methylcellulose, and Eudragit® RS 12.5 was found to be stable at 25 °C for up to 26 weeks. The addition of microspheres to the films reduces mucoadhesive capacity, peak detachment force, tensile strength, and elasticity but improves stability at room temperature (Patlolla et al., 2021).

Therefore, buccal films demonstrate that a residual amount of doxycycline observed after three days provides sustained local antibiotic release, which is a promising clinical perspective, especially for patients who lack discipline in wound care during treatment.

3.5. Biopolymers

Bioactive polymers are increasingly important in wound healing. Biopolymers are natural biomolecules synthesized by microorganisms, plants, and animals with the highest degree of biocompatibility. Their biologically active properties, such as antimicrobial, immunomodulatory, cell-proliferative, and angiogenic properties, create a favorable microenvironment for the healing process. Biopolymers such as cellulose, alginate, hyaluronic acid, collagen, chitosan, gelatin, chitin, fibrin, etc., are used in the development of parenteral formulations for wound treatment (Sahana et al., 2018).

The functional and structural characteristics of biopolymers improve wound care and enhance tissue regeneration, restoration of tissue integrity, and scarless healing. The accumulation of microbial biofilm around the wound surface can increase the risk and physically impede wound healing. Therefore, for the healing of both acute and chronic wounds, the combination of known biomaterials with antimicrobial agents is a promising approach (Prasathkumar et al., 2021).

Recent studies have described the investigation of doxycycline (DOX)-loaded collagen-chitosan non-crosslinked (DOX-NCL) and crosslinked (DOX-CL) scaffolds and evaluated their wound healing properties for diabetic wounds. In vitro experiments showed that the DOX-CL scaffold was biocompatible and enhanced cell proliferation compared to cells treated with the CL scaffold and control groups, indicating its potential for local treatment of diabetic wounds (Sanapalli et al., 2021). Thus, biopolymer-loaded DOX systems are promising for skin regeneration, restoration, and bio-printing in the treatment of skin wounds.

3.6. Implants

Implants represent a promising drug delivery form and offer a novel approach to the treatment of periodontal wounds, bone tissue regeneration, and peri-implant wound healing (Sculean et al., 2015). Previous studies show that the placement of the implants in the alveolar bone

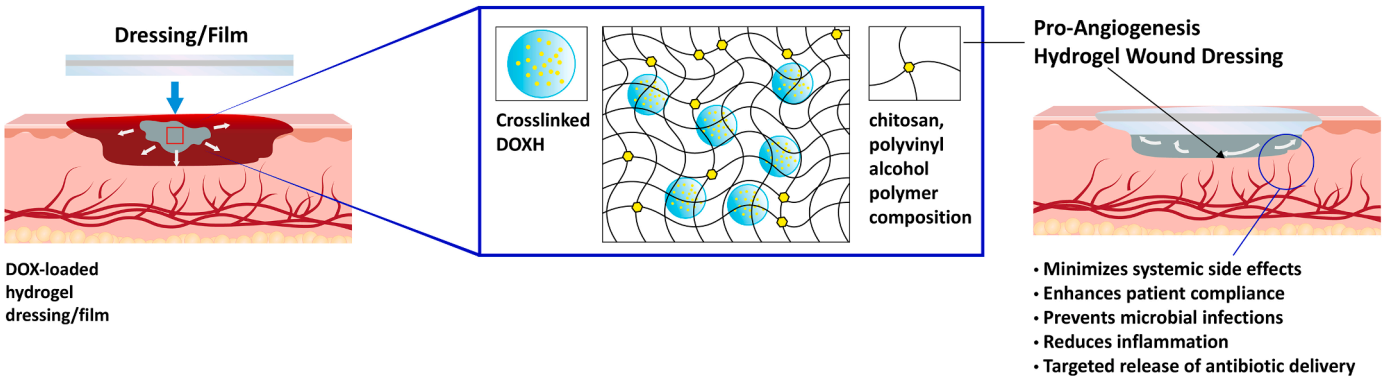

Fig. 1. Mechanism of the release of DOX-loaded hydrogel dressing/film.

triggers a cascade of healing events, starting with clot formation and continuing with bone maturation in contact with the implant surface or attachment to soft tissue (epithelium and connective tissue) (Carcuac et al., 2014). Although, despite the significant progress in implant development, infection remains the most common complication after implantation surgery. Therefore, antibiotic coatings on the implant surface can be a promising solution to reduce infection by providing local antibiotic delivery at the site of surgical intervention as implants with antibiotic coatings do not require additional removal surgeries or wound closure delay (Rahmati et al., 2020).

Studies on the selection of carriers for implant systems with doxycycline show the latest technologies, such as the synthesis of a copolymer of trimethylene carbonate/ε-caprolactone (TMC/CL) and glycolide/caprolactone (GL/CL) in the form of small elastic rings with a doxycycline content of 5 wt% and 10 wt% or in the form of flakes obtained by the electroforming technique (Kopytynska-Kasperczyk et al., 2015), where a coating of doxycycline solution is applied to the surface of titanium-zirconium alloys at a constant current of 0.65 mA. (Rahmati et al., 2020), coating of doxycycline on titanium alloy using Polymer-Lipid Encapsulation MatriX (PLEX) technology (Metsemakers et al., 2015). All doxycycline bolus injections have shown the ability to reduce the rate of implant-related infection, but there are still unsolved problems in increasing the stability of doxycycline implants, as exposure to air leads to attraction of carbon from the environment and degradation of doxycycline on the implant surface, where most test samples were stored in a hermetic container under gas to ensure stability.

3.7. Denticap

Denticap are soft polymer-based forms with the appropriate consistency to adhere to the tooth and are designed to provide sustained release of medication to provide better relief for dental patients. Most denticaps contain antibiotics and analgesic agents, including auxiliary substances such as Eudragit L, carbopols, Commiphora myrrha resin, Tragacanth gum, ethyl cellulose, hydroxypropyl cellulose (HPC), PEG-400, and others (Mukherjee et al., 2009). The development and evaluation of a novel sustained-release formulation containing DOXH and lidocaine hydrochloride for the treatment of pain sensitivity and early wound healing after non-surgical periodontal therapy have been investigated. Ethylcellulose, Tragacanth gum, HPC, PEG-400, and carbopol 934 were used to prepare the denticaps as a soft gummy-forming material. The drug release studies showed a cumulative release percentage of approximately 99.58 % of DOXH within 12 h. The antimicrobial activity of the formulation was determined by the disk diffusion method, and the highest inhibition zone was observed at a concentration of 100 μg/mL. The denticap was found to be stable and satisfactory in terms of the release of drugs for prolonged local wound healing and analgesic effects (Birtia et al., 2020).

3.8. Transdermal patches

Transdermal patches are adhesive patches placed on the skin to deliver a specific dose of a drug through the skin into the bloodstream. The primary objective of drug delivery through this dosage form is the localized release of drugs to enhance their presence and absorption at the desired site, which can be referred to as a drug release control system. Painless application, less frequent replacement, and greater dosing flexibility have led to the research and development of transdermal patches for the treatment of skin wounds. Biopolymers have been extensively studied for the manufacture of transdermal patches due to their safety, biocompatibility, low toxicity, and controlled degradation by human enzymes (Santos et al., 2022).

In the development of transdermal patches with controlled release for drugs with polar properties and higher molecular weight, such as DOX HCl, specialized delivery systems are required to achieve optimal therapeutic effects, such as water-organic partitioning. The rate of DOX transport through porous membranes in water-organic partitioning systems was studied, initially with release in 1-octanol and subsequently in light mineral oil, using appropriate organo-water partition coefficients. The creation of the patch was modeled by using mouse skin adjacent to a microporous membrane in the absence or presence of enhancers such as linoleic acid (Fan et al., 2004).

The results demonstrated that a porous polyvinylidene fluoride (PVDF) membrane and light mineral oil were suitable for in vitro testing of DOX with prolonged release for up to 120 h, achieving satisfactory release profiles of the polar antibiotic doxycycline HCl with higher molecular weight after optimization.

A controlled-release system for transdermal application based on a chitosan matrix loaded with DOX nanoparticles and selenium has been investigated and developed. Chitosan-based transdermal patches retain water in the underlying skin layers and promote cell flow. The inclusion of selenium nanoparticles in the transdermal patch formulation enhanced the wound healing action of this dosage form and provided better control of DOX dosage (Oyarzun-Ampuero et al., 2015). The prepared transdermal patch exhibited a uniform soft surface without bubbles, was opaque and purple, had acceptable appearance, disintegration time, thickness, swelling, surface pH, film adhesion, and in vitro release characteristics. The results indicated that this patch can be tested and used for the treatment of various skin conditions, such as skin wounds (Altememy et al., 2022).

3.9. Films (Hydrogel, bacterial)

Antimicrobial hydrogel films used for wound care absorb excess exudate through their gel-like structure, provide a moist microenvironment for enhanced cell growth and wound protection against pathogen attacks, and have enormous potential for controlling the increasing prevalence of microbial infections associated with impaired wound

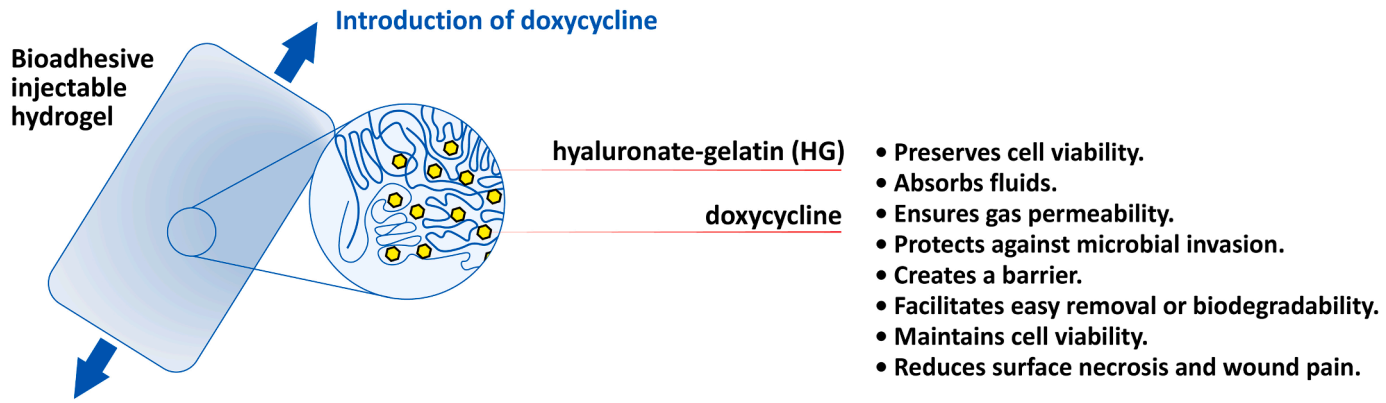

Fig. 2. Example of DOX-loaded bioadhesive injectable hydrogel dressing.

healing. The functional properties of hydrogel films depend significantly on the polymer composition and interactions within the network (Kamoun et al., 2017). Modern excipients and technologies with a wide range of synthetic and natural polymers, as well as hydrophilic polymers such as chitosan, carboxyl group-modified chitosan, polyvinyl alcohol (PVA), polyacrylamide, etc., are used for the production of these films (Zhang et al., 2015).

Films composed of chitosan, polyvinyl alcohol, and DOXH increased the rate of wound closure, collagen content, dermis, and epidermis volume (Fig. 1). Additionally, they increased the number of fibroblasts and basal epidermal cells, and vessel length, and decreased the number of neutrophils. Pro-inflammatory cytokines and MMP-2 were reduced, while anti-inflammatory interleukin-10 (IL-10) and tissue inhibitor of metalloproteinase (TIMP-1) were increased. DOX improves wound healing by reducing pro-inflammatory cytokines and matrix metalloproteinase and increasing TIMP. The combination of chitosan/PVA/DOXH may be a promising diabetic wound healing film during the proliferation phase (Hedayatyanfard et al., 2020).

Films have the ability to absorb a large amount of biological fluids, making them ideal parenteral delivery systems for antibiotics like doxycycline (DOX) for the development of wound care films.

The presence of bacterial biofilms in wounds is a major problem in the healing process. A novel approach combining bacteria-sensitive nanoparticles (NPs) loaded with DOX, incorporated into dissolvable microneedles (MNs), has been introduced for enhanced penetration of the biofilm and targeted delivery of DOX to the infection site. Poly (lactic-co-glycolic acid) (PLGA) and poly(ε-caprolactone) (PCL) were used as polymeric matrices for the NPs, which were coated with chitosan. Preparation of PLGA (NP-1) and PCL NPs (NP-2) with DOX loading was carried out using the double-emulsion (water-in-oil-in-water) (W/O/W) solvent evaporation method. The release behavior of DOX from the NPs was evaluated in the presence and absence of bacteria commonly associated with chronic wounds, namely *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Subsequently, the NPs were further loaded into dissolvable MNs. DOX release was significantly enhanced in the presence of bacteria-producing biofilms, up to 7 times. The incorporation of these NPs into dissolvable MNs greatly improved the dermatokinetic profiles of DOX, as evidenced by a longer retention time compared to patch formulations (Permana et al., 2020).

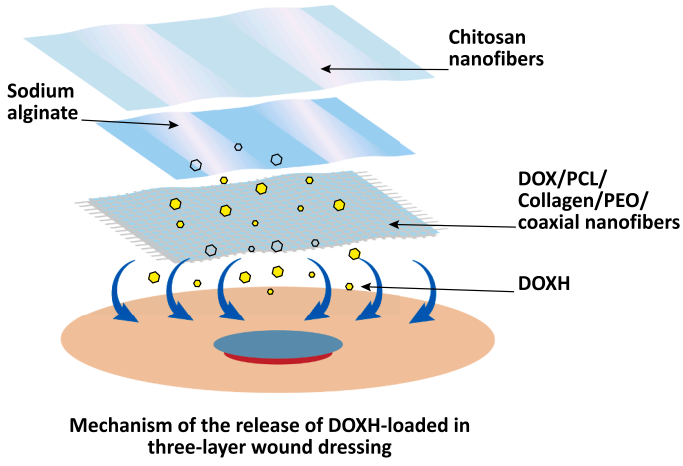

Fig. 3. Mechanism of the release of DOXH-loaded in three-layer wound dressing.

3.10. Wound dressings

The diversity of wound types has led to a wide range of antibiotic-containing wound dressings, which are often introduced to target various aspects of the wound healing process (Boateng et al., 2008). For effective clinical performance, wound dressings should possess characteristics such as moisture retention in the wound environment while absorbing or removing excess fluids and exudates, gas permeability, protection against microbial invasion, creating a barrier to shield the wound from external trauma, easy removal or biodegradability to avoid painful removal and damage to newly formed tissue, preservation of cell viability, reduction of surface necrosis, wound pain, and cost-effective facilitation (Firlar et al., 2022). Wound dressings may be applicable for improving wound healing in skin and eye injuries caused by sulfur mustard (SM) (Caffin et al., 2023). Dressings for wound management are used as primary treatment in patients not suitable for skin grafting. Modern excipients such as hydrocolloids, alginates, polyurethane, hydrogels, collagen, and hyaluronic acid are employed in the production of dressings (Dhaliwal et al., 2018), as well as Polyox and carrageenan (Boateng et al., 2013).

The initial dressings with DOXH were developed based on collagen-containing gelatin microspheres modified with 2,3-dihydroxybenzoic acid and filled with DOXH. The potential benefits of the dressing were investigated on an excision wound model in rats infected with *Pseudomonas aeruginosa*. The developed dressing attenuated both infection and MMP levels, thus demonstrating potential application for wound healing (Adhirajan et al., 2009).

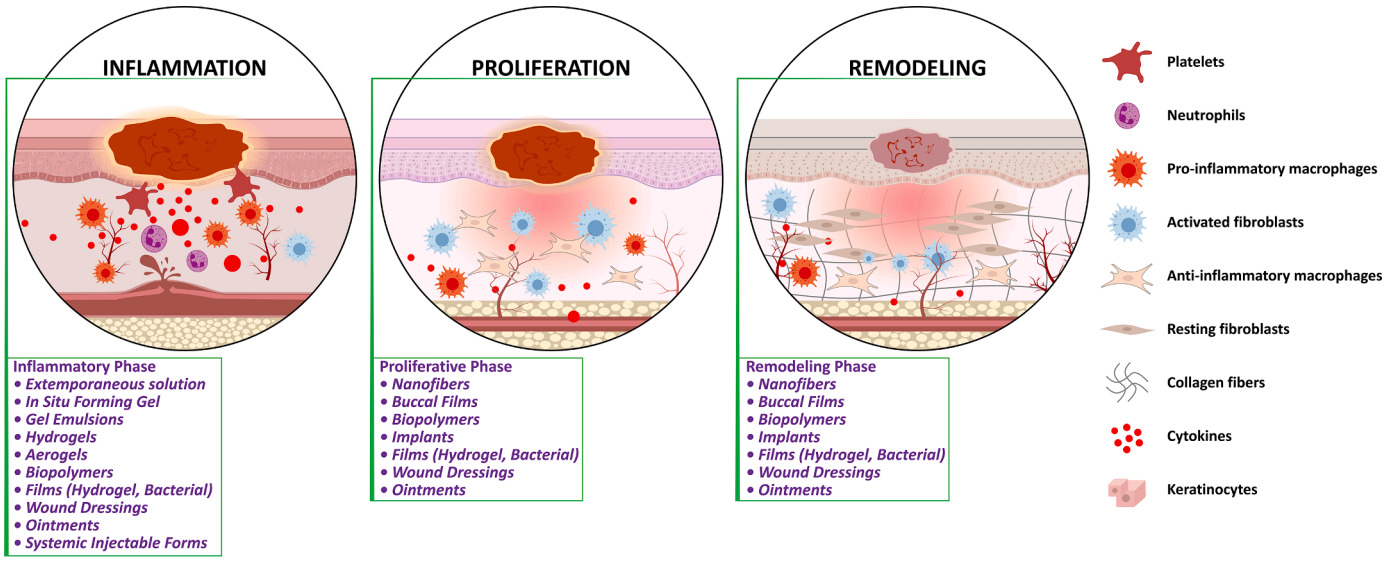

Fig. 4. Spectrum of action of DOXH-loaded delivery systems on different stages of wound healing.

A bioadhesive injectable hydrogel dressing for the treatment of burn injuries has been developed and investigated. The dressing is cross-linked by dynamic boronate ester bonds between modified hyaluronate and gelatin (HG). Doxycycline is encapsulated within the HG network for antibiotic delivery to wound areas (Fig. 2). The HG/Doxy hydrogel dressing demonstrates biocompatibility and antibacterial activity against gram-positive and gram-negative bacteria while promoting skin structure regeneration, including collagen deposition, angiogenesis, and hair follicle formation, ultimately reducing burn wound healing periods (Hu et al., 2023).

A promising approach in wound healing is a three-layer nanofiber wound dressing. The first and second layers are sodium alginate and chitosan nanofibers, respectively. The third layer, constituting the core of the coaxial nanofibers, contains 1 % polycaprolactone and 4.5 % collagen, while the shell consists of 2.5 % DOX and 2.5 % polyethylene oxide (Fig. 3). The developed dressing exhibits aligned nanofibers with a contact angle of 38°, a bioadhesion work value of 0.485 mJ/cm2 on rat skin, a tensile strength of 2.76 MPa, an elongation at break value of 7.65 %, a specific surface area of 9.65 m^2/g, and a porosity of 52.3 %. The DOX content is determined to be 260 μg/cm^2, and complete drug release is achieved within 15 min. The DOX content remains stable for 12 months at 4 °C (Tort et al., 2017). A three-layer nanofibrous biopolymer wound dressing loaded with DOX was evaluated for its antibacterial and anti-inflammatory properties from histological, biochemical, and immunohistochemical perspectives on full-thickness wound models in normoglycemic rats (acute model) and diabetic rats (chronic model) compared to a commercial product. Immunohistochemical analyses revealed a decrease in matrix metalloproteinase enzyme levels and an increase in tissue inhibitor of MMP, attributed to the antibiotic use and the fact that the dressing improved angiogenesis and shortened the inflammation phase. Biochemical analyses confirmed the effectiveness of the wound dressing in the inflammation and proliferation phases, while immunohistochemical and histological analyses demonstrated comparable strength and safety of the wound dressing for both acute and chronic wounds compared to the commercial dressing (Tort et al., 2020).

An in vitro and in vivo studied wound dressing with immunomodulatory and electrical conductivity properties has been created. [2-(acryloyloxy)ethyl] trimethylammonium chloride (Bio-IL) and gelatin methacrylate (GelMA) were 3D printed onto doxycycline hydrochloride (DOXH)-loaded reactive oxygen species (ROS)-degradable polyurethane (PFKU) nanofibrous membrane, followed by UV exposure to obtain electrically conductive hydrogel stripes. The dressing promoted endothelial cell migration and macrophage proliferation to an anti-inflammatory (M2) phenotype in vitro (Cao et al., 2022).

Wound dressings with DOX demonstrate the greatest effectiveness for accelerating wound healing by the mechanisms of collagen deposition, revascularization, and re-epithelialization by downregulating ROS and inflammatory factor levels, as well as by increasing the ratio of M2 macrophages.

3.11. *Ointments*

Ointments are semi-solid and homogeneous in appearance and are used for external application to the skin or mucous membranes. Classical ointments are predominantly hydrophobic in nature, poorly absorbable, and traditionally used as wound healing agents. Due to their (semi) occlusive nature, ointments are not well-suited for all biomedical applications in wound treatment, such as exudative dermatoses. However, wound healing ointments are frequently used for superficial injuries (Rüther et al., 2021).

DOX and amoxicillin (AMOX) were loaded into a nanocomposite of magnesium-aluminum layered double hydroxide (Mg-Al LDH) through co-precipitation. An experimental wound was aseptically created on the anterior-dorsal side of each rat. Acute toxicity, wound closure rate, percentage of wound healing, ulcer index, protection level, and histopathological investigations were evaluated after the application of DOX and AMOX-loaded LDH nanocomposite ointments. Rats treated with topical application of DOX/LDH (G4) and AMOX/LDH (G2) nanocomposites ointment showed a significant increase in the wound healing percentages and in the wound closure size at 4 ($p < 0.001$, $p < 0.00$), 8 ($p < 0.001$, $p < 0.001$),12 ($p < 0.001$, $p < 0.00$) and 16 day ($p < 0.001$, $p < 0.001$) compared to the untreated control group at the wound site. Nanocomposites (Mg/Al LDH) of DOX and AMOX in ointment form hold great potential for wound healing within a shorter timeframe (El-Ela et al., 2019).

3.12. *Systemic injectable forms*

Systemic injectable forms and bolus dosage forms of DOX can be beneficial in rapidly developing infections (such as cutaneous anthrax, plague) where the infection penetrates mucous membranes through an existing wound, and prompt DOX administration can be life-saving (Patel et al., 2023). As aqueous DOX solutions are unstable, lyophilized powder form allows for a stable formulation for long-term preservation and commercial availability.

Table 1
Examples of doxycycline-based drug delivery systems and their respective therapeutic target in wound healing.

Drug delivery system	Delivery vehicle	Wound healing	Reference
Extemporaneous solution	Phosphate-buffered saline	Corneal erosions	Horwitz et al., 2014
In situ forming gel	Cholesterol, benzyl benzoate 10 %	Wound periodontitis processes	Phaechamud al., 2017
In situ forming gel	Gallic acid, tamarind seed polysaccharides	skin wounds	Gupta et al., 2021
Gel-emulsion with in-situ forming microspheres	Eudragit® RS PO Dimethyl sulfoxide 2-pyrrolidone Olive oil Camellia oil	Wound periodontitis processes	Chuenbarn al., 2022
Gel-emulsion with microspheres	Chitosan, soybean oil n-octanol, span 80	infected wounds	Shanmuganathan al., 2008
Gel emulsion	Glycerol monooleate sesame oil	infectious periodontitis	Singh al., 2021
In situ forming gel- emulsion	Ibuprofen, dimethyl sulfoxide N-methylpyrolidone	infectious periodontitis	Puyathorn al., 2023
Hydrogel	peroxide water, S-thiopiridyl glycerin, PVP, PEG 600	Skin wounds	Anumolu, al., 2011
Hydrogel	Polymer 8-arm-PEG-SH (20 kDa), PVP, PEG 600	Dermal wound	Anumolu al., 2010
Hydrogel	Collagen, glutaraldehyde	Dermal wound, superficial burns, surgical wounds	Ghica al., 2012
Aerogel in form polymeric microcapsules	Alginate, amidated pectin	Dermal wound	De Cicco al., 2016
Nanofibers	Polylactide	chronic wounds	Cui al., 2019
Nanofibers	Kefiran	chronic wounds	Dadashi al., 2019
Mucoadhesive Buccal Film	HPMC, Carbopol 940	early process periodontitis	Dinte al., 2023
Mucoadhesive Buccal Film	β-cyclodextrin, MgCl2, sodium thiosulfate, High-Quality Hydroxypropylmethyl cellulose, Eudragit® RS	early process periodontitis	Patlolla al., 2021
Biopolymer	Collagen-Chitosan Composite	Diabetic Wounds	Sanapalli al., 2021
Implant	ε-caprolactone copolymers with trimethylene carbonate or glycolide	infectious periodontitis	Kopytynska-Kasperczyk et al., 2015
Implant	Titanium alloy	Surgical rani	Rahmati et al., 2020
Orthopedic implant	titanium alloy	Infections after surgical intervention	Metsemakers et al., 2015
Denticap	Ethylcellulose, tragacanth gum, HPC, PEG-400, and carbopol 934	early confinement run after non-surgical periodontal therapy	Birtia al., 2020
Transdermal Patch	1-octanol, mineral oil Linoleic acid, hydrophobic Celgard 2400 of polypropylene, hydrophilized polyvinylidene fluoride (PVDF)	Dermal wound	Fan al., 2004
Transdermal Patch	scaffold with chitosan, Nanoparticles selenium	skin-wound	Altememy al., 2022
Hydrogel Films	Chitosan, PVA	Diabetic Wound	Hedayatyanfard al., 2020
Bacterial biofilm	Poly (milk-co-glycolic acid (PLGA), poly (Ɛ- caprolactone) Bacterially sensitive nanoparticle	skin-wound	Permana al., 2022
Wound dressing	Collagen, 2,3-dihydroxybenzoic acid	skin-wound	Adhirajan al., 2009
Hydrogel Wound dressing	Hyaluronate, gelatin	burn injury	Hu al., 2023
Three-layered nanofiber wound dressing	nanofibers alginate sodium and chitosan, polycaprolactone, collagen, polyethylene oxide	skin-wound	Tort al., 2017
Three-layered wound dressing	Nanofiber Collagen, chitosan, alginate	skin-wound	Tort al., 2020
Hydrogel Wound dressing	Dimethylformamide, Gelatin, Hexamethylene diisocyanate (HDI), 1,1,1,3,3,3-hexafluoroisopropanol (HFIP), [2-(acryloyloxy) ethyl] trimethylammonium chloride (Bio-IL)	Diabetic Wound	Cao al., 2022
Ointment	magnesium aluminum layer double hydroxide (Mg-Al)/LDH nanocomposite	wound and ulcer	El-Ela al., 2019
intravenous bolus	2-hydroxypropyl-β- cyclodextrin (HP-β-CD)	in emergency situations, when fast intervention is required	Kiss et al., 2020

A new intravenous (IV) bolus dosage form of DOX was obtained using electrospinning. 2-hydroxypropyl-β-cyclodextrin (HP-β-CD) was utilized as a solubilizer for water-insoluble DOX, and lyophilized DOX-HP-β-CD was obtained using a high-speed electrospinning technique (HSES). The samples contained 100 mg of DOX equivalent (similar to currently available market products). The dissolution properties of the prepared complexes were compared by adding a small volume of water (1.5 mL) to the electrospun and lyophilized samples containing 100 mg DOX, yielding solutions without visible particles. The study confirmed that continuous high-speed electrospinning can be a viable high-productivity alternative to sublimation drying (Kiss et al., 2020).

Research has shown that DOX exerts potential benefits in various stages of the wound-healing process through multiple complex mechanisms. While its action is primarily in the inflammatory and proliferative phases, has been proven that DOX acts on each stage of wound healing. Modern DOX-loaded delivery systems can be selected case-on-case basis regarding the wound specifics and enable the consideration of such factors as wound location, the degree of infection, etc. The appropriate selection of a drug delivery system can significantly enhance wound healing efficacy (Fig. 4).

Table 1 summarises the examples of doxycycline-based drug delivery systems and their respective target activities in wound healing.

4. Future perspectives

The high effectiveness and absence of widespread resistance to DOX have opened up extensive possibilities for the development of parenteral antibiotic delivery systems. From the above review, it is evident that the formulation of DOX in many forms is still in the early stages of formula and technology development, as there are limitations in the stability of

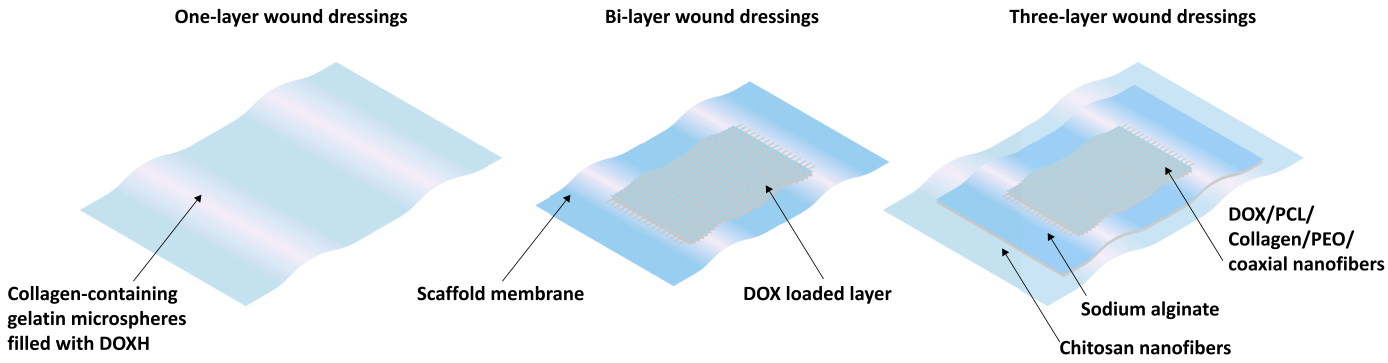

Fig. 5. Evolution of DOXH-loaded wound dressings from mono- to three-layered systems.

DOX, especially in long-term storage in a dissolved form. However, the number of studies searching for new formulations is increasing, as indicated by scientific sources, particularly in the last 5 years. Research has shown that careful selection of excipients for stabilization and protection of DOX from light and avoiding the use of water in formulations can lead to successful outcomes. The prospects of research lie in continuing the stabilization of DOX for long-term storage and scaling up the technology and industrial production to provide the market with commercial forms of modern DOX delivery systems for wound treatment. Wound dressings are considered the most common and practical form of DOX delivery, demonstrating the evolution from mono- to three-layered systems and maximizing effectiveness in accelerating the healing of skin wounds of various etiologies (Fig. 5). As a result of this report, DOX is currently being considered as a strategy for the development of a suitable convenient parenteral antibiotic delivery for wound treatment with consumer-friendly properties that do not require the involvement of medical personnel, and with the possibility of non-contact wound treatment using spray and aerosol forms, which can be useful in emergencies and the absence of access to medical care.

5. Conclusions

The wound-healing action of DOXH is attributed to the synergy of tissue regeneration through the inhibition of matrix metalloproteinases (MMPs), reduction of anti-inflammatory cytokines, and suppression of protein synthesis by binding to bacterial ribosomes. The development of new parenteral pharmaceuticals based on DOXH is a promising direction aimed at limiting systemic side effects associated with high doses of DOXH and avoiding antibiotic resistance. Local application of doxycycline significantly accelerates wound healing, and no side effects have been observed with local application of DOXH. Therefore, doxycycline in parenteral forms can be an acceptable alternative to the widely used oral doxycycline for the treatment of wounds of various etiologies.

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Data availability

No data was used for the research described in the article.

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