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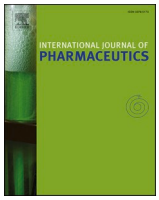

Review

Delivering hydrosoluble compounds through the skin: what are the chances?

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1. Introduction

As an application site for drugs, the skin has old-standing origins. The first evidence dates back to the ancient Egyptian populations reporting the use of natural products for skin care and cosmetics. Some thousands of years later, Galen introduced a vegetable oil-based cream for skin wounds, burns and joint pain. Since then, copious investigation on skin drug delivery has been documented and keeps gaining momentum (Ramadon et al., 2022). Such attractiveness results from the advantages offered by the cutaneous (epidermal and dermal delivery) and percutaneous (transdermal delivery) absorption of drugs. In the case of the epidermis and dermis, the cutaneous route allows direct access to the target tissues avoiding systemic administration and its shortcomings (Demartis et al., 2021b). In the case of transdermal delivery, the percutaneous absorption escapes the pre-systemic first-pass metabolism, often responsible for drugs low bioavailability and the significant frequency and severity of adverse effects. Furthermore, transdermal delivery offers a relatively consistent drug concentration in the bloodstream over a prolonged time. This undoubtedly facilitates reducing the frequency of administration and ameliorating patient's compliance and therapeutic adherence (Benson et al., 2019; Roberts et al., 2021). However, the most significant challenge derives from encountering a drug with a potent pharmacological activity that presents a biopharmaceutical profile suitable for skin penetration (Alkilani et al., 2015). The Scientific Committee on Consumer Safety (SCCS) has suggested that substances with a molecular weight>500 Da, a high degree of ionization, and a Log P lower than −1 or >4 provide an insignificant cutaneous absorption, mainly due to the brick and mortar structure of the *stratum corneum* (SC) (Ates et al., 2016). As a result, at the time of writing this review, the drugs approved by the FDA for transdermal administration mostly present low molecular weight, lipophilicity and relatively high potency thus requiring low doses (nicotine, rivastigmine, fentanyl and hormones, among others) (Benson et al., 2019; Wong et al., 2023). Instead, hydrosoluble, ionised, and hydrophilic molecules are rarely formulated in transdermal dosage forms as the percutaneous absorption of those compounds is challenging and they have often been left aside. Some hypotheses may be framed to explain this phenomenon:

I. There are not, or too few, hydrosoluble compounds helpful in treating skin diseases and alterations or that would benefit from a transdermal delivery.
II. Low aqueous solubility is among the significant issues encountered with formulation development, and the inadequate solubility of the desired dose results in incomplete absorption of administered drugs (Bhattachar et al., 2006; Savjani et al., 2012). Conversely, formulation and delivery of hydrosoluble compounds may be perceived as less tricky, resulting in less attraction to investigate.

However, both hypotheses can be easily confuted. Examples of hydrosoluble compounds employed for epidermal and dermal delivery are topical antibiotics (gentamicin and bacitracin, among others) and anaesthetics (lidocaine), amino acids, peptides or biological molecules for skin regeneration, and hydrosoluble vitamins (Chawla and Kvarnberg, 2014; Juhaščik et al., 2022; Nasca et al., 2014; Solano, 2020). In terms of percutaneous absorption, the increasing relevance of biological molecules (nucleic acids, proteins), largely hydrosoluble (e.g., insulin), to treat a wide range of diseases is evident, as well as the advantages of

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being administered transdermally (Chaulagain et al., 2018; Münch et al., 2017).

Regarding formulation and delivery strategies of hydrosoluble compounds, there are multiple aims to pursue, first of all, higher therapeutic adherence and safer therapy. As an example, local anaesthetics typically lead to sharp fluctuating levels of drug plasma concentration, causing patients to experience severe side effects based on the drug release into the aqueous biological media (Wang et al., 2019). Therapies based on the delivery of hydrosoluble molecules are limited by the low bioavailability or inappropriate biodistribution due to the poor stability against proteolytic and hydrolytic degradation in the case of macromolecules (proteins, peptides), low permeability across barriers, and a short biological half-life in the bloodstream; thus, delivery systems able to improve drug stability and biopharmaceutical profile are required (Demartis et al., 2021a; Labie et al., 2019). However, efficient delivery systems for hydrosoluble compounds are not easy to prepare as the rapid migration into the aqueous phase often leads to poor encapsulation efficiency alongside the *in vivo* undesired drug loss and burst release (Michely et al., 2022).

The current article proposes to revise the state of the art regarding complex formulation and delivery strategies adopted to enhance hydrosoluble compounds cutaneous and percutaneous absorption. Mainly, this review considers lipid and polymer micro- and nanoparticles, hydrogels, microneedles, 3D-printed devices and iontophoretic delivery systems presented in studies published in the last five years, including at least *ex vivo* studies. Although they are many and

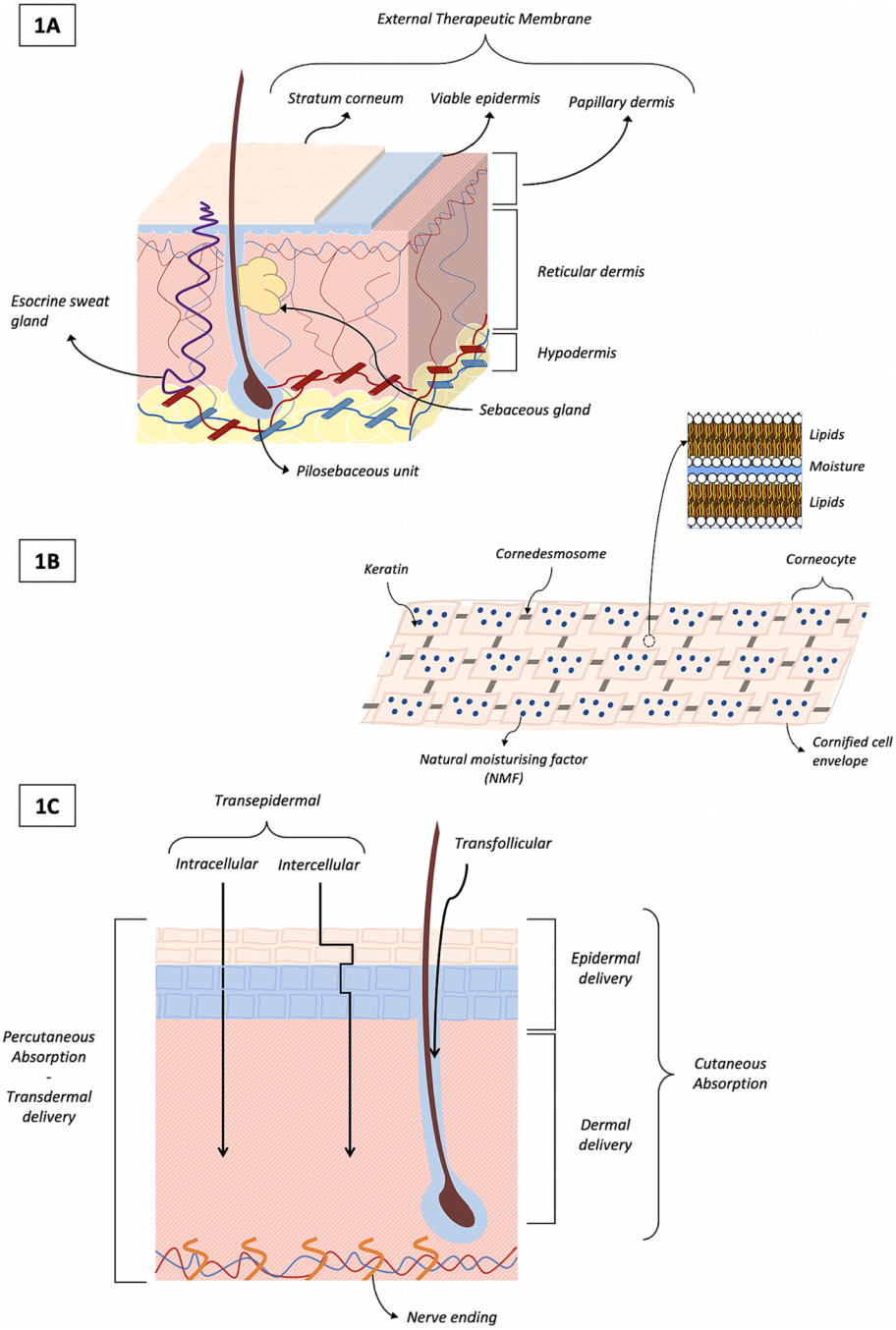

Fig. 1. Skin structure and skin drug delivery pathways. 1A) Simplified representation of the skin structure. **1B)** Zoom on stratum corneum structure. **1C)** Drug penetration pathways and representation of skin drug delivery targets.

have different therapeutic activities, little space is dedicated to hydrosoluble molecules in the current literature; notably, to the best of our knowledge, a review article dedicated to improving the performance of those drugs when administered to or through the skin is missing.

2. Considerations for the delivery of hydrosoluble compounds through the skin

The skin represents a dynamic system responsible for the interactions between the individual and the external environment. In this view, the absorption or deposition grade of a substance mainly depends on the skin status and the chemical-physical profile of the molecule (Zoabi et al., 2021).

2.1. Influence of the skin properties on drug delivery

Among the several factors that can affect drug delivery across the skin, the health condition in paramount (De Oliveira et al., 2021).

Cutaneous drug absorption is normally mediated by the so-called "external therapeutic membrane" (ETM), formed by three most superficial skin layers (SC, vital epidermis and papillary dermis) alongside the annexes (hair, glands, nails) **(**Fig. 1**A)**. In an adult individual presenting undamaged skin, the ETM measures about 200–300 μm in thickness. Among ETM layers, the SC is the main regulator of drug absorption. With an average thickness of 10–20 μm (Van Smeden et al., 2014), the SC is similar to a wall made of "bricks" organised in a running bond pattern surrounded by a lipid intercellular "mortar" (Michaels et al., 1975) **(**Fig. 1**B)**. SC has a very high density (1.4 g / cm^3 in the dry state) and is mainly composed of proteins (70%), lipids (20%) and water (Walters and Brain, 2002). Underneath the SC, the vital epidermis and papillary dermis represent the second-line barrier. The vital epidermis, 50–100 μm thick (Andrews et al., 2013), is comprises of keratinocytes and its composition changed to approximately 40% protein, 40% water and 20% lipids (Jepps et al., 2013). The basal lamina is located just below the vital epidermis; it is a 500 Å thick structure with fibrils extending into the dermis to anchor the skin layers and support the epidermis. The papillary dermis, 100–200 μm thick (Andrews et al., 2013), is the upper layer of the dermis, made up of thin bundles of collagen and elastin, fibrocytes, water, electrolytes, plasma proteins and polysaccharide-polypeptide complexes. Immediately beneath the epidermis is an extensive network of micro-blood vascularisation, an essential target in the administration of drugs, which originates from a superficial dermal plexus (Zgraggen et al., 2013). In this regard, dermal vascularisation is particularly developed and 1/3 of the total blood is estimated to circulate in it.

Local or systemic drug delivery can be achieved when medicated formulations are applied to ETM. However, the profound changes in skin physiology and structure (Liu et al., 2014), due to skin injures such as burns and cuts, not only exposes to the penetration of exogenous compounds that could be toxic, microorganisms that can cause infections and loss of water that leads to the alteration of the hydration gradient but also drastically change permeability to drugs (Giménez-Arnau, 2016; Qassem and Kyriacou, 2019). The removal of the SC significantly increases skin permeability, but if the entire epidermis is removed, permeability would further increase by 1–2 orders of magnitude (Andrews et al., 2013). Indeed, the basal membrane and tight junctions, which typically limit the absorption of hydrosoluble therapeutics, could have been damaged, allowing excessive drug absorption.

Wound healing is a complex process aiming at restoring the skin barrier function. After a minor or severe injury, the wound healing process initiates and consists of four overlapping and integrated phases: haemostasis, inflammation, proliferation, epithelialisation and tissue remodelling (Chin et al., 2019). Haemostasis occurs immediately after injury. If the alteration is relatively small, constriction of blood vessels, platelet plugs and formation of a blood clot result in an initial sealing of the wound, which prevents further blood loss and temporarily substitutes the skin barrier function. This triggers a molecular cascade and cellular events leading to the formation of a provisional extracellular matrix that functions as a scaffold for cellular attachment and subsequent proliferation (Wang and Yang, 2023). During the inflammation phase, the permeability of blood vessels increases. On one hand, this allows enzymes and immune cells to reach the injury site; however, on the other hand, the distribution volume of hydrosoluble molecules significantly increases and consequently causes undesired effects (Liu et al., 2014). The release of various growth factors and cytokines during the inflammation phase initiates the recruitment of fibroblasts, keratinocytes, and endothelial cells to repair the damaged blood vessels. Then, the inflammatory phase diminishes, and the proliferative phase succeeds. Angiogenesis and reepithelialisation occur, and the extracellular matrix undergoes remodelling. Collagen gets extensively remodelled and replaced but never achieves healthy skin structure; instead, it tends to form scar tissue (Diller and Tabor, 2022).

The pathophysiological changes described significantly affect the pharmacokinetics and pharmacodynamics of administered drugs (Souto et al., 2022). For example, it is very challenging to achieve a systemic effect following the application a conventional cream on intact skin. Conversely, ETM of injured skin promotes, to a great extent, the systemic absorption of the applied drug. On the other hand, wounds altered vasculature prevents proper drug delivery to the skin when the drug is administered systemically. A higher dosage of therapeutics is often required for systemic delivery and may induce undesirable side effects. Furthermore, hydrosoluble compounds have short half-lives. Due to the complexity of injured skin, an advanced drug delivery system is required (Saghazadeh et al., 2018). The application of topical drugs on damaged skin mainly focuses on accelerating the wound healing process and preventing infection. Therefore, topical hydrosoluble therapeutics in these cases mainly consist of growth factors and antimicrobial agents, but anaesthetics may also be used for pain relief (Eriksson et al., 2023).

The requirements for local and systemic drug absorption when considering intact skin are significantly different; in particular, systemic effects can be satisfactorily achieved when ETM provides minimal resistance to the penetration, marginal binding, and local metabolism of drugs (Choi, 2020; Souto et al., 2022). On the contrary, local delivery, including epidermal and dermal deposition, requires relatively high concentrations of drugs at the skin's outmost level and minimal absorption in the bloodstream. For both local and systemic drug delivery, the absorption may occur either following the solubilisation of low-molecular-weight and liposoluble molecules in the epidermis (transepidermal pathway) or by their migration through the annexes (transappendage pathway) (Schuetz et al., 2005). The transepidermal absorption of drugs may occur by the intercellular or transcellular routes **(**Fig. 1**C)**. The intercellular course involves the migration of the drug through the corneodesmosome (tight lipid junctions) between the corneocytes, representing the preferred pathway for small hydrophobic molecules. Conversely, in the transcellular (or intracellular) route, the drug passes through the corneocytes themselves, enabling the migration of small hydrophilic or amphiphilic molecules since herein drugs migrate through the water-filled openings existing because of imperfections in corneocytes (Iqbal et al., 2018; Zoabi et al., 2021).

The transappendage route can work for macromolecules and substances that cannot permeate through the transepidermal pathway. (Baroli et al., 2007) **(**Fig. 1**C)**. Hydrosoluble molecules appear as outstanding candidates for absorption through this route. Until recently, skin appendages were considered to play a minor role in cutaneous absorption of drugs even if it was consistently assumed that hair follicles and sweat glands could offer shunt routes through the SC (Otberg et al., 2008). The permeation efficiency through this route is strongly correlated to the region of the body. The pilosebaceous units are irregularly distributed in the body, and their density, the diameter of the orifice and the volume of the follicular infundibulum differ in each region (Souto et al., 2022). The total area of these openings was estimated to amount to just 0.1% of ETM, which is approximately 0.002 m^2 (Iqbal et al., 2018;

Zoabi et al., 2021). These numbers were revised after measuring follicular orifice size and distribution of hair follicles in different skin areas (Otberg et al., 2008). Recent studies concluded that the percentage of 0.1% is adequate for the inner side of the forearm, generally employed for *in vivo* permeation tests (Jain et al., 2016). The lowest permeability was detected on the forearm in contrast to the forehead, which showed the highest absorption rates (Otberg et al., 2008). Indeed, the forehead was found to have the maximum hair follicle density; furthermore, combining the scalp and face area, the number of follicular openings can cover>10% of the total skin area. Relating to the reservoir capacity of the SC, all follicles on the forehead are open for the penetration process in the follicular reservoir (Jain et al., 2016).

2.2. Transport of hydrosoluble molecules through the skin

Despite the complex structure of the skin, Fick's law appears suitable to describe the transport through this type of physiological membrane (Eq. (1)):

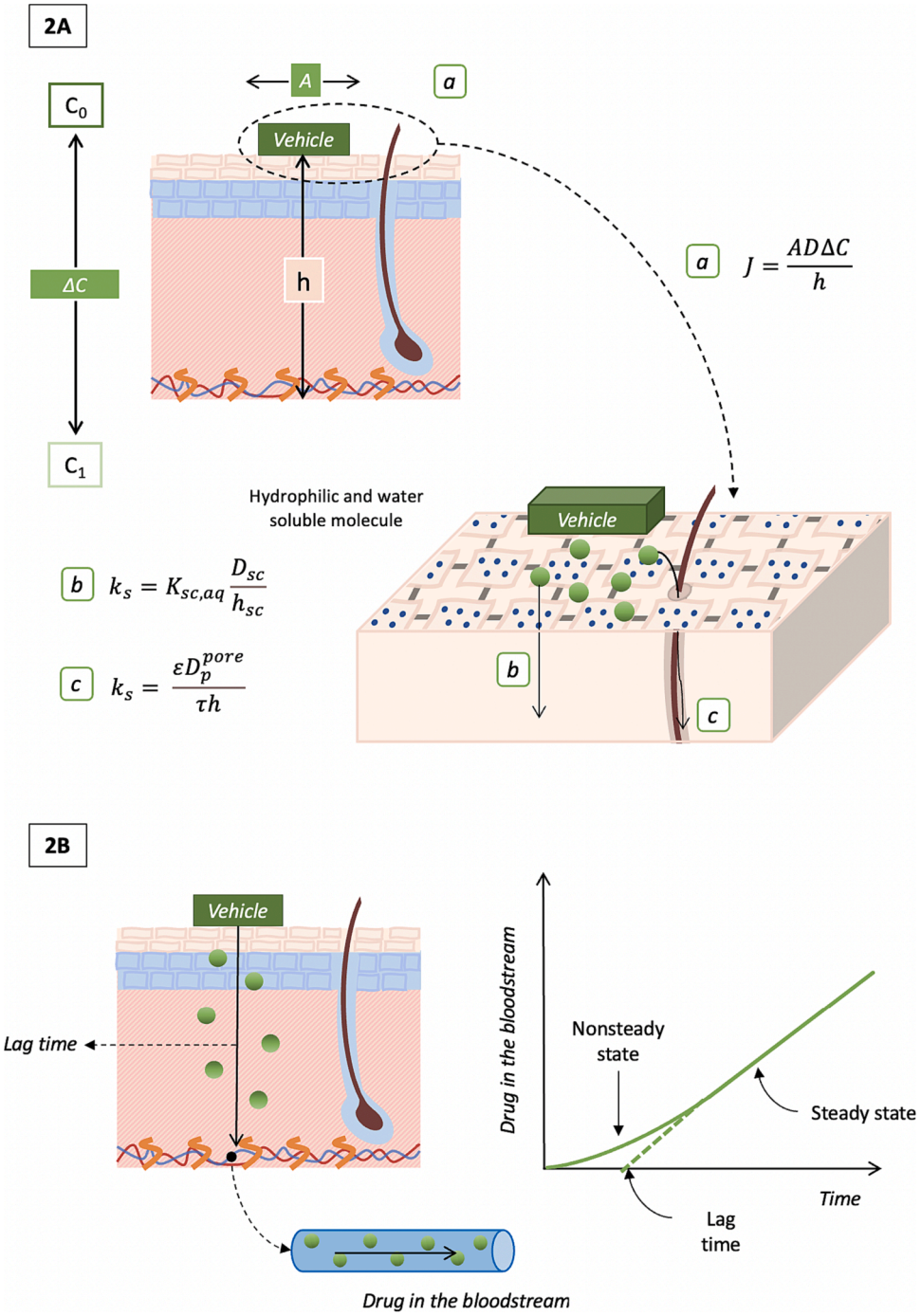

Fig. 2. Drug transport mechanisms through the skin. 2A) Fick's law diffusion (a): *J* is the drug flux through the skin, *A* the application area, D the drug diffusion coefficient, *ΔC* the concentration gradient across the two sides of the skin, *h* the skin's thickness; Transepidermal pathway of hydrosoluble drugs (b): ks is the human skin permeability, $K_{sc,\ aq}$ is the SC-water partition coefficient; Transappendage pathway of hydrosoluble drugs (c): where ε is the porosity, τ the tortuosity, D_p^{pore} is the diffusion coefficient of the drug in the liquid-filled pores of the skin. **2B)** Conventional drug permeation profile at steady state conditions, when a vehicle with constant drug concentration is applied to the skin.

$$J = \frac{AD\Delta C}{h} \quad (1)$$

where J is the drug flux through the skin, A is the area of the skin where the formulation is applied, D is the diffusion coefficient of the drug through the skin, ΔC is the concentration gradient across the two sides of the skin, h is the diffusional pathlength or skin thickness (Keservani et al., 2020) **(**Fig. 2**A)**. Even if the skin area and diffusional pathlength influence the drug flux through the skin, the driving force defining J is the concentration gradient ΔC. Skin drug delivery systems should be formulated to provide the maximum driving force for passive diffusion across the skin layers, this could be achieved by saturation of the delivery system with drug to ensure a sustained concentration gradient that drives delivery to the target site (epidermis, dermis, bloodstream), on the basis of the steady state (SS) conditions (Alkilani et al., 2015). In this case Eq. (1) can be written as

$$J_{ss} = Ak_sC_v \quad (2)$$

where J_{ss} is the steady-state flux, k_s is the effective diffusion constant, related to skin permeability of drugs and C_v is the drug concentration in the vehicle applied on the skin (Keservani et al., 2020).

Hydrosoluble molecules may dissolve in skin-hydrated regions, such as regions including keratin, and further diffuse within them. In the case of aqueous solutions, k_s is described in Eq. (3):

$$k_s = K_{sc,aq}\frac{D_{sc}}{h_{sc}} \quad (3)$$

where $K_{sc,\ aq}$ is the SC-water partition coefficient, D_{sc} is the drug diffusion coefficient in SC, and h_{sc} is its pathlength. In a saturated aqueous solution, $K_{sc,\ aq}$ is given by the concentration ratio between aqueous environments and SC (C_{sc}/C_{aq}) (Roberts et al., 2021) **(**Fig. 2**A)**.

SC hydration is essential for drug delivery efficiency. The permeability of hydrosoluble drugs in the hydrated areas of the SC depends on the mobility of the water molecules surrounding the tissue. Polar molecules of simple molecular complexity and lacking ionisable groups can cross the SC at about the same speed as water. As the polarity of the functional group increases, the permeability constant decreases. Beyond SC-hydrated regions hair follicles or sweat ducts are considered appropriate channels for the penetration of hydrosoluble drugs. In this case, a porous pathway model can be used to predict skin permeation. In particular, the k_s of a hydrophilic solute can be obtained by Eq. (4):

$$k_s = \frac{\varepsilon D_p^{pore}}{\tau h} \quad (4)$$

where ε is the porosity, τ is the tortuosity, D_p^{pore} is the diffusion coefficient of the drug in the liquid-filled pores of the skin, and h is its thickness (Peck et al., 1994) **(**Fig. 2**A)**. This approach suggests that pores can be created using chemical or physical techniques (Polat et al., 2011a, 2011b).

Eq. (2) can be used for a formulation applied to the skin, producing a consistent drug flux absorbed in the bloodstream at steady-state conditions, where the rate of drug entering the bloodstream becomes equal to the elimination rate of the drug from the body. The drug elimination from the body most often displays a first-order kinetic. In this case, Eq. (2) allows to obtain (Roberts et al., 2002):

$$C_p^{ss} = \frac{J_{ss}}{Cl_{body}} \quad (5)$$

where C_p^{ss} is the plasma concentration of the drug under steady-state conditions and Cl_{body} is the total body clearance of the drug. According to Eq. (5), a formulation applied to the skin's surface that generates a constant drug flux toward the bloodstream has a plasma concentration under steady-state conditions C_p^{ss} equal to the ratio of drug flux across the skin and the clearance of the drug. Eq. (5) can be used to predict the plasma levels of a drug under steady-state conditions from *in vitro* patch release data (Hadgraft and Lane, 2007). For example, the C_p^{ss} values of sinomenine hydrochloride encapsulated in liposomes or transfersomes were predicted by using J_{ss} *ex vivo* data. They correlated with the values obtained by *in vivo* kinetic experiments where transdermal delivery systems were applied to rats. The *ex vivo* and *in vivo* results evidenced that transfersomes had the highest ability to induce the permeation of sinomenine hydrochloride across the skin of rats (Fan et al., 2022). The C_p^{ss} values of chlorpromazine hydrochloride formulated as pluronic lecithin organogels evidenced the poor ability of the gels to increase the drug permeability across the skin (Alsaab et al., 2016).

When applied to the skin, several topical formulations can be characterised by significant solute depletion, with consequent plasma concentration–time profiles sensibly modified concerning steady-state conditions. In the case of slow-releasing patches are applied to the skin, the plasma concentration of the drug (C_p) during time can be described, over a long time as:

$$C_p = \frac{Fk_a dose}{V_d(k_{el} - k_a)}\left(e^{-k_a(t-lag)}\right) \quad (6)$$

where F is the cutaneous bioavailability of the dose, k_{el} is the first-order elimination rate constant of the drug, k_a is the first-order absorption rate constant of the drug and lag is the lag time related to skin permeation (Roberts et al., 2002).

Regarding the local skin delivery, cutaneous pharmacokinetics aims to study a given skin region as a drug target. In this case, the skin target concentrations can be deduced by knowing the drug flux to the site and drug clearance from the site. Under sink conditions, the steady-state concentration, $C_{ss,\ local}$, is determined as per Eq. (7) (Roberts et al., 2021):

$$C_{ss\ local} = \frac{J_{ss}F_{local}A}{Cl_{skin}} = \frac{F_{local}Q(t)_{ss}/t_{ss}}{Cl_{skin}} \quad (7)$$

where J_{ss} is the steady-state skin flux (i.e., the steady-state amount $Q(t)_{ss}$ permeating per unit area over a steady-state time (t_{ss})), F_{local} is the local bioavailability (which may be less than 1 due to epidermal metabolism and sequestration), and CL_{skin}/A is the drug clearance from that site per unit area.

The values of the steady-state free drug (or unbound) concentration C_{ss}* at a given skin site are defined as the product of the total concentration of the drug (C_{ss}) and its free fraction at that site (f_u*). Consequently, $C_{ss}^* = f_u^* C_{ss}$ results from the input flux of the drug and its removal clearance from that site. This approach can be extended by estimating the target site's free drug concentration (C*) by *in vitro* flux data. The following Eq. (8) enables us to calculate the C_{SS}* of a drug in a given area of the skin from its permeability coefficient and dermal clearance, also taking into account the corrections on permeability coefficient by partition coefficient effects:

$$C_{ss}^* = f_u^* C_{ss} = \frac{f_u^* k_p C_v}{\frac{Cl^*}{A} + k_p\frac{K^*}{K_m}} \quad (8)$$

Where k_p is the permeability coefficient constant to the site, C_v is the drug concentration in the vehicle, Cl^* is the drug clearance from the site, A is the area of allocation of the vehicle, K* is the SC–site partition coefficient ($K^* = C_{sc}/C_{ss}$), K_m is the SC-vehicle partition coefficient ($K_m = C_{SC}/C_v$). Eq. (8) can be used to estimate C_{ss}* values of drugs by using *in vitro* skin permeation values and *in vivo* dermal clearances (Roberts et al., 2002).

3. Complex drug delivery systems

The so-called "conventional" formulations cannot modulate drug release after administration. These formulations present some important advantages: the technological processes for their preparation are well-

known and well-established; costs for their design and manufacturing at the industrial level are usually low or relatively low. However, they are unsuitable for drugs that have low bioavailability, short half-life, *in vivo* degradability (e.g., peptide and protein drugs), need to reach a specific site of action (drug targeting) and have chronobiological requirements (when the therapeutic need of the drug is dependent on circadian rhythms). In all these cases, conventional formulations, despite their wide use, cannot be utilized because they fail the therapeutic goal. In recent decades, remarkable efforts have been spent developing complex drug delivery systems to improve patient safety, efficacy, and compliance. These systems should ideally fulfil different requisites. Firstly, these complex formulations can deliver the drug, throughout the treatment, at controlled rates; different drug release profiles can be achieved from these dosage forms, suitable for different therapeutic situations. Secondly, they can target the drug to a specific site. Lastly, they should have additional requisites such as the capacity to protect the drug from *in vivo* biodegradation, or the ability to improve the bioavailability of highly soluble drugs. Thus, developing complex drug delivery systems can be the only chance to improve or obtain therapeutic goals.

During the development of a delivery system for skin application, it is crucial to perform *ex vivo* permeation tests meant to evaluate drug permeation across the skin layers. Guidelines for standard experimental protocols for skin permeation studies were defined by the Organization for Economic Cooperation and Development (OECD) in 2004; they state that, depending on the target of the delivery system, trypsin-treated SC sheet, heat-separated epidermis, dermatome-treated skin, and intact skin can be used (Neupane et al., 2020). The skin model employed is the first parameter that affects this type of experiment and influences the conclusions that will be drawn. Human skin is considered the gold standard for assessing the delivery efficiency of skin drug delivery systems. Nevertheless, ethical and economic reasons pose a significant problem to its accessibility and use. Thus, isolated skin from animals, including pig, rodent, rabbit, and snake, is regularly employed as replacements since it can be obtained effortlessly, excised fresh before studies, and display lower variability than human skin due to using inbred animal lines (Neupane et al., 2020; Todo, 2017).

According to the European Medicinal Agency (EMA), it is not necessary to establish a correlation of *ex vivo* permeation results to that of *in vivo* permeation; furthermore, six or more replicates from at least two or more donors are suggested to be used to minimize the influence of skin variability. The species of the animal and the body part from where the skin was obtained need to be detailed (Neupane et al., 2020). It is important to note that no comparisons of permeation profiles and parameters of the same drug can be made when using different animal species due to other skin characteristics. Among the skin models used, the porcine one is generally preferred due to its structural similarity to the human skin in terms of hair growth density, thickness, and content of the different skin layers. In the same way, rat skin presents some similarities with human skin, and it is the most used rodent model. Nevertheless, several reports indicate that rat skin is generally more permeable than human skin. Likely, rabbit skin is more permeable, possibly due to the high density of hair follicles (Pereira et al., 2023).

3.1. Polymer and Lipid-based particulate carriers

The topical application of drug-loaded micro (MPs) and nanoparticles (NPs) has been extensively explored for local (epidermis and dermis) and transdermal delivery. As a general rule, particulate systems may facilitate skin drug delivery since the unique properties of high surface area, reduced size and smart surface chemistry enable efficient interaction with skin components to mediate transport and create depots for a controlled drug release. In this review, we provide an overview of the application of polymer and lipid-based particles to enhance the skin deposition and permeation of hydrosoluble molecules (Fig. 3, Table 1).

3.1.1. Polymer-based particles

Polymer-based particles have been rarely used for hydrosoluble drugs; they are preferably used for skin delivery of slightly soluble or water-insoluble drugs (Asad et al., 2021; Jeon et al., 2019; Mao et al., 2017; Reis et al., 2017). In the literature, only a few articles reported *in vivo* studies.

Rasagiline mesylate is a hydrosoluble drug clinically employed to treat Parkinson's disease. To overcome the low oral bioavailability, Bali and Salve proposed PLGA NPs in a gellan gum film for its transdermal delivery (Bali and Salve, 2020). PLGA NPs showed moderate drug entrapment efficiency (29.20 ± 1.82%), small size (221.7 ± 5.71 nm) and negative charge (−36.08 ± 4.37 mV). The *in vivo* pharmacokinetic studies in Wistar rats demonstrated a curve characterised by an initial lag time followed by sustained drug release for>72 h and the AUC was 7- and 11-fold higher than that obtained after oral and i.v. drug

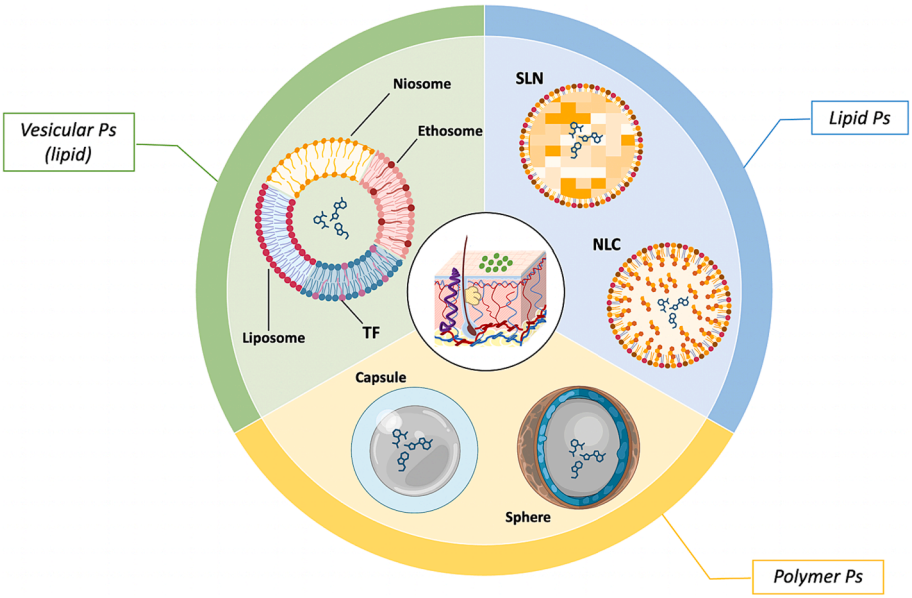

Fig. 3. Polymer and Lipid particulate carriers employed as hydrosoluble compound delivery systems for skin-related administration routes. Ps: particles; TF: transfersomes; SLN: solid lipid nanoparticles; NLC: nanostructured lipid carriers. Part of the image was created with Biorender.com.

Table 1
Polymer and Lipid-based micro and nanoparticles employed for skin-related delivery of soluble molecules as defined by European Pharmacopoeia 11 ed. (water solubility > 0.03 g/mL).

Delivery	*Drug*	*Solubility in water (g/mL)*	*Particulate carrier*	*Study*
Local	L-ascorbic acid	0.33	Spanlastics	(Elhabak et al., 2021)
	ascorbic acid	0.33	Chitosan MPs	(Di Filippo et al., 2022)
	nicotinamide	1		
	ascorbic acid	0.33	Ethylcellulose NPs, incorporated into an HPMC gel	(Duarah et al., 2017)
	Cefazolin	0.05	Niosomes	(Mansouri et al., 2022)
	polynucleotide	–	poly(β-amino ester)s NPs	(Duran-Mota et al., 2021)
	β-arbutin	–	Chitosan NPs	(Sahudin et al., 2022)
	VEGF-A mRNA	–	Ionisable lipid NPs (LNP)	(Zha et al., 2023)
Follicular	Arginine	0.18	Nanostructured lipid carriers (NLCs)	(Yazdani-Arazi et al., 2017)
Topical Immunisation	Ovalbumin	0.04	Liposomes, Transfersomes and Ethosomes	(Zhang et al., 2017)
Transdermal	Cytarabine	0.048	Ethosomes	(Raj et al., 2018)
	Rasagiline mesylate	0.053	PLGA NPs included in a gellan gum film	(Bali and Salve, 2020)
	plasmid DNA	–	PLGA NPs coated with chitosan glutamate	(Bansal et al., 2020)

administration, respectively. No skin irritation was observed during the application time. Moreover, after *in vivo* transdermal administration, NPs and the drug were able to diffuse through swollen film and to reach the blood and, after crossing the blood–brain barrier (BBB), the brain. Here, the rasagiline mesylate reduced Parkinson-like symptoms in the animal model (Bali and Salve, 2020).

Di Filippo et al. proposed spray-dried chitosan MPs for skin topical delivery of ascorbic acid and nicotinamide (Di Filippo et al., 2022). The MPs, with a size of 7.53 μm, spherical shape and low encapsulation efficiency (3.65 ± 0.82% and 6.94 ± 1.65%), were dispersed in an emulsion and applied on excised pig skin. The tape stripping technique demonstrated that MPs permitted nicotinamide to overcome the SC and deposited it in the epidermis without permeating the acceptor medium. No data were reported for ascorbic acid as its concentration was due to the permeated amount below the detection limits of the analytical method employed (Di Filippo et al., 2022). On the contrary, the *ex vivo* permeation profile was shown by Duarah and collaborators (Duarah et al., 2017); they demonstrated that ascorbic acid was released in a controlled manner for 8 h from ethyl cellulose-based NPs (258 ± 12 nm), incorporated into hydroxyl propyl methyl cellulose (HPMC) gel, and slowly and steadily permeated the excised goat skin membrane (40% of the drug up to 8 h) (Duarah et al., 2017). This result supports the feasibility of the topical delivery of ascorbic acid to treat hyperpigmentation.

Bansal et al. studied the *in vivo* immune response induced by intradermal administration of PLGA NPs coated with chitosan glutamate and loaded with plasmid DNA by electrostatic interaction (Bansal et al., 2020). This vaccine induced the production of antibodies against the rabies virus and the gonadotropin-releasing hormone inducing sterility in animals. After dispersion in a poloxamer gel with or without adjuvants, NPs (size of 350–450 nm and positive charge (+50.0 ± 5.0 mV)) were applied on mice skin treated with a microporation laser device. The results demonstrated that the laser-assisted skin delivery of pDNA NPs induced an immune response comparable to that obtained after intramuscular administration, using a four-fold lower dose. Adding adjuvants was helpful for obtaining infection control and immunocontraception (Bansal et al., 2020).

Duran-Mota and co-workers studied the *in vitro* transfection efficiency of polynucleotide-loaded poly(β-amino ester)s NPs on human dermal fibroblasts for the treatment of chronic cutaneous wound by gene therapy (Duran-Mota et al., 2021). The NPs (size between 99 and 206 nm, and positive zeta potential) were included in an *in situ* forming hydrogel based on poly(β-amino ester)s and PEG; the gel degraded within 240 h, releasing in a controlled manner the NPs that maintained the capability to transfect dermal fibroblasts without any cytotoxicity (Duran-Mota et al., 2021).

Sahudin and collaborators proposed chitosan NPs to enhance permeation through the skin of β-arbutin (Sahudin et al., 2022). Chitosan NPs, crosslinked with sodium triphosphate (size from 211 nm to 289 nm, zeta potential around + 50 mV), effectively increased the amount of β-arbutin penetrated and permeated through the excised rat skin to the free β-arbutin (Sahudin et al., 2022).

3.1.2. Lipid-based particles

Lipid-based nanoparticle systems (LBNS) are colloidal nano-sized carriers that encapsulate therapeutic molecules for biomedical purposes (Kumar et al., 2022). LBNS are more frequently employed as hydrosoluble drug delivery systems than polymeric particles. Furthermore, there is a significant number of research articles, including *in vivo* outcomes.

Unilammelar vesicles (ULVs) have been developed as a flexible vehicle able to squeeze through the gaps between the cornified cells reaching the deeper skin layers more efficiently (Cevc et al., 2002). Elhabak et al. designed Tween® and Span®-based ULV systems for topical delivery of L-ascorbic acid (LAA) to prevent skin ageing and tested them in an *in vivo* rat model (Elhabak et al., 2021). Tween 60®-based ULVs measured of 642.6 ± 16.54 nm, and presented a higher entrapment efficiency (42.75 ± 1.56%) than other prototypes. The tape-stripping technique revealed a deeper SC deposition of the drug compared to an aqueous solution. Furthermore, UVB radiation exposure produced an overexpression of MMP2 and MMP9 genes and their related proteins (matrix metalloproteases) that were downregulated by the treatment with LAA-based ULVs because of LAA antioxidant properties. Histological analysis of skin samples showed abnormalities like atrophy and epidermis thickness decrease, disorganisation of collagen fibres and atrophied skin appendages that were mitigated in animals treated with LAA nanosystems. As stated, ULVs exhibit higher drug permeability rates than classical liposomes (Elhabak et al., 2021).

Using nanotechnology also brings the opportunity to treat skin appendages or wounds locally. Yazdani-Arazi et al. delivered arginine (ARG) via Precirol®-oleic acid-based nanostructured lipid carriers (NLCs) as an alternative approach for treating alopecia (Yazdani-Arazi et al., 2017). Their carriers showed a particle size and entrapment efficiency rate of 87 nm and 68 ± 3.4%, respectively. In an *in vivo* model, first, rhodamine B-loaded NLCs confirmed their ability to achieve a follicular accumulation compared to free-rhodamine B in fluorescence microscopy observations. Then, after 8 weeks of treatment, an enlargement of the hair follicles area only was observed when ARG was encapsulated in NLCs (Yazdani-Arazi et al., 2017).

Chronic wounds are among the most challenging skin conditions that need a practical therapeutic approach to avoid related medical complications. The ideal treatment of these injuries requires a dual action: tissue regeneration and bacterial infection prevention. Mansouri et al. developed cefazolin (CEF)-loaded niosomes incorporated in a chitosan nanofibrous scaffold with an average diameter of 184 ± 8 nm (Mansouri et al., 2022). Applying the scaffolds in an *in vivo* wound model showed the ability of chitosan nanofibers to enhance skin regeneration by improving re-epithelialisation, tissue remodelling, and angiogenesis.

Specifically, CEF-loaded niosomes played a crucial role in preventing bacterial infections (*S. aureus* and *P. aeruginosa*) during the healing process because of the gradual release of CEF (Mansouri et al., 2022). Recently, ionizable lipid NPs (LNP) have attracted much attention as an optimized mRNA delivery carrier (Mansouri et al., 2022). Ionizable LNP are positively charged at low pH, while at physiological pH, they remain neutral. The pH-sensitivity of ionizable LNP is advantageous for mRNA delivery *in vivo*: the positively charged ionizable lipids can boost membrane destabilization and improve the endosomal escape of the NPs (Mansouri et al., 2022). Moreover, they effortlessly combine with mRNA (negatively charged) by electrostatic force and form LNP with a general surface charge close to neutral. Zha and co-workers developed LPN based on ionizable lipids to efficiently deliver VEGF-A mRNA for diabetic wound healing treatment. NPs were produced by applying microfluidic technology and tested *in vitro* and *in vivo* (Zha et al., 2023). Ionizable lipid L546-1, helper lipid (DSPC), cholesterol, and lipid-anchored PEG (DMG-PEG2000) were used to prepare the carrier at a 50: 10: 38.5: 1.5 M ratio. Obtained loaded LPN, LNP/VEGF-A mRNA, showed regular spherical morphology, a small size (101.17 nm), a narrow PDI (0.17) and negative Zeta potential (-3.05 mV). LNP/VEGF-A mRNA possessed an excellent mRNA delivery efficiency, protected mRNA from rapid degradation, possessed excellent biosecurity and promoted endothelial cell proliferation and cell migration validating their ability to promote *in vitro* wound healing. Finally, *in vivo* studies were performed in a diabetic mouse model; 8-mm diameter full-thickness dorsal cutaneous wounds were created on the shaved backs of diabetic mice. Intradermal injection of loaded LNP gave rise to protein production at the injection site and generated epithelialization, increased vessel density and produced abundant collagen. Therefore, it was concluded that the LNP/VEGF-A mRNA formulation accelerates diabetic wound healing (Zha et al., 2023). A specific study was carried out to understand the effect of the formulation parameters as the composition and content of lipids on the performance of LNP for loading sRNA and their efficacy (Blakney et al., 2019). The type of lipid, their charge (cationic, ionizable and zwitterionic) and their concentration, the ratio of total lipids to RNA, the ratio of cationic to zwitterionic lipids and the concentration of particles were evaluated. Through a DoE (design of experiment) study, saRNA-containing LNP formulations were optimized and tested on skin explants to evaluate saRNA-induced luciferase expression. Results demonstrated that lipid type and concentration significantly influence saRNA-induced luciferase expression in human skin. In particular, cephalin, a zwitterionic lipid used as a helper lipid, proved to be the most effective in complexation. The experimental design was very effective because it identified formulations that were 7 times more effective than the initial one. Flow cytometry studies showed that all formulations improve the expression of eGFP in human skin cells, particularly those with cephalin. In addition, the results showed that the immune cells express more RNA than the total cell population. This study demonstrates the efficacy of LNPs in delivering RNA and enhancing its effects; with the help of the experimental design, the optimization of the formulations yields optimal results (Blakney et al., 2019).

Lipid vesicles have also been used for transdermal approaches to achieve systemic effects in treating various diseases. Raj et al. used cytarabine (CYT) ethosomes to achieve an optimal transdermal absorption of CYT for treating leukaemia without topical side effects, to achieve better patient compliance as an alternative to oral delivery of the drug due to its poor bioavailability (Raj et al., 2018). It was observed that varying the ethanol and drug concentration led to changes in particle size from 118 to 201 nm and entrapment efficiency from 38.13 ± 2.42% to 63.46 ± 2.36%; however, all formulations showed narrow size distribution as indicated by low polydispersity (<0.24 ± 0.02). The *in vitro* permeability studies showed around a 5-fold increase in transdermal flux for ethosomes compared to liposomes and hydroethanolic solution; thereafter the safety profile of CYT ethosomes for healthy lymphocytes and their concentration-dependent efficacy in the growth inhibition of human promyelocytic leukaemia cells (HL 60) was assessed. *In vivo* studies were performed on rats after transdermal administration to determine the bioavailability of CYT and safety profiles. From the safety point of view, skin irritation results revealed a significant difference between the free drug ethanolic solution and the vesicular formulations in terms of skin erythema. *In vivo* pharmacokinetic studies reasonably correlated with *in vitro* findings since blood profiles revealed that CYT plasma concentration from ethosomes was 21.6 ± 2.6 ng/mL and 49.4 ± 2.5 ng/mL after 1 and 8 h, respectively. Rigid liposomes gave no detectable amount after 1 h and transported only 3.5 ± 0.01 ng/mL 8 h later (Raj et al., 2018).

Lipid vesicles have also been used transdermally for immunisation. Zhang et al. encapsulated ovalbumin (OVA) in liposomes, transfersomes and ethosomes containing stearyl amine or saponin as edge-activators (Zhang et al., 2017). Among the vesicular prototypes developed, ethosomes showed the best stability after 2 months of storage (mean diameter was maintained around 160 nm) and one of the highest entrapment efficiency rates (70.5 ± 0.9%). In addition, ethosomes provided the most elevated *in vivo* increase in the titre of serum anti-OVA IgG antibodies, specifically saponin-containing ethosomes. However, they exhibited a more limited entrapment efficiency (around 30 %). This effect points out that possibly a high level of incorporation of antigen into the formulation is not the critical point to trigger an immunological response *via* transdermal immunisation.

3.2. Hydrogels

Hydrogels are semisolid formulations consisting of highly cross-linked polymeric networks able to absorb their dry weight in water several times without dissolving (Tozzi et al., 2016). A wide variety of natural and synthetic polymers can be employed in the formulation of hydrogels (Li et al., 2022). The crosslinking can be obtained by physical entanglement, covalent or non-covalent chemical bonding (e.g. ionic interactions, hydrophobic interactions). Reversible crosslinking, triggered by changes in environmental conditions such as pH, ionic strength, electrical stimulation, temperature or presence of specific molecules, can be exploited in the design of smart hydrogels (Quan et al., 2022); these hydrogels either shrink or swell on cue affording a mechanism for controlled drug release (Sastri et al., 2022). Loading of highly hydrosoluble drugs into hydrogels is easily obtained by diffusion; controlling the release of these drugs and limiting burst release is more challenging, and several strategies have been developed to overcome this limitation, for example, the formulation of composite hydrogels containing MPs (Labouta and El-Khordagui, 2010), liposomes (Ciobanu et al., 2014), lipid NPs, micelles (Hu et al., 2020) or other components (Morsi et al., 2016). The use of hydrogels for the topical local and transdermal delivery of hydrosoluble drugs herein reported is summarised in Table 2.

3.2.1. Hydrogels for local delivery

One of the most common applications of hydrogels in topical drug delivery is to deliver drugs to the site of a wound, particularly as part of a dressing **(**Fig. 4**).**

Hydrogels are mostly suited for this application as they help maintain the injured tissue hydrated, thus supporting cell migration and production of extracellular matrix components that will favour the tissue regeneration at the wound site (Bustamante-Torres et al., 2021). At the same time, they can be exploited for the controlled delivery of antibiotics to prevent or treat bacterial and fungal infections and anti-inflammatory drugs to reduce inflammation and pain. The change in pH associated with a wound active infection can be used as a trigger for the specific release of antibiotics; this can be achieved by designing hydrogels that change in polarity or conformation depending on the environmental pH. One such example is the co-formulation of sulfasalazine, with slow antifungal activity, and tobramycin, a fast-acting antifungal. The combination of these two drugs has been chosen for

Table 2
Hydrogels employed for skin-related delivery of soluble molecules as defined by European Pharmacopoeia 11 ed. (water solubility > 0.03 g/mL).

	Drug	*Solubility in water (g/mL)*	*Hydrogel*	*Study*
Local	Tobramycin	0.05	Oxidised alginate-based smart hydrogel	(Zhang et al., 2021)
	Silver sulphadiazine	0.08	Acetal polymers-based smart hydrogel	(De Silva et al., 2019)
	Ciprofloxacin	0.036	pH-sensitive agar hydrogel	(Bustamante-Torres et al., 2021)
	DNAenzyme	–	Chitosan Hydrogel (polyplexes)	(Eicher et al., 2019)
Transdermal	metronidazole	0.06	Polymeric nanofibers-based gel	(Dahlizar et al., 2018)
	levamisole hydrochloride	0.48	Azone-enhanced hydroalcoholic gel	(Chen et al., 2018)

two main reasons. Firstly, because the two drugs act with different mechanisms of action, affording a higher efficacy when used together. Secondly, because the amino groups of tobramycin can react with the aldehydes of the main component of the hydrogel, oxidised dextran and form a Schiff base, thus crosslinking the gel, this crosslinking, as well as the link between sulfasalazine and the oxidised dextran, has been designed to obtain on-demand release of the two drugs, as degradation of the Schiff bases occurs at acidic pH as found on the skin when an active bacterial infection is present. This pH-sensitive crosslinking strategy limits the release of the drugs when they are not required and triggers their release, as well as the degradation and dissolution of the gel, in case of skin infection (Zhang et al., 2021).

Furthermore, controlling the degree of crosslinking, the gelation time of the formula and the self-healing properties of the gel can be tuned, allowing for the development of not only topical formulations but also injectable ones. Hu et al. previously employed a similar strategy using oxidised dextran, carboxymethyl cellulose, alginate and chondroitin sulphate as the gel bases and aminoglycosides antibiotics as the crosslinking agents (Hu et al., 2017). De Silva et al. have developed a smart formulation that responds to pH and temperature (De Silva et al., 2019). They employed polyacetal polymers that respond to temperature by changing their hydrophobicity. At room temperature, the hydrophobicity of the polymer can be exploited to hinder the removal of the formulation from the skin and help maintain moisture in the tissue. At the same time, washing off is aided by simply using cold water, as at lower temperatures, the polymer mixture becomes more hydrophilic. Furthermore, a lowering in pH induces degradation of the polymers, and fine-tuning of the degradation can be exploited to control the rate of release of the drugs. When highly hydrophilic drugs are loaded, the release rate can be reduced by manipulating the number of polyacetals used in the hydrogel formulation. *In vivo* studies have proven the efficacy of this smart system, showing a prolonged action compared to current formulations of silver sulphadiazine, fast wound healing and non-toxic behaviour. A pH-dependent hydrogel was also developed for ciprofloxacin (Bustamante-Torres et al., 2021; Kyriacos et al., 2009). In this case, the pH dependency of the hydrogel behaviour was exploited to maintain a sustained release of the drug rather than trigger it. Agar was crosslinked with acrylic acid, initiating the vinyl monomer radical polymerisation by gamma irradiation. Changing the concertation of the monomer and the power of the irradiation allowed to produce hydrogels with different degrees of crosslinking, tuneable swelling and mechanical properties, and controllable drug loading and release properties (Bustamante-Torres et al., 2021).

Hydrogels have been proposed likewise to entrap and deliver nucleic acid-based therapeutics. The investigation of Eicher et al. (2019) focused on the dermal delivery of 10–23 DNAenzyme entrapped in a chitosan hydrogel. In this study, the electrostatic interaction of negative DNAenzyme and positive chitosan was exploited to form DNA/chitosan polyplexes, further protecting the entrapped material from enzymatic degradation. Finally, skin penetration assays performed on fresh dissected porcine ear skin samples demonstrated the higher penetration of DNAenzyme when delivered by the chitosan hydrogel (Eicher et al., 2019).

3.2.2. *Hydrogels for transdermal delivery*

In order to obtain transdermal drug delivery, hydrogels are mainly used as a vehicle to support systems with specific mechanisms of penetration enhancement. Hydrogels can, for example, be used in combination with chemical penetration enhancers or as drug reservoirs for microneedles or iontophoresis. Various chemical skin penetration enhancers have been studied: azone, sulfoxides, pyrrolidones, alcohols, ether alcohols, glycols, fatty acid esters, surfactants and terpenes (Chen et al., 2022; Dahlizar et al., 2018). The different classes display different mechanisms of action or combinations of them, including enhancement of lipid fluidity, solubilisation of α-keratin, enhancement of drug

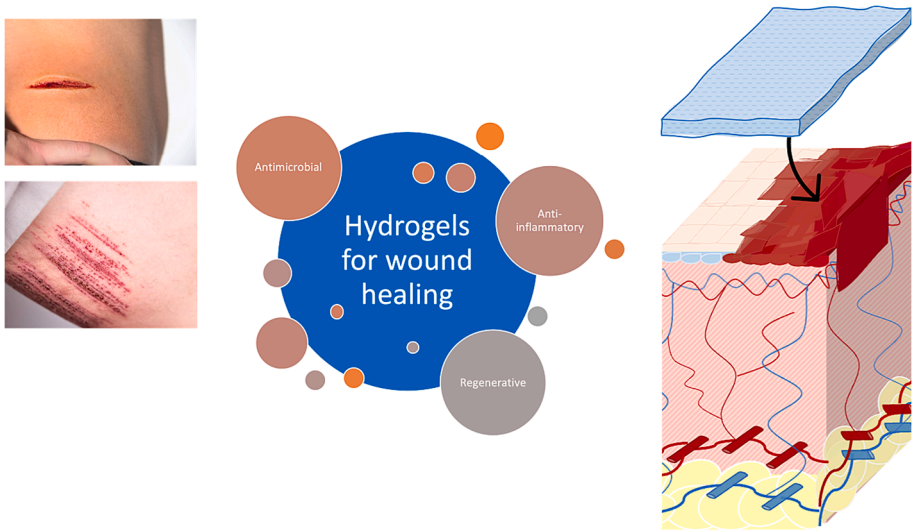

Fig. 4. Exemplification of Hydrosoluble drug-loaded hydrogel used for wound healing.

solubility in the skin lipids and changes in the hydration levels of the skin (Dahlizar et al., 2018). Dahlizar et al. studied the effect of several different penetration enhancers within *N*-Palmytoyl-Glycine-Hystidine (Pal-GH) gel on the penetration of the hydrophilic drug metronidazole (Dahlizar et al., 2018). Pal-GH forms nanofibers that assemble to form gels due to their amphiphilic nature. The concentration of Pal-GH was crucial in defining the release profile of the drug from the gel and could be used to fine-tune how tight the nanofiber network was and, therefore, the extent to which the drug could diffuse through it. While these structures can enhance skin permeation of metronidazole in comparison with an aqueous solution of the drug, they presented better results when either isopropyl myristate or propylene glycol were added to the formulation, with flux values 1.5 times higher. Interestingly these penetration enhancers did not have the same efficacy on their own, so it is suggested that Pal-GH nanofibers self-assembled into micellar structures that, in combination with the penetration enhancers, increase drug partition into the SC and affect the ordered structure of the phospholipid bilayer. The nature of the penetration enhancer can change the skin penetration efficacy of the formulation and the physical state the penetration enhancer is in within the formulation, as demonstrated by Chen et al. (Chen et al., 2022). Azone, a highly lipophilic penetration enhancer that increases the fluidity of the lipids in the SC, has been added to hydroalcoholic gels at different concentrations and in the presence of different amounts of Tween® 80. At low concentrations, azone was homogeneously dispersed in the gel; as the concentration increased, the formation of micelles, followed by oil droplets, was observed. Azone was effective in enhancing the steady-state flux of the hydrophilic drug levamisole hydrochloride when dispersed in the molecular or micellar state but not when dispersed as bigger oil droplets. The bigger droplets might also hinder the diffusion of the drug through the formulation. Dahlizar et al. and Chen et al. demonstrate the complexity of selecting penetration enhancers and evaluating the most effective dose.

3.3. Microneedles

Microneedle arrays/microarray patches (MN) are the basis of an attractive group of technologies that bypass the barrier of biological membranes and deliver drugs close to the target tissue or blood supply for systemic absorption, enabling enhanced delivery efficiency. Originally, MN were developed to facilitate transdermal drug delivery, as they can dramatically enhance skin permeability of various molecules, especially hydrosoluble drugs and biomolecules. Based on the inherent characteristics of MN, they are long enough to penetrate the SC and short enough to avoid significant damage to the dermis, dermal microcirculation and nerve endings, making application painless and blood-free. Several MN systems have been developed for the local, intradermal, and transdermal delivery of hydrosoluble drugs **(**Fig. 5**,** Table 3**)**.

Ruan et al. (2018) have developed coated MN containing cell-penetrating peptide octaarginine (R8)/siRNA nanocomplexes for targeted anti-melanoma treatment. This MN system deposited 90% of its payload into the epidermis and upper dermal layers of nude mouse skin after 5 min application (Ruan et al., 2018). In a murine melanoma model, the R8)/siRNA nanocomplex coated MN inhibited tumour growth, induced apoptosis and inhibited proliferation. This proves that coated MN do have therapeutic potential when incorporated with potent hydrosoluble compounds for localised drug delivery. Drug potency is, therefore, a key factor with coated MN, as the small needle size and the fact that only drug molecules on the needles themselves can be delivered, the loading capacity is typically quite low. This has been highlighted by Bhatnagar et al. (2018), in which the transdermal delivery of gemcitabine using drug-coated zein-based MN was investigated. These MN were prepared using a dip-coating approach, with 83.07 ± 3.24 μg of gemcitabine coated onto the MN (Bhatnagar et al., 2018). Considering the high doses of this anti-cancer agent, skin permeation of ~ 35% *in vitro* indicates that the low drug loading in this MN system would result in subtherapeutic levels. In addition to the low drug loading, coating uniformity is another critical limitation associated with this MN design and their industrial scale-up. As such, significant focus has been placed on dissolving and hydrogel-forming MNs as these have the potential to deliver sufficient concentrations of hydrosoluble drugs for a wide range of therapeutic applications.

Dissolving MN are made of hydrosoluble polymers and are typically prepared by integrating drug molecules into biocompatible polymers. For instance, Champeau et al. (2020) developed a hyaluronic acid (HA) dissolving MN for deep skin delivery of 5-aminolevulinic acid (5-ALA). Notably, the authors reported that the manufacturing method could be easily scaled up, with HA also selected due to its biocompatibility, fast solubility and biodegradation (Champeau et al., 2020). In contrast to the coated MN platform, this dissolving MN contained up to 100 mg 5-ALA, indicating that a single MN application is sufficient to deliver therapeutically relevant concentrations. HA-based MN have been

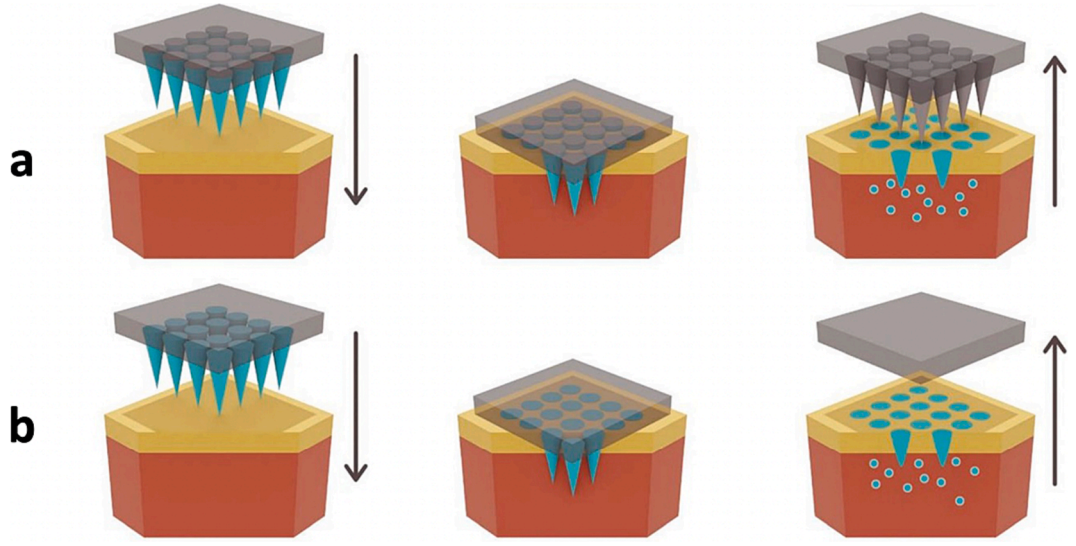

Fig. 5. Schematic representation of different types of MNs applied to the skin to achieve enhanced hydrosoluble drug delivery. (A) Coated MNs following the 'coat and poke' strategy. (B) Dissolving MNs following the 'poke and release' strategy. Adapted from (Kirkby et al., 2020) with permission from Springer, Copyright [2020].

Table 3
Microneedle arrays employed for skin-related delivery of soluble molecules as defined by European Pharmacopoeia 11 ed. (water solubility > 0.03 g/mL).

Delivery	*Drug*	*Water Solubility (g/mL)*	*Microneedle*	*Ref.*
Local	LiH	0.05	Dissolving MNs	(Yu et al., 2020) (Zhang et al., 2017) (Ramadon et al. 2022) (Lee et al. 2020)
Dermal	Octaarginine/ siRNA nanocomplex	1	Coated	(Ruan et al 2018)
	5-aminolevulinic acid HCl	0.05	Dissolving MNs	(Champeau et al. 2020;2021) (Requena et al., 2021)
	Ascorbic acid	0.33		(Zhang et al. 2020)
	Placental Bioactives miRNA-218	–		(Tansathien et al. 2022) (Zhao et al. 2023)
	Rose Bengal loaded TF	0.1		(Demartis et al. 2022)
	siRNA-loaded lipid vesicles	–	Haliclona sp. Spicules	(Liang et al., 2020)
Transdermal	Gemcitabine	0.04	Coated MNs	(Bhatnagar et al. 2018)
	Amifostine	1	Dissolving MNs	(Yu et al. 2020)
	Neurotoxin	–		(Yao et al. 2019)
	Calcein	0.05		(Kim et al. 2022)
	Staphylococcal enterotoxin B	0.1		(Liu et al. 2019)
	Lixisenatide	0.1		(Zhu et al. 2021)
	FITC-dextran	0.05		(Panda et al. 2022)
	Methylene blue	0.04		(Vora et al., 2019)
	Fluorescein Sodium	0.1		
	Sumatriptan succinate	0.1		(Ronnanden et al. 2018; 2019)
	Exenatide	1		(Liu et al 2023)
	Tranexamic acid	0.167		(Xing et al. 2021)
	Vitamin B12	0.03		(Donnelly et al. 2019)
	Ovalbumin	0.1		
	Bevacizumab	–		(Courtenay et al 2018)
	Anti-TNF- α VNAR	–		(Hutton et al 2022)

manufactured for the delivery of other hydrosoluble compounds such as ascorbic acid (Zhang et al., 2020), lidocaine (Ramadon et al., 2023), placental bioactives (Tansathien et al., 2022) calcein (Kim et al., 2022), amifostine (Yu et al., 2020) and miRNA (Zhao et al., 2023). Polyvinylpyrrolidone (PVP) is another widely available polymer with good biocompatibility and film-forming properties. As such, it has been used in several dissolving MN designs (Demartis et al., 2022; Ramöller et al., 2019; Yao et al., 2019; Zhu et al., 2021). For example, Ronnander et al. (2018) developed a sumatriptan-loaded polyvinylpyrrolidone (PVP) hydrosoluble MN for migraine treatment. These MN displayed sufficient mechanical strength for skin insertion with diffusion studies using Gottingen minipig skin, indicating that a 10.7 cm^2 MN loaded with 118.8 mg sumatriptan succinate would provide the required therapeutic plasma concentration when applied for 60 min. In addition, Xing et al. (2021) prepared tranexamic acid-loaded PVP dissolving MN to treat the chronic pigmentary disorder melasma. *In vitro* permeation of tranexamic acid across the skin resulted in a cumulative release of 44.43 ± 6.55% (Xing et al., 2021). Pharmacokinetic investigations in Sprague Dawley rats showed that the relative bioavailability of MN was 132.5% compared to the oral route when applied to the skin for 8 h. This suggests that the MN system can offer once-daily administration compared to the current oral dose of 250 mg two–three times daily. Several other hydrosoluble and biodegradable materials have been used to fabricate dissolving MN including carboxymethylcellulose (Lee et al., 2020), poly (methyl vinyl ether-maleic acid) (Requena et al., 2021), chondroitin sulfate (Liu et al., 2019), poly (D, L-lactic co-glycolic acid (PLGA) (Panda et al., 2022) and poly(vinyl alcohol) (Liu et al., 2023). Vora et al. (2020) also investigated the film-forming carbohydrate biopolymer pullulan. Using methylene blue, fluorescein sodium and FITC-BSA as model hydrosoluble compounds, the authors suggest that this pullulan-based MN could be used for the transdermal delivery of both small and large biomolecules (Vora et al., 2020). Furthermore, as pullulan is inherently biocompatible, repeat application to the skin would not result in polymer accumulation or long-term side effects.

Hydrogel-forming MN are composed of physically- or chemically-crosslinked hydrophilic, swellable polymer matrices. Donnelly et al. (2012) were the first to report the use of hydrogel-forming MN, proving that the creation of aqueous pores in the *stratum corneum* offered the potential for high-dose delivery of hydrosoluble drugs, including the ever-growing list of biological macromolecules. In this study, the authors successfully delivered metronidazole, caffeine and methylene blue across dermatomed (350 μm) neonatal porcine skin (Donnelly et al., 2012). Upon this initial work, Donnelly's team developed a 'super-swellable' Gantrez® S-97 based hydrogel-forming MN (Donnelly et al., 2014). In this study, ibuprofen sodium and ovalbumin were successfully administered to Sprague Dawley rats. For ibuprofen sodium, a maximum concentration of 179 ± 19 μg/mL was achieved at 6 h, which was deemed to be approximately 18 times greater than human therapeutic blood levels. The authors concluded that an ibuprofen sodium MN patch of < 32 cm^2 could be therapeutic in human subjects. The successful delivery of ovalbumin, a macromolecule, was also a significant finding as systemic absorption is typically slow when administered intradermally. Developing this work further, Courtenay et al. (2018) investigated the transdermal delivery of bevacizumab (BEV), an anti-cancer monoclonal antibody, using hydrogel-forming MN. Due to the high MW, BEV was found to drain into the lymphatic system following MN administration to female Sprague Dawley rats. Based on these findings, the authors suggested that MN-mediated delivery of BEV could be used to treat lymphoma carcinoma or secondary metastasis. More recently, Hutton et al. (2022) have shown that hydrogel-forming MN technology can also be used to successfully deliver a multivalent hydrosoluble anti-hTNF-α variable new antigen receptor (VNAR) derived from nurse sharks which is considered as a 'next generation' biotherapeutic (Hutton et al., 2022).

A study by Liang and collaborators focused on the formulation of small interfering RNA (siRNA) for skin delivery (Liang et al., 2020). RNAi therapy has great potential to treat skin diseases raised from abnormal gene expression. To overcome the skin barrier and intensify skin absorption of hydrophilic macromolecules, they developed formulations combined with sponge Haliclona sp. Spicules (SS), microneedle-like, with flexible liposomes (FL) or cationic flexible liposomes (CFL) loaded with siRNA.

Obtained liposome formulations were both homogeneously nano-sized unilamellar spherical vesicles with a mean diameter of around 100 nm. Loaded FL (FL-siRNA) possessed a neutral ζ-potential, while CFL-siRNA showed a negative zeta potential of −31.4 ± 1.1 mV. The optimum formulation was identified as CFL (0.05%)-siRNA (0.05% of

lipids, w/v). It possessed better deformability (94.08% ± 1.01%, $p < 0.001$) which strongly influenced the capability of CFL to deliver siRNA into the skin after SHS treatment. Moreover, studies using confocal microscopy demonstrated that labelled CFL- siRNA were not cytotoxic and favoured fluorescent siRNA cell entry, while siRNA alone could not enter the cells by itself. The topical application of SS in combination with CFL enhanced siRNA skin penetration *in vitro* by 72.95 ± 2.97-fold compared to the control group. Once more, the cell transfection effect was the best. The topical administration of SS combined with CFL resulted in the *in vivo* RNAi efficiency statistically comparable to that of the subcutaneous injection but also almost equally and homogeneously available to all topically applied areas. These results suggest that SS, in combination with CFL, can be suitable for large-area topical application for RNAi therapy. The combined system (SS and CFL (0.05%)-siRNA) permits to delivery of siRNA in skin deep layers by creating microchannels and facilitating the liposome cell internalization and consequently significantly inhibiting the protein expression *in vivo,* offering new opportunities to siRNA-based treatment for dermatological diseases (Liang et al., 2020).

3.4. Iontophoretic delivery systems

Iontophoresis is a drug delivery technique that exploits electric current to enhance the permeation of neutral and ionised drugs into the skin (Wang et al., 2022). Therefore, it is an ideal technique for the transdermal delivery of hydrosoluble compounds (Hasan et al., 2022). An iontophoretic system consists of a cathode, an anode and a power supply (Phatale et al., 2022). This technique relies on two transport mechanisms to enhance drug permeation across the skin: electromigration and electroosmosis (Wang et al., 2022). During electromigration, ionised drug molecules move in the presence of an external electric field due to electronic pair repulsion (Wang et al., 2022). Therefore, the permeation of cationic and anionic drugs through the skin can be enhanced by using positively and negatively charged electrodes, respectively (Wang et al., 2022). On the other hand, electroosmosis is the movement of fluid in the direction of an externally applied electric field (Pikal, 2001). In this way, hydrated ions can be driven inside the skin due to fluid movement. The skin presents a slight negative charge (Pikal, 2001), therefore, electroosmotic flow occurs from the anode to the cathode (Pikal, 2001). Electroosmosis plays a crucial role in enhancing the permeability of neutral drugs (Wang et al., 2022). A diagram of an iontophoretic device can be seen in Fig. 6. Table 4 summarises applications described in the literature for delivering hydrosoluble molecules to the skin using iontophoresis.

Puri et al. described the use of iontophoresis to deliver 3-fluoro amphetamine hydrochloride (Puri et al., 2017). This compound is a substitute-agonist therapy for cocaine use disorder (Puri et al., 2017). The transdermal route is appropriate for this particular application considering that individuals suffering from this condition present limitations with treatment compliance (Puri et al., 2017). In this study, the permeation of 3-fluoro amphetamine hydrochloride was evaluated *ex vivo* using excised human skin. This compound's passive permeation was around 4 μg/cm^2 when the drug was topically administered as an aqueous solution. Interestingly, when iontophoresis was applied, the permeation rate increased 548 fold, up to around 2160 μg/cm^2. However, a lag time of around 3.5 h was observed before permeation increased significantly.

Table 4
Iontophoretic patches employed for skin-related delivery of soluble molecules as defined by European Pharmacopoeia 11 ed. (water solubility > 0.03 g/mL).

Drug	*Solubility in water (g/mL)*	*System*	*Ref.*
3-fluoro amphetamine hydrochloride	0.01–0.03	Iontophoretic patch	(Puri et al. 2017)
Memantine hydrochloride	0.03–0.1		(del Río-Sancho et al. 2017)
Pramipexole dihydrochloride	0.1–1		(Kalaria et al. 2018)
Rasagiline mesylate	0.1–1		
oligodeoxynucleotide	–		(Fukuta et al. 2021)

Dementia and Parkinson's disease can highly benefit from transdermal drug delivery systems. Patients suffering from them could experience treatment adherence problems, inconsistent intestinal absorption and dysphagia (Larrañeta and Singh, 2022; Larsson et al., 2017; Nyholm and Lennernäs, 2008). Therefore, several research groups have reported iontophoretic systems to treat these conditions. Río-Sancho et al. described a memantine hydrochloride iontophoretic delivery system (Del Río-Sancho et al., 2017). In their study, the use of occlusive transdermal patches made of poly(vinyl pyrrolidone) and poly(vinyl alcohol) was evaluated (del Río-Sancho et al., 2017). They included *in vitro* drug permeation results using excised porcine skin. The polymeric composition of the matrix tailored the properties of the resulting patches. The higher permeation achieved was around 9 μg cm^{-2}h^{-1} for passive patches. This formulation contained 24% poly(vinyl pyrrolidone), 5% sorbitol, 17% of commercial adhesive Plastoid E35H, 5% of a permeation enhancer (limonene), 0.5% memantine and water up to 100% (del Río-Sancho et al., 2017). The maximum permeability achieved for this formulation using iontophoresis increased to around 46 μg cm^{-2}h^{-1}. However, iontophoresis required longer times than in previous studies (8 h). Similarly, Kalaria et al. described an iontophoretic system for simultaneously delivering rasagiline mesylate and pramipexole dihydrochloride to treat Parkinson's disease (Kalaria et al., 2018). Again, this study was completed using excised porcine skin and diffusion cells (Kalaria et al., 2018). In this case, the formulation was not

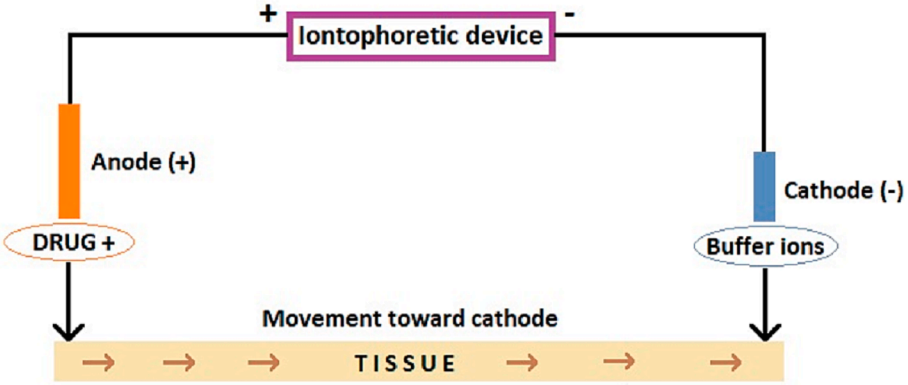

Fig. 6. Schematic of an iontophoretic device.
Reproduced with permission from: Karpiński, 2018

a patch but a solution (20 mM for each drug) in sodium metabisulfite buffer (pH 5.3). Passive permeation after 6 h was *ca.* 16 μg cm^{-2} for both drug molecules (Kalaria et al., 2018). The permeation of these two drug molecules increased up to *ca.* 613 μg cm^{-2} for pramipexole dihydrochloride and 441 μg cm^{-2} for rasagiline mesylate (Kalaria et al., 2018). These were a 38- and 27-fold increase, respectively, compared to passive diffusion (Kalaria et al., 2018).

Fukuta et al. (2021) investigated the transdermal delivery of oligodeoxynucleotide for psoriasis treatment. Psoriasis is an immune-mediated disease-causing chronic skin inflammation characterised by epidermis hyperplasia, suitable to be treated by topical application of therapeutics. The authors demonstrated the utility of iontophoresis using a weak electric current (0.3–0-5 mA/cm^2), mainly when delivering hydrophilic macromolecules, including nucleic acids therapeutics and antibodies. However, the thickened barrier of psoriasis skin hampered the permeation of the mentioned hydrophilic macromolecules (Fukuta et al., 2020). In this view, the authors focused on combining the iontophoresis technique with a functional peptide, AT1002, which can reversibly open the tight junctions and enhance penetration efficiency, with interesting and successful outcomes (Fukuta et al., 2021).

3.5. 3D-printed drug delivery systems

The use of 3D printing (or additive manufacturing) to develop drug delivery systems and medical devices has risen considerably during the last decade (Seoane-Viaño et al., 2021). This type of technology has been used to produce a wide variety of drug delivery systems, such as oral dosage forms, suppositories, implantable devices or transdermal/intradermal drug delivery systems, among many others (De Oliveira et al., 2022; Domsta and Seidlitz, 2021; Korelidou et al., 2022; Okafor-Muo et al., 2020; Seoane-Viaño et al., 2021; Stewart et al., 2020). This section will focus on 3D-printed transdermal/intradermal systems for delivering hydrosoluble drugs. To start, it is essential to highlight that 3D printing is a broad family of manufacturing techniques that produce 3D objects via the addition of layers of materials based on computer designs generated previously. The main difference between 3D-printing techniques is the layer-by-layer addition mechanism. Table 5 summarises the studies describing the use of 3D printing to deliver hydrosoluble molecules to the skin.

3.5.1. Extrusion-based 3D-printing

Extrusion-based 3D-printing techniques are based on the addition of layers of material via extrusion through a nozzle. The extrusion process can be done at room temperature with gels and pastes (Khaled et al., 2018, 2015; Picco et al., 2022) or at higher temperatures using thermoplastic polymers (Domínguez-Robles et al., 2022, 2021; Shaqour et al., 2021). This is family of several techniques, including the popular fused deposition modelling (FDM) and semisolid extrusion (SSE). The former is widely spread across research laboratories and hospitals due to its low cost and ease of use (Wang et al., 2020). This technique uses thermoplastic polymer filaments to prepare 3D objects. In order to incorporate drugs into the 3D-printed objects, the drug can be incorporated into the filament or just added later into the 3D-printed object (De Oliveira et al., 2021). The first option is preferable, but it is tricky as the drug and the polymer should be combined before producing the filaments using hot-melt extrusion this technique usually requires higher temperatures that could damage the drug cargo. FDM has been used to prepare wound dressings (Domínguez-Robles et al., 2019; Muwaffak et al., 2017), scaffolds (Azadmanesh et al., 2021) and MN patches (Khosraviboroujeni et al., 2022; Luzuriaga et al., 2018). However, most of these devices were not used to deliver hydrosoluble drugs. One of the few works that describe the use of FDM 3D-printing for the delivery of hydrosoluble drugs was published by Domínguez-Robles et al. (Domínguez-Robles et al., 2019). This work combined poly(lactic acid) (PLA) with lignin, a natural antioxidant macromolecule, and tetracycline to prepare wound dressings with antioxidant and antimicrobial properties. The authors prepared circular meshes (25 mm in diameter and 0.4 mm thick) with two different mesh sizes, 1 and 1.5 mm. The resulting dressings were tested *in vitro*, showing antibacterial properties against *S. aureus*.

SSE has been extensively used for the preparation of topical drug delivery systems. This technique is similar to FDM, but no filaments are required. The materials are loaded in cartridges and extruded using a piston (Domínguez-Robles et al., 2021a) or pneumatic pressure (Sjöholm et al., 2022). The cartridge can be heated to melt the cargo or print using pastes or gels. The main advantage of this technique over FDM is that it does not require the preparation of filaments. However, this type of 3D printing is more complicated than FDM and does not provide the same resolution (Domínguez-Robles et al., 2022). The use of this technique to print aqueous gels makes it ideal for the preparation of hydrogel-wound dressings loaded with hydrosoluble drugs. Long et al. developed 3D-printed hydrogel wound dressings loaded with lidocaine hydrochloride. These devices were meshes (mesh size 2.5 mm/thickness 1 mm). The dressings were prepared using chitosan and pectin (Long et al., 2019). This type of dressing could provide up to 6 h of sustained lidocaine release. Teoh et al. described a combination of two hydrosoluble drugs loaded into wound dressings to treat thermal burns (Teoh et al., 2021). The combination of these two drugs will prevent infection due to the presence of levofloxacin while providing some pain relief because of lidocaine. These wound dressings were prepared using methacrylate chitosan so they could be crosslinked via-UV irradiation. The resulting dressings were prepared layered, and the drugs were loaded in different layers. The dressings could provide drug release for up to 6 h for both types of drugs. Due to the presence of levofloxacin, these wound dressings presented antimicrobial properties against *S. aureus* and *P. aeruginosa*. Finally, chitosan dressings loaded with both drugs were tested using a rat animal model. The resulting dressings provided enhanced wound healing properties compared to the control. Moreover, they did not cause any adverse effects demonstrating their biocompatibility and safety. Finally, Akkineni et al. used an advanced 3D-printing methodology based on coaxial needles to prepare core–shell devices (Akkineni et al., 2021). In this way, the drug can be encapsulated within the gel instead of dispersed in the matrix (Fig. 7). For this purpose, a combination of alginate and methylcellulose or laponite was employed. This type of formulation was printed using core–shell needles. Blank formulations were loaded in the shell needle, while equivalent formulations loaded with hydrosoluble antibiotics (gentamicin, vancomycin and clindamycin) were loaded in the core needle. The resulting alginate-based gels were crosslinked using Ca^{2+} ions to obtain core–shell dressings containing antibiotics which were released for up to a week.

Table 5
Summary of the studies describing using 3D-printing to prepare cutaneous delivery systems for soluble molecules as defined by European Pharmacopoeia 11 ed. (water solubility > 0.03 g/mL).

Drug	*Solubility in water (g/mL)*	*3D-printed system*	*Study*
Tetracycline hydrochloride	0.1–1	Wound Dressing	(Domínguez-Robles et al. 2019)
Lidocaine hydrochloride	>1	Wound Dressing	(Long et al. 2019)
Levofloxacin	0.03–0.1	Wound Dressing	(Teoh et al. 2021)
Lidocaine hydrochloride	>1		
Vancomycin hydrochloride	0.03–0.1	Core-Shell Wound Dressing	(Akkineni et al. 2021)
Clindamycin hydrochloride	0.1–1		
Gentamicin sulphate	0.1–1		

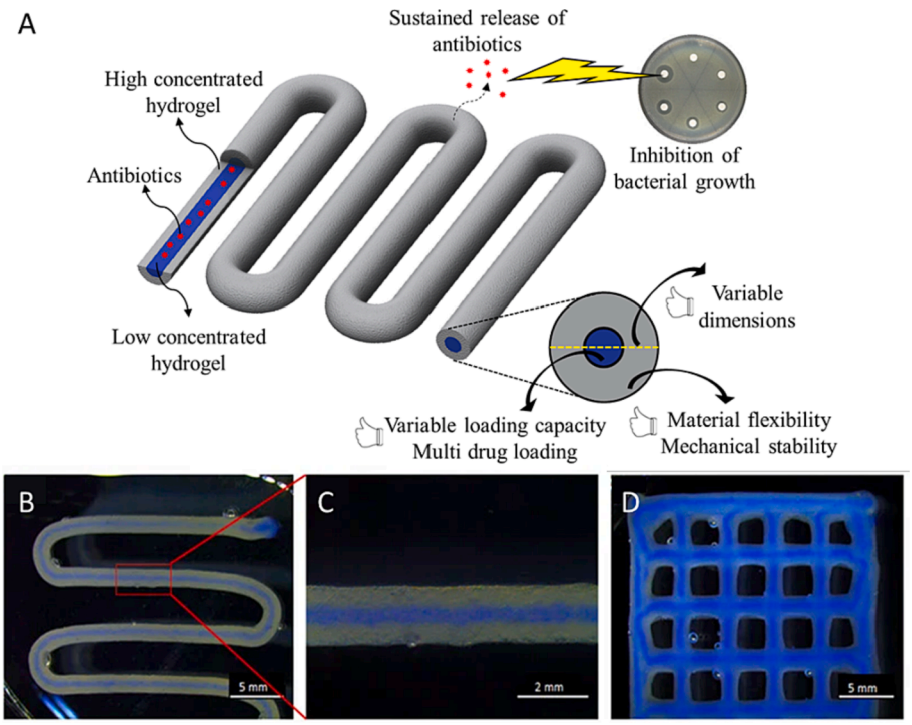

Fig. 7. Diagram of a core–shell hydrogel dressing loaded with antibiotics (A). Images of 3D-printed core–shell hydrogel dressings loaded with a blue die in the core section (B-D).
Reproduced with permission from: Akkineni et al., 2021

3.5.2. Vat photo-polymerisation 3D-printing

Vat photo-polymerisation is characterised by forming solid objects by solidifying liquid resin contained in a vat, or large tank, by light irradiation (Xu et al., 2021). Currently, there are different types of vat photo-polymerisation technologies available in the market, and their main advantage over other 3D-printing techniques is their higher resolution (Detamornrat et al., 2022). Therefore, this type of technology has been extensively used for microneedle (MN) manufacturing (Detamornrat et al., 2022). A possible way of using this type of technology is to prepare MN moulds that can be used subsequently for manufacturing MNs (Balmert et al., 2020; Cordeiro et al., 2020). Moreover, 3D printing has been used to prepare solid MNs coated with drug formulations (Uddin et al., 2020, 2015) or hollow MNs capable of injecting different formulations inside the skin (Economidou et al., 2021). MN-based technology has been described and discussed in previous sections of this review article. This technology has also been used to prepare other types of topical drug delivery systems (de Oliveira et al., 2021). Goyanes et al. developed personalised patches for treating acne loaded with salicylic acid (Goyanes et al., 2016). These patches were prepared using stereolithography, a vat photo-polymerisation technique that uses a laser beam to crosslink each resin layer. The resin was prepared using a combination of poly(ethylene glycol) (PEG) and poly(ethylene glycol) diacrylate (PEGDA). Salicylic acid was added to the mixture, and this formulation was used to prepare devices adapted to the patient's skin. Prior to printing, the skin of the patient was 3D-scanned (Fig. 8**A-B**). Different ratios of PEGDA/PEG were tested to evaluate the effect of the

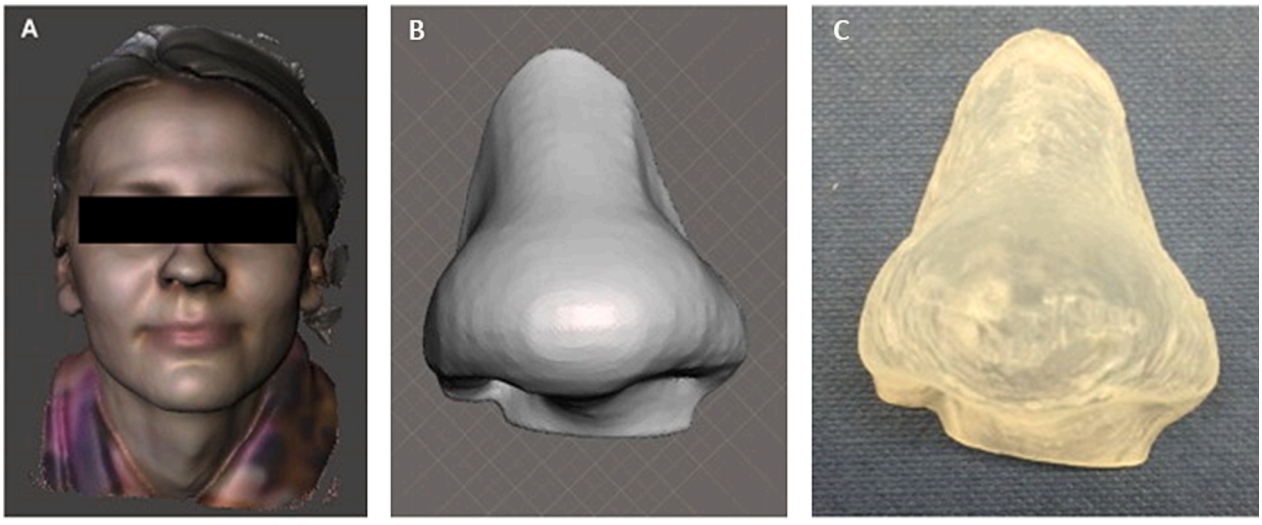

Fig. 8. Scanning image of a volunteer (A). 3D model of the nose of the patient (B). A nose-shaped patch containing salicylic acid prepared using stereolithography. ().
Reproduced with permission from: Goyanes et al., 2016

composition on drug release. All the formulations tested contained 2% (w/w) of salicylic acid. The resulting device was tested using Franz cells and cellulose nitrate membranes. The results suggested that patches prepared with higher PEG content presented higher drug permeation as they generated pores in the structure that accelerate drug permeation. Fig. 8**C** shows an example of one of these customised patches containing salicylic acid prepared and adapted to a patient's nose.

4. Conclusions

The current review article focused on the strategies recently explored to promote the skin permeation and absorption of hydrosoluble compounds. Data reported herein proved that different strategies are being evaluated in order to assess the delivery challenge posed by the increase availability of novel hydrosoluble macromolecule-based therapy, which are destined to continue to rise. The strategies reported include the use of already known systems and techniques, such as those of second generation (liposomes and iontophoresis among others) extended to the delivery of hydrosoluble macromolecules. On the other hand, the role of third-generation strategies, such as microneedles, and combination of second-generation ones (e.g., polymeric nanofiber-based gel) has been highlighted.

In terms of materials, lipids are more frequently employed to prepare particulate carriers, mainly liposomal vesicles and their modifications. On the other hand, polymer materials take the first stand when considering other types of delivery systems, such as hydrogels and 3D-printed systems. Furthermore, polymers are commonly employed for microneedle development, which represents the evolution of conventional transdermal delivery systems alongside iontophoretic patches. However, most of the published articles evaluates the delivery efficacy of the developed formulation or techniques in terms of *in vitro* and *ex vivo* studies on cells, synthetic and biological membranes; still, *in vivo* studies are currently lacking, and future research endeavors should focus on *in vivo* testing and clinical translation of these systems.

CRediT authorship contribution statement

S. Demartis: Conceptualization, Writing – original draft, Writing – review & editing. **G. Rassu:** Conceptualization, Writing – original draft. **V. Mazzarello:** Writing – original draft. **E. Larrañeta:** Writing – original draft. **A. Hutton:** Writing – original draft. **R.F. Donnelly:** Writing – original draft. **A. Dalpiaz:** Writing – original draft. **M. Roldo:** Writing – original draft. **A.J. Guillot:** Writing – original draft. **A. Melero:** Writing – original draft. **P. Giunchedi:** Writing – original draft. **E. Gavini:** Supervision, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

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

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