

REVIEW ARTICLE


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Topical and Transdermal Drug Delivery: From Simple Potions to Smart Technologies


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ARTICLE HISTORY

Received: November 22, 2018
 Revised: January 16, 2019
 Accepted: January 25, 2019

DOI:
 10.2174/1567201816666190201143457



CrossMark

Keywords: History, skin delivery, nanotechnology, physical enhancement, microneedles, minimally invasive, transdermal technologies, targeted delivery, stratum corneum, follicular transport, wearable devices.

1. SKIN DELIVERY PAST

Ointments and potions made of animal, mineral or plant extracts were in common use in ancient Egyptian and Babylonian medicine in 3000 BC [1]. For example, a Sumerian clay tablet dates back to 2100 BC, describing a formulation made of pulverised snake and bat's dung incorporated into an aqueous paste of plant extracts and earths for the treatment of skin disease [2]. Topical remedies that were anointed, bandaged, rubbed or applied to the skin for the treatment of skin conditions in ancient Egypt are detailed in the Ebers Papyrus, dating from 1550 BC [3]. The Greek physician Claudius Galenus (commonly known as Galen, 129-199 AD), introduced the compounding of herbal drugs to Western medicine and developed a formula for a cold cream (Galen's Cerate) which was similar to those available today. The Persian physician Ibn Sina (best known as Avicenna, 980-1037 AD), in his treatise *The Canon of Medicine*, proposed that topical drugs possess two spirits or states: the soft part that penetrates the skin and the hard part that does not [4]. Avicenna thus provided the first known mechanistic approach that underpins our current knowledge and pharmaceutical development in topical and transdermal delivery.

Our understanding of skin permeation has evolved from early observations of adverse systemic effects following skin

Abstract: This overview on skin delivery considers the evolution of the principles of percutaneous absorption and skin products from ancient times to today. Over the ages, it has been recognised that products may be applied to the skin for either local or systemic effects. As our understanding of the anatomy and physiology of the skin has improved, this has facilitated the development of technologies to effectively and quantitatively deliver solutes across this barrier to specific target sites in the skin and beyond. We focus on these technologies and their role in skin delivery today and in the future.

exposure, such as systemic poisoning after belladonna plaster applications [5] and headaches after occupational exposure to nitroglycerin in explosives factories [6]. The observation that skin was relatively permeable to lipid-soluble substances but not to water and electrolytes [7] was explored in systematic *in vitro* studies conducted by Rein in the 1920s [8]. Moore *et al.* [9] showed that testosterone, testosterone propionate or estradiol applied to animal skin led to an enhancement in various hormonal responses. Zondek [10] described the cutaneous application of follicular hormone for amenorrhoea in 1938 and successful management of urogenital infections following topical application of an ointment containing the disinfectant chloroxyleneol, in the early 1940s [11]. In 1948, a nitroglycerin ointment was successfully applied to treat Raynaud's disease, by utilising its ability to permeate to and dilate the cutaneous blood vessels [12, 13]. Systematic studies identified that important determinants of skin permeability included drug solubility [14], partition coefficient and thermodynamic activity [15], drug-vehicle-skin interactions [16, 17], and skin temperature [18, 19]. In 1975, Michaels and co-workers [20] described a mathematical model of the stratum corneum and demonstrated that the skin diffusion coefficients of ten model drugs correlated with their water solubility and lipid-protein partition coefficient.

In the early 1970s, the Alza Corporation, through their founder Alejandro Zaffaroni, filed the first US patents describing transdermal delivery systems for scopolamine, nitroglycerin and nicotine. These delivery systems offered ad-

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vantages including: (i) overcoming oral delivery limitations of vomiting and low bioavailability (e.g. due to a high liver and gut wall first-pass); (ii) providing an alternative to parenteral administration, which can be invasive, painful and risks bruising and infection; (iii) acting as a convenient, non-invasive means to achieve relatively constant and reliable blood levels over 24 to 72 hrs, whilst also being able to cease the delivery at any time by removing the patch [21].

2. SKIN DELIVERY PRESENT

In addition to the range of drugs applied for local effects in the skin and associated tissues, nearly twenty drugs have been successfully developed for transdermal delivery using various topical dosage forms such as patches, gels and ointments and cutaneous solutions [21]. Four drugs using transdermal patches for the management of central nervous system disorders were approved in a two-year period: methylphenidate (Daytrana®, 2006), selegiline (Emsam®, 2006), rivastigmine (Exelon®, 2007) and rotigotine (Neupro®, 2007) for the management of attention deficit hyperactivity disorder, depression, Alzheimer’s disease and Parkinson’s disease, respectively [22]. Interestingly the rotigotine patch is the first patch that has been originally developed for transdermal delivery while other drugs now delivered through the skin have previously been administered using more conventional oral and parenteral dosage forms [23]. Fig. (1) summarizes the key transdermal drugs used today and their recommended application sites [21]. Continued development for topical and transdermal delivery is providing new technologies for controlled dosing, targeted site-specific delivery, and/or enhanced skin penetration, thereby extending the range of therapeutic compounds that can be applied *via* the skin.

3. SKIN AS A TARGET SITE

The skin is a complex multilayered structure (Fig. 2) extending over an area of 1.5 to 2 m² in adults. Its primary role

is to act as a barrier between the body and the relatively hostile external environment. The stratum corneum is a highly structured lipid-rich region that minimises the ingress and egress of water, oxygen and chemicals. Topically applied products may target sites in one or more different skin layers (i.e. epidermis, dermis and hypodermis), the skin appendages (e.g. hair follicles with associated sebaceous glands, sweat glands and nails), and underlying tissues. The key target region for the majority of topical products is the viable epidermis and sites targeted here include nerves, keratinocytes, melanocytes, Langerhans cells and hair follicles (Fig. 2). Transdermal products target the systemic circulation.

Most solutes suitable for transdermal delivery are characterized by low molecular weight, low melting point, moderate lipophilicity, and few hydrogen bonding sites. Table 1 summarizes the properties of current transdermally delivered drugs.

The time course of the drugs in the body is defined by the flux of the solute’s entry into the skin, and for local effects, clearance from the skin, or for systemic effects, clearance from the systemic circulation. Steady state transport flux (*J*) across a plane sheet membrane of area *A* and thickness *h* with a constant diffusivity *D*, and diffusion path length *h* can be described in terms of the interior membrane concentrations; *C*, at the interior interface adjacent to the product and 0, adjacent to a sink receptor [24]. Applying this to transport across the stratum corneum membrane, and assuming that the regions below that major diffusion barrier offer essentially sink conditions, the steady-state flux can be described by the expression:

$$J = \frac{ADC}{h} \tag{Eq. 1}$$

Non-sink conditions can slow down skin permeation as well as increase local skin concentrations of solutes [25].

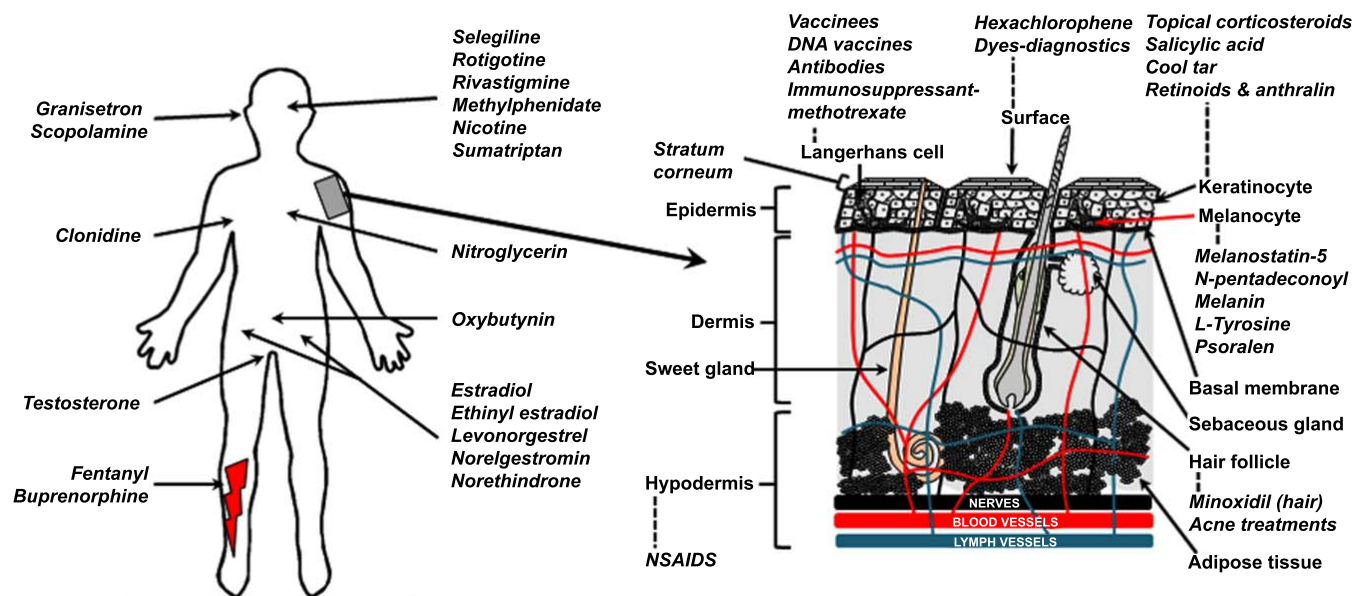


Fig. (1) A. Approved and underdevelopment transdermal drugs and their recommended site of application. B. Close-up of human skin and various sites and targets within the skin.

Table 1. Physicochemical, pharmacokinetic properties and indications of currently marketed transdermal drugs.

Drug	Dose/day (mg)	MW (Da)	MP (°C)	Log P	S _{aq} (mg/mL)	Clearance (cl) L.h ⁻¹	t _{1/2} (h)	Oral F (%)	Plasma Concentration	Estimated J _{skin} Required (µg/h)	In vivo J _{skin} (µg/cm ² /h)	Product name	Indication
Buprenorphine	0.12-1.68	468	209	3.8	0.047, 0.008 (32°C)	77 i.v. 55 i.m.	22-36	51 sl 28 bc	0.1-0.4	7.7	0.8	Butrans	Moderate to severe pain
Clonidine	0.1-0.3	230	130	2.7	0.17, 13.58	15	6-20	95	0.2-2.0	3-30	1.2	Catapres-TTS	Hypertension
Ethinyl oestradiol	-	296	141-146	4.3	0.039, 0.0092 (25°C)	70	7.7 p.o, 17 t.d.	55	0.025-0.075	1.75-5.25	0.07	Ortho Evra	Female contraception
Fentanyl	0.288-2.400	337	83-84	3.9	0.15, 0.2 (30°C), 0.2 (25°C)	27-75	3-12	50	1-3	27-225	2.4	Duragesic	Chronic pain
Granisetron	3.1	312	152-154	2.6	0.017	33-76	4-6	60	3.9 (t.d. Mean C max)	129-296	2.5	Sancuso	Chemotherapy-induced emesis
Glyceryl trinitrate	2.4-15.0	227	13, liquid	1	0.66, 1.3 (30°C)	216-3270	0.03-0.05	< 1	0.1-5	4.32-1308	20,30	Nitro-Dur	Angina
Estradiol and Levonorgestrel	0.050, 0.0007-0.015	272, 312	173-179 235-237	4.2 3.8	0.003 (30°C) 0.0015 (25°C) 0.017	600-800 5.7	Approx. 1p.o. 28t.d.19.3p.o.	3-5	0.03-0.05 0.1-0.2	24-48 1	0.63, 0.09, 0.12, 0.18, 0.14, 0.23, 0.42, 0.17 0.2 0.03	Climara Pro	Female contraception
Methylphenidate	26.0 - 80.0	233	74-75, liquid	2.1	1.8	12(d); 21(1) (children 30kg)	1.5-5	5-20	5-25	60-315	88	Daytrana	ADHD
Nicotine	7.0-21.0	162	-79, liquid	1.1	62,1085 (30°C)	77	2	20-45	5-30	385-2310	29, 69, 31, 40	Nicoderm	Smoking cessation
Norelgestromin	0.2	327	110-130	3.67	0.0043	-	28	-	0.6-1.2	-	0.31	Ortho Evra	Female contraception
Norethindrone acetate	0.125-0.250	341	161-162	3.2	0.0065	20.6	6-8	60	0.5-0.8	10.3-16.5	0.65	Combipatch	Female HRT
Oxybutynin	3.9	358	56-58	4.3		10-64	2	6	0.5-5	5-192	4.2	Oxytrol	Enuresis
Oestradiol	0.025-0.100	272	173-179	4.2	0.003 (30°C) 0.0015 (25°C)	600-800	0.05	3-5	0.04-0.06	24-48	0.2,0.17,0.14, 0.12,0.42,0.18, 0.63,0.23, 0.09	VivelleDot	Female HRT
Rivastigmine	4.6-9.5	250	Oil at 25°C	2.3	25	108	1.5	36	2.5-20	270-2160	39	Exelon	Alzheimer's disease
Rotigotine	1.0-3.0	316	75-77	4.7	0.017	600	5-7	-	0.4-2	240-1200	8.3	Neupro	Parkinson's disease & restless leg syndrome
Scopolamine	0.3	303	55, liquid	0.8	1.8, 75 (30°C)	65-121	1-5	4-27	0.04	3.25-6.05	5.6	Transderm Scop	Travel sickness
Seligiline	6.0-12.0	187	Liquid at 25°C	2.7	0.73	84	10	4,10	2	168	12.5	Emsam	Depression
Testosterone	0.3-5.0	288	155	3.6	0.02	41	0.17-1.7	<1, 7	3-10.5	123-430.5	13.9	Androderm	Hypogonadism

Abbreviation: i.v. intravenous, i.m. intramuscular, p.o. per oral, t.d. transdermal, s.I. sublingual, bc buccal.

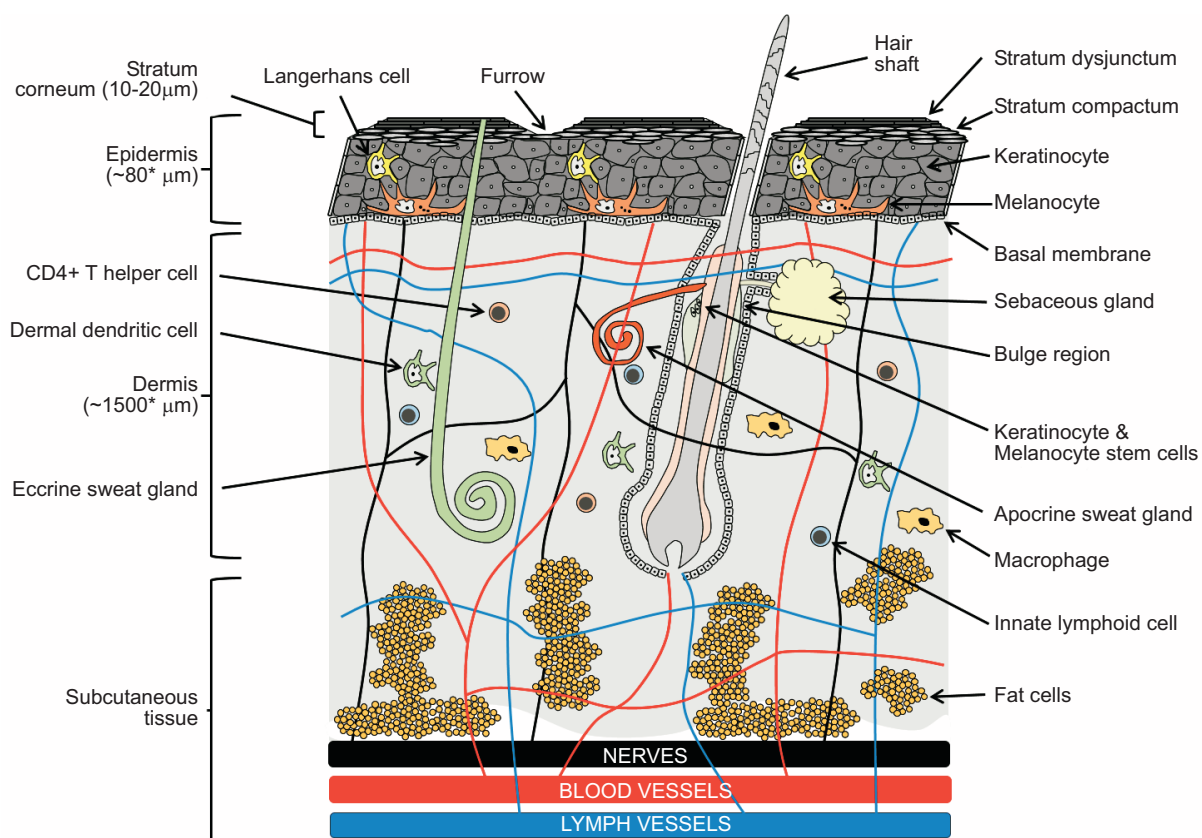


Fig. (2). Diagrammatic illustration of human skin. Barriers, sites of action and drug delivery opportunities.

The maximum, or saturated flux J_{max} , in turn, relates to the solubility of the solute in the stratum corneum (S_{sc}):

$$J_{max} = \frac{ADS_{sc}}{h} \quad (\text{Eq. 2})$$

4. SKIN DELIVERY TECHNOLOGIES FOR PENETRATION ENHANCEMENT AND SITE-SPECIFIC TARGETING

The primary challenge to optimising skin delivery and extending the range of compounds that can be effectively delivered *via* the skin is overcoming the barrier properties of the skin. The stratum corneum is an effective barrier that limits skin penetration and consequently the extent to which the skin can be effectively used to deliver compounds for therapeutic outcomes in the skin, underlying tissues or systemically. Many compounds do not possess the physico-chemical criteria [*i.e.* low molecular weight, adequate lipophilicity ($\log P_{o/w} \cong 1-3$) and low melting point] to passively permeate the skin in therapeutic quantities thus limiting the topical and transdermal market. Development of technologies to enhance delivery into the skin has been a major research focus for over half a century. ‘Passive’ technologies involve the use of formulation excipients and chemical penetration enhancers, and various types of micro and nano-delivery systems [26-28]. Technologies using an external driving force (‘active’ or ‘physical’ enhancement methods) have employed electrical (iontophoresis and electroporation), thermal (laser and radiofrequency thermal ablation), ultra-

sound, mechanical (microneedles), and velocity (jet injector) based approaches [29].

5. COLLOIDAL NANOSYSTEMS

Colloidal systems used in skin delivery are typically lipid-based (Fig. 3) and include nanoemulsions (NE), liposomes or flexible vesicles, nanostructured lipid carriers (NLC) and solid lipid nanoparticles (SLN). These nanosystems can offer the potential to improve bioavailability and efficacy, target delivery to skin regions and follicles, increase the stability of active, and facilitate the formulation of lipophilic, poorly water-soluble compounds. They may also have a role in combination with other penetration enhancement technologies.

5.1. Nanoemulsions (NE) and Microemulsions (ME)

Are transparent, monophasic, optically isotropic colloidal dispersions composed of oil, water, surfactant and cosurfactant with droplet sizes less than 100 nm and a low polydispersity [27]. ME are thermodynamically stable whilst NE are kinetically stable, with both providing a large surface area and uniform distribution on the skin. The primary difference between NE and ME is their thermodynamic stability [30], which also results in the higher energy required to form NE compared to ME. NE typically contain a relatively low surfactant content with reduced irritancy to the skin. They enhance permeation into the skin by: (i) their high solubilization capacity for both lipophilic and hydrophilic compounds increase the loading capacity and dose application; (ii) their

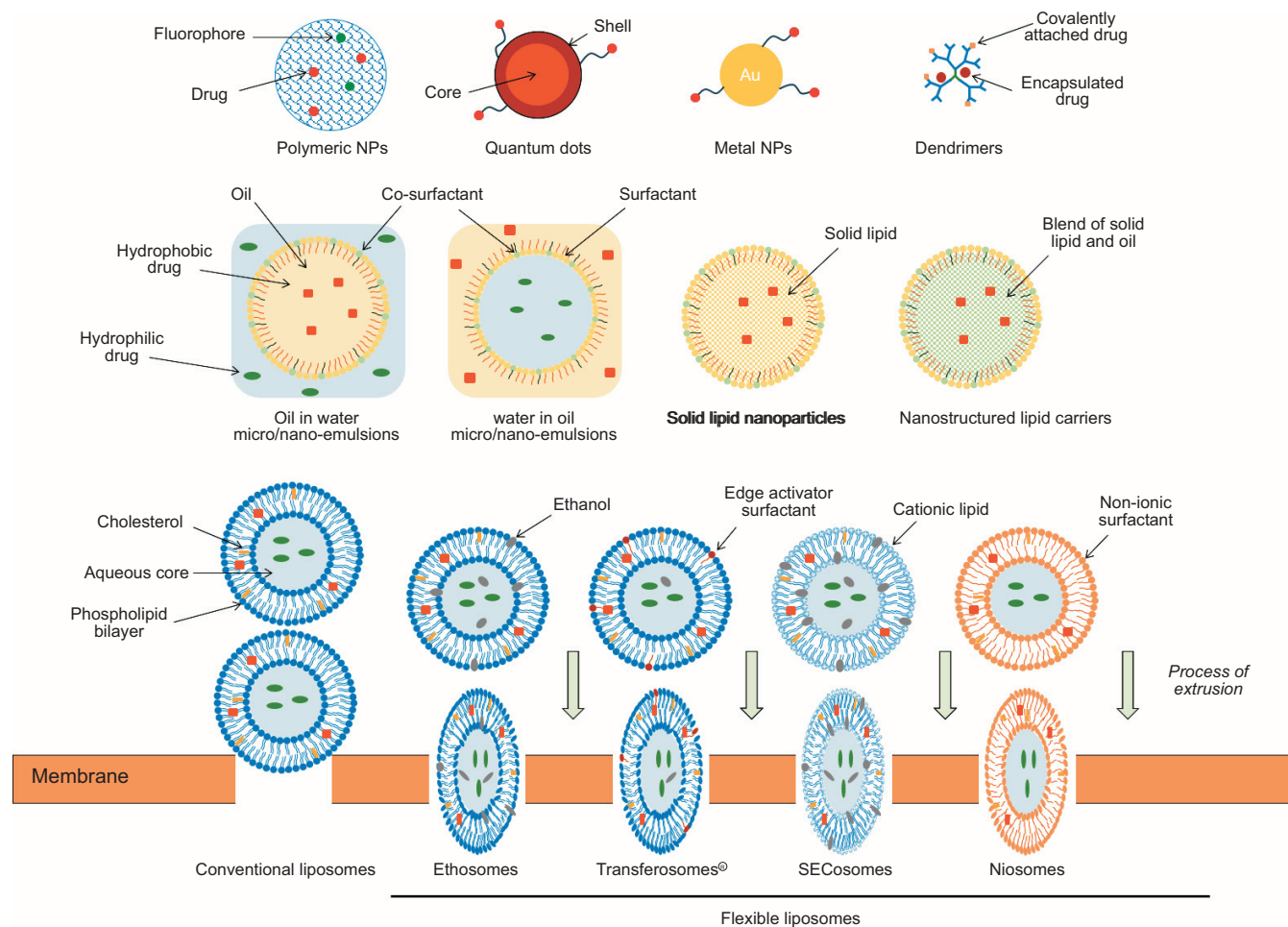


Fig. (3). Nanodelivery systems for topical and transdermal drug delivery. (Adapted with permission from Roberts *et al.* 2016 [26]).

large surface area and good skin contact, coupled with their occlusive nature ensures surface contact with the stratum corneum; (iii) their oil and surfactant components may have a direct permeation enhancement effect on the stratum corneum lipid structure. We showed significantly enhanced flux of caffeine and naproxen across human epidermis *in vitro* applied as a NE containing the skin penetration enhancers oleic acid or eucalyptol [31]. The NE provided a modest increase in solubility within the stratum corneum of hydrophilic caffeine, therefore the increase in flux was due primarily to the direct effect of the NE excipients reducing the diffusional resistance for caffeine penetration within the stratum corneum. In contrast, increased flux of the lipophilic naproxen was primarily due to NE increased solubility of naproxen in the stratum corneum, with only a modest increase in diffusivity.

5.2. Solid Lipid Nanoparticles (SLN)

Are composed of lipids that are solid at room temperature, that are stabilised as a nanodispersion by a surface covering of surfactant [32]. They facilitate the formulation and increase the stability of lipophilic compounds such as retinol that are prone to decomposition in the presence of light and oxygen [33], increase the deposition in the stratum corneum and reduce flux [34]. Enhanced stratum corneum permeation

is attributed to: (i) prolonged contact with the skin surface; (ii) their occlusive nature due to formation of a film on the skin surface that combines with the skin lipid film to reduce water loss thus hydrating the skin [35]; and (iii) interaction between formulation lipids and stratum corneum lipids facilitating permeation of lipid-soluble compounds [36]. Their primary applications are for skin targeting such as that demonstrated by curcumin SLNs which showed controlled release over 24 hrs, and effective deposition for the reduction of skin pigmentation and inflammation in Balb/c mouse skin [37]. The use of cationic lipids offers the potential for charge interaction with the negatively charged skin surface. For example, highly positively charged (51 mV) SLN using the cationic phospholipids 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP) and dioleoylphosphatidylethanolamine (DOPE), Tween 20 as surfactant, tricaprין as a solid lipid core, and encapsulating plasma DNA, showed enhanced *in vitro* permeation into mouse skin and expression of mRNA *in vivo* in mice after topical application [38].

5.3. Nanostructured Lipid Carriers (NLC)

Are composed of a fluid lipid phase embedded into a solid lipid matrix or localized at the surface of solid platelets and the surfactant layer [32]. The lipids spatial structure allows greater drug loading and better stability compared to

SLN [39]. NLC and SLN are reported to have a similar mechanism of permeation enhancement, *via* occlusion and mixing between the formulation and stratum corneum lipids, however the liquid lipid component in NLC increases solubilization and thereby loading of the active, resulting in greater skin deposition [40, 41]. Surface modification with the cell-penetrating peptide transactivating transcriptional activator (TAT-NLC) showed greater epidermal deposition of a fluorescent probe and the lipophilic drug celecoxib, compared to control NLC, that were located mainly in the hair follicles following topical administration to rat skin *in vitro* [42]. They subsequently showed that surface modification of the NLC with a cell-penetrating peptide containing 11 arginines had significantly greater skin permeation enhancing ability compared to other polyarginines and TAT peptides [43].

5.4. Flexible Liposomes or Vesicles

Are composed of materials that will aggregate into bilayer structures to form spherical vesicles but have the ability to deform in shape. Various vesicle compositions have been developed, including compositions with materials known to have penetration enhancement properties including transfersomes (phospholipids with the surfactant sodium cholate) [44-47], ethosomes (phospholipids with a high proportion of ethanol) [48, 49], niosomes (flexible non-ionic surfactant vesicles) [50, 51], invasomes (phosphatidylcholine, ethanol and a mixture of terpene penetration enhancers) [52, 53], SECosomes (surfactant, ethanol and cholesterol) [54, 55], PEVs (penetration enhancer containing vesicles) [56-58]. Multiple mechanisms by which flexible vesicles enhance skin penetration have been suggested. First, that the vesicles disintegrate on the skin surface, their components penetrate into the stratum corneum, mixing with and modifying the lipid bilayers to indirectly enhance permeation [59, 60]. Cevc [61] suggested that intact flexible vesicles penetrate through the stratum corneum due to the transepidermal osmotic gradient, deforming to fit through 'pores' within the lipid bilayers. This hypothesis remains controversial, with recent evaluations of vesicle skin interactions using high-resolution technologies such as multiphoton excitation fluorescence microscopy imaging and cross correlation-raster image correlation spectroscopy (CC-RICS) [60] and stimulated emission depletion microscopy (STED) and CC-RICS [59] failing to show evidence of intact vesicles within the skin. STED provides image resolution to 20 nm, whilst CC-RICS allows tracking of two fluorescent probes so that intact vesicles can be resolved. For example, following unoccluded application of vesicles and flexible vesicles incorporating sodium cholate (mean diameter 96 nm) to freshly excised human skin for 4-8 h, a large number of vesicles remained at the skin surface, particularly in the skin furrows, with very few intact vesicles in the stratum corneum. A smaller proportion of the flexible vesicles remained intact on the skin surface, and there was significantly more fluorescent probe in the deeper layers of the stratum corneum. However, the fluorescent signal was not associated with any vesicle-like shape, leading the authors to conclude that the flexible vesicle formulation components were contributing to the penetration enhancement. They also suggested that the choice of mouse skin with its thinner stratum corneum contributed to vesicle penetration reported in earlier studies.

Regardless of the mechanism, flexible nano-vesicles have been shown to enhance skin penetration of many compounds

including photosensitizers for topical photodynamic therapy (PDT) [62], which requires effective delivery of compounds such as 5-aminolevulinic acid and temoporfin (mTHPC) that typically have very poor passive permeation, to cancer cells within the skin, followed by light activation to kill the cells. Dragicevic-Curic *et al* [52, 63] showed that the surface charge of flexible liposomes influenced the physicochemical properties, stability and permeation in human epidermal membranes *in vitro*, with skin deposition of mTHPC enhanced by all flexible vesicles in the following order: cationic > neutral > anionic > conventional liposomes. They suggested that cationic lipids most effectively interact with the negatively charged stratum corneum. Multiphoton tomography with fluorescence lifetime imaging microscopy (MPT-FLIM) showed that SECosomes (58 nm nanosomes composed of the cationic lipid 1,2-dioleoyl-3-trimethylammonium propane chloride (DOTAP), cholesterol, sodium cholate and 30% ethanol) delivered fluorescent siRNA into human epidermis after 1h, with NADH lifetime changes in the epidermal tissue suggesting siRNA cell penetration [54]. SECosome based delivery of siRNA to target human beta-defensin (hBD-2), which is highly up-regulated in psoriasis, in a bioengineered skin-humanized mouse model for psoriasis, lead to the recovery of transglutaminase activity, filaggrin expression and stratum corneum appearance that was similar to normal regenerated human skin [55]. Clearly flexible vesicles have demonstrated penetration enhancement for a range of compounds and provide useful formulations in skin delivery.

5.5. Application of Future Nanotechnologies

There is the potential to adopt a number of promising nanotechnologies to skin delivery. These include nanofiber-based dispersions generated by electrospinning [64, 65]. These amorphous solid dispersions (ASD) can not only improve dissolution behaviour of poorly water-soluble compounds but also provide programmable drug release profiles [66]. Electrospun fiber-based ASDs can maintain an incorporated active ingredient in the amorphous physical form for prolonged periods of time [64]. Whilst the primary focus of these ASDs is likely to be the generation of oral dosage forms for poorly water-soluble drugs, they also have potential for dosage forms delivered to the skin.

6. PHYSICAL ENHANCEMENT APPROACHES

6.1. Indirect Physical Methods

Indirect physical enhancement technologies (Fig. 4) involve the application of electrical (iontophoresis and electroporation), acoustic (sonophoresis), laser and magnetic energy have advanced through experimental evaluation to commercial development [67]. They apply an energy source to the skin surface to increase diffusivity of an applied solute in the stratum corneum.

6.1.1. Electroporation

Applies high intensity, high voltage (50-1500 V) electric pulses of short duration (10 μ s to 10 ms) to form aqueous pores in the lipid bilayers of the stratum corneum, thus facilitating drug diffusion across the skin [68]. Enhanced skin penetration has been reported for drugs ranging from small *e.g.*, fentanyl, doxorubicin [68-70], to high molecular weight

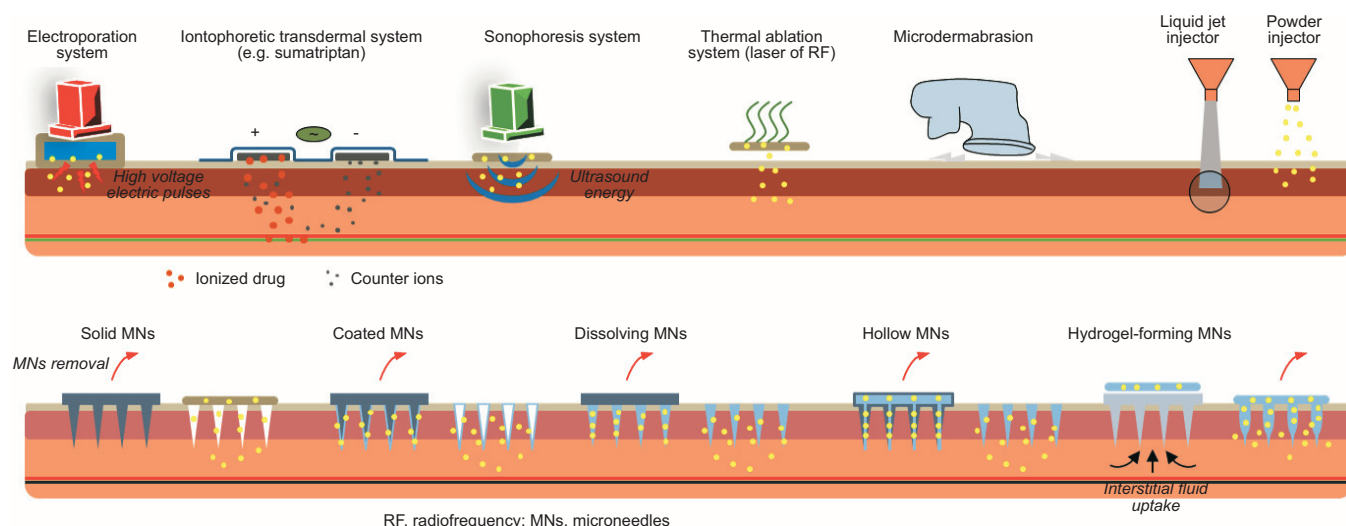


Fig. (4). Physical penetration enhancement techniques.

such as LHRH, calcitonin, heparin, vaccines and FITC-dextrans with molecular weights up to 40 kDa [69, 71-76]. The main drawbacks of electroporation are the lack of quantitative delivery, cell death with high fields, pain and muscle contraction at the application site and potential damage to labile drugs, particularly peptides and proteins [77].

6.1.2. Iontophoresis

Applies a mild electric current (typically 0.1 to 1.0 mA/cm²) to increase skin permeation by three mechanisms: electromigration (ordered movement of ions within an applied electric field as described by Faraday's law), electroosmosis (convective solvent flow in the anode-to-cathode direction due to the isoelectric point pI of 4 - 4.5 and negative charge of the skin) and enhanced passive diffusion (relatively minor role) [78-81]. Enhancement is greatest for suitably charged molecules. Iontophoretic molecular transport is determined by the intensity and duration of the applied current including the current density and continuous/pulsed waveform, and the area of electrode contact with the skin. Transport is also influenced by solute and vehicle factors including the solute concentration and molecular factors (charge/molecular mass ratio; volume, shape and charge distribution of the molecule), and the vehicle pH which influences both the solute and skin ionization (solute ionization affects mobility and electromigration; skin ionization determines the electroosmotic solvent flow) [82]. Iontophoresis is generally well tolerated, particularly when applied as a pulsed current profile that can allow time for the skin to depolarize and return to its resting state prior to initiation of the next pulse. Iontophoresis has been extensively investigated for enhancement of a wide range of molecules and in combination with other vehicle-based and physical enhancement methods [67, 78, 83]. A number of transdermal iontophoretic products have received regulatory approval including the LidoSite™ Topical System (lidocaine for local anaesthesia: Vyteris Inc., Fairlawn, NJ, USA), Ionsys™ (fentanyl iontophoretic transdermal system for patient-controlled analgesia; developed by Alza Corporation then acquired in sequence by

Johnson & Johnson, Incline Therapeutics and The Medicines Company) and Zecuity™ (6.5 mg sumatriptan delivered over 4 h for migraine: NuPathe Inc./Teva). The Ionsys system was launched in Europe in 2008 but withdrawn due to corrosion. The Zecuity patch was introduced in September 2015 but sales were halted in June 2016 due to reports of burns at application site. There is clearly further development required to effectively exploit this technology.

6.1.3. Sonophoresis

Sonophoresis (phonophoresis) is the application of acoustic waves to facilitate transdermal delivery by cavitation (formation and oscillation of microbubbles), acoustic streaming, thermal effects and direct ultrasound pressure effects on the stratum corneum bilayers [84]. Polat *et al.* proposed that a microjet formed by the collapse of the cavitation bubbles in the application medium generates shock waves in the stratum corneum that perturb the barrier and increase permeability [85]. Krasovitsi *et al.* focused on modelling the applied ultrasonic pressure waves and suggested that their oscillation between compression and rarefaction transiently interrupts the continuity of the stratum corneum lipid bilayers to form pores through which applied drugs can diffuse [86]. Ultrasound-mediated skin delivery was initiated with high frequency ultrasound similar to that used in the treatment of musculoskeletal injuries [87-89] and progressed into low frequency ultrasound (20-100 kHz) which is reported to enhance permeation of a range of molecules including macromolecules such as insulin, interferon- γ and erythropoietin (5.8, 17 and 48 kDa respectively) across human skin [90]. The intensity and duration of ultrasound treatment is limited by the thermal effects on the skin therefore the combination of low and high-frequency ultrasound has been applied to generate a synergistic pressure effect without overheating the skin. Schoellhammer *et al.* reported that a 4 min pre-treatment with dual high and low ultrasound frequencies (20 kHz and 1 MHz), in combination with the surfactant 1% sodium lauryl sulfate, enhanced the permeation of inulin and glucose across porcine skin by 3.81 and 13.6-fold respectively, compared to single frequency sonophoresis [91]. A bulky sonophoresis device named Sonoprep® was

named Sonoprep[®] was launched by Sontra Medical in 2004 and withdrawn 2007, then acquired by Echo Therapeutics who are also developing the Prelude[®] skin prep system but it has yet to be launched. Their application has been focused on preparing the skin for local anaesthetic drugs or glucose monitoring in diabetes management. Attempts have been made to develop wearable sonophoretic devices [92] but are yet to reach commercial applications.

6.1.4. Laser-assisted Delivery

Involves the generation of a photomechanical wave by laser ablation of a target medium consisting of a polymer such as black polystyrene placed on the surface of the skin. The medium undergoes rapid phase change (*e.g.* degradation or plasma formation) resulting in the production of a shock-wave through the adjoining skin [93]. Unlike sonophoresis that oscillates between positive and negative pressure, there is no cavitation observed. The photomechanical wave is a unipolar compressive wave that disrupts the lipid arrangement within the stratum corneum intracellular space and also the cellular membranes. The technique has not progressed, with few publications in recent years.

6.1.5. Magnetophoresis

Is the enhancement of drug permeation by the application of a magnetic field, utilising diamagnetic repulsion of the molecule into the skin and possibly also causing transient stratum corneum barrier reduction [94, 95]. We have shown that pulsed electromagnetic fields (PEMF) increased the permeation of naltrexone hydrochloride across human epidermis by 6.5x compared to passive administration [95]. Gold nanoparticle (10 nm)-treated human skin exposed to a PEMF had 200x more gold nanoparticle positive pixels when visualised by multiphoton tomography with fluorescence lifetime imaging microscopy (MPM-FLIM), than skin exposed to gold nanoparticles without PEMF suggesting that the channels through which the nanoparticles move must be larger than 10 nm in diameter. An alternative magnetophoresis fabrication is a thin flexible polymer matrix containing multiple magnetic elements arranged to produce complex 3-dimensional magnetic gradients (40 mT peak magnetic field strength; 2 T/m² total magnetic gradient: OBJ Ltd., Perth, Australia). In a recent pilot clinical trial, magnetophoresis enhanced transdermal delivery of key ground substance components (Lubricen[™] gel containing chondroitin, glucosamine, hyaluronic acid) was shown to be equivalent to a commercially available transdermal non-steroidal anti-inflammatory formulation in controlling pain and facilitating movement in males with prior knee injury [96]. The first commercially available magnetophoresis-based enhancer consists of the magnetic array housed in an applicator that is used to enhance delivery of cosmetic actives in skin care products (SK-II and Olay brands, Procter & Gamble, Cincinnati, Ohio). Synergistic permeation enhancement of peptides has been demonstrated where it was hypothesised that the magnetic array increased flow in stratum corneum pores generated by microneedle pretreatment of human skin [97].

6.2. Direct Physical Methods

Direct physical enhancement methods can also be described as ‘minimally invasive’ methods and have in common an approach that involves creating a pore or hole in the

stratum corneum barrier through which a drug molecule can be forced or flow to access the epidermis. All direct physical enhancement techniques increase skin permeation but their methods vary and include thermal, mechanical and pressure-based technologies (Fig. 4).

6.2.1. Microdermabrasion

Is utilised in dermatological and cosmeceutical practices for skin rejuvenation. It involves superficial disruption of the skin by instruments that generate air-propelled particles to ‘sandblast’ and simultaneously remove the skin by vacuum [98]. Microdermabrasion systems are designed for local disruption of the stratum corneum and superficial viable epidermis, resulting in minimal to no pain or bleeding and relatively fast recovery time [99, 100]. Although microdermabrasion is generally used for cosmetic skin rejuvenation, it has been applied with topical cosmetic products such as retinoic acid, vitamin C, and nicotinamide [101] and has also been investigated as a tool for enhancing delivery of therapeutic compounds into the skin including 5-fluorouracil, aminolevulinic acid, and clobetasol 17-propionate for local effects [102] and systemic drugs such as insulin [103]. Interestingly, whilst microdermabrasion increased the delivery of the hydrophilic drugs 5-fluorouracil and aminolevulinic acid (log partition coefficient -1.5 and -0.89 respectively), the flux of the lipophilic clobetasol 17-propionate (logP of 3.5) decreased following microdermabrasion [102]. This suggests that whilst removal of the lipid rich stratum corneum benefits penetration of hydrophilic compounds, it reduces penetration of lipophilic compounds. Drugs delivered post dermabrasion rely on passive diffusion within the viable skin tissue, potentially excluding lipophilic compounds that partition into the now removed stratum corneum lipid bilayers. Whilst removal of the stratum corneum by controlled microdermabrasion may be an effective means to enhance skin penetration and have utility in specific procedures such as PDT [104], it is unlikely to be a practical approach in most skin delivery applications.

6.2.2. Thermal Ablation

Applies extreme heat at the skin surface ($\approx 300^{\circ}\text{C}$ for microseconds) with no significant temperature rise in the underlying viable epidermis and deeper skin tissues [105], to vaporize portions of the stratum corneum to create micron-scale channels similar to those created by a microneedle array. This can be achieved by the application of arrays of microfabricated resistive [106] and radiofrequency [107, 108] electrical heating elements and by lasers. Examples are the ViaDor[™] system (developed by TransPharma Medical, now owned by Syneron Medical Ltd., Israel) which creates 144 microchannels in 1cm², and the Passport[®] system (developed by Altea Therapeutics, now owned by Nitto-Denko, Japan). In both cases, the device is used to ablate the skin prior to the application of a transdermal patch. Laser ablation is routinely applied in skin rejuvenation for dermatological (scarring, stretch marks) and cosmetic outcomes. In skin delivery investigations erbium:yttrium-gallium-garnet (Er:YAG) and yttrium scandium gallium garnet (YSGG) lasers, emitting at 2,790 nm and 2940 nm respectively, are most commonly used as they offer effective ablation of the stratum corneum with reduced thermal damage to the underlying viable tissue [109]. Whilst full coverage ablation provides greater penetration enhancement partial, coverage is more clinically rele-

vant. Fractional laser ablation is applied to sub-millimetre regions to generate spots (typically 40 to 300 μm with densities between 50 and 600 cm^{-2}) mimicking a microneedle array-type pattern [110-112]. The overall effect is to cause minimal thermal damage and promote fast healing times due to migration of neighbouring viable keratinocytes into the ablated cavities.

Penetration enhancement following fractional ablation with an Er:YAG laser of the relatively lipophilic imiquimod (logP 2.7) and hydrophilic aminolevulinic acid (logP -1.5) was 8.83-fold and 41.23-fold respectively [113], again demonstrating that removal of the stratum corneum is more effective for hydrophilic than lipophilic compounds. Fractional laser ablation has also been shown to allow effective delivery of large molecules such as 10 kDa dextran, siRNA, plasmid DNA and vaccines [110, 114]. There will need to be significant technological developments as current bulky and expensive laser devices are a significant limitation for their further development in the transdermal delivery field.

6.2.3. Biolistic Injectors

Deliver a liquid or particulate payload to the skin surface at high velocity to breach the stratum corneum. Liquid jet injectors apply high pressure to eject through a small nozzle, at velocity 100-200 m/s, with the delivered volume and penetration depth dependent on the velocity and microjet size [115]. Deeper penetration is achieved with higher velocities, but there is also greater potential for splash back of solution from the skin surface and damage to the skin tissues [116]. Volumes in the nanolitre range minimise damage and splashback [117]. The skin is pre-ablated to facilitate penetration of the propelled liquid. Jang and colleagues used a 250 μs ER:YAG laser pulse laser to generate vapour bubbles and downstream shockwaves that propelled a microjet of liquid containing epidermal growth factor and human growth hormone into pre-ablated pig skin [118, 119]. Microjet velocities of 23.0 to 50.6 m/s provided effective delivery of epidermal growth factor and human growth hormone in porcine skin, as determined by gene expression of keratinocyte laminin and fibroblast elastin, respectively. A subsequent refinement of the procedure has involved a pre-ablation step prior to microjet generation, both achieved by the same laser [120]. The advantage is that pre-ablation is targeted to finite areas, with the microjet then bombarding those defined areas. Effective drug delivery efficiency is achieved with much reduced tissue damage and more rapid reepithelialisation.

The original biolistic particle delivery system, or gene gun, was designed for delivering exogenous DNA (transgenes), typically as a particle of a heavy metal coated with plasmid DNA, into plant cells. This has been developed into biolistic injectors delivering a 'shot gun' burst of nano- or micro-particles into the skin, that have been effective for the immunisation of antigens including influenza, malaria and also anti-cancer applications [121-124]. Solid or dense spheroidal particles in the sub-micron size range are typically used for biolistic particle delivery [125], although porous particles can provide options for increased drug loading and controlled release [121]. For both liquid and particulate biolistic injection technologies, their most likely adoption is an alternative to conventional injection such as vaccination. Examples of their clinical role in this field are described later.

6.2.4. Microneedle

Arrays are the direct penetration enhancement approach that has garnered most attention as an alternative to conventional hypodermic and subcutaneous injections. They have been the subject of extensive investigation in academia and industry and their development towards the clinic is well advanced, particularly for vaccination [126, 127]. Microneedle array fabrications vary from the so called 'poke and patch' to the inclusion of their payload on the surface or within the projections, but they have in common the mechanism of poking an array of small, sharp projections into the skin to breach the stratum corneum barrier. Microneedle projections typically range from 25 to 2000 μm in height, 50 to 250 μm in base width and 1 to 25 μm in tip diameter [29], so that they do not contact the nerve endings or cutaneous blood vessels thereby providing pain-free administration [128] and avoiding the potential of transmitting blood-borne infection [129]. The shape, size and material of the microneedles must be such that the projections will overcome the elasticity of the skin to enter the epidermis to sufficient depth and will remain intact to facilitate removal, unless they are designed to dissolve within the skin. Pores caused by microneedle projections of a range of geometries have been shown to close within 2h unless occluded, which can increase closure time to over 24h [130]. There are five main types (Fig. 4): solid, coated, dissolvable, hollow and hydrogel swellable microneedles, the latter designed to remain *in situ* and facilitate diagnosis.

- Solid microneedles employ the 'poke and patch' approach whereby the microneedle array is used as a pre-treatment to create microchannels in the epidermis, then removed and a drug-laden patch or other suitable formulation is applied to release drug that can diffuse into the viable epidermis *via* the transient channels. Solid microneedles have been fabricated from a number of materials including metals, silicon and polymers [126].
- Coated microneedles provide the advantage of a one-step application but dosage is limited to the quantity that can be coated onto the total surface area of the microneedle projections [131]. Coated microneedles have attracted particular interest for macromolecules such as vaccines [132-136].
- Dissolvable microneedles approach, are composed of materials that form sharp projections with the payload dissolved or suspended within, allowing them to be poked into and then dissolve within the skin to release their payload. At completion there is no need to remove and dispose of the microneedle array. Dissolving microneedles composed of varied materials have been very widely investigated for a range of applications from caffeine delivery to combat obesity [137] and 5-aminolevulinic acid for PDT [138] to macromolecules such as insulin [139, 140] and vaccines [141-143], and diagnostic monitoring [144].
- Hollow microneedles are akin to an array of ting hypodermic needles that allow 'poke and flow' of drug solution into the skin [145]. They require more complex microneedle fabrication and pressurized assistance to generate and regulate flow [129].
- Swellable microneedles consist of suitable hydrogels that can be poked into the skin and will then swell and remain *in situ*. Water or skin interstitial fluid diffuses

into the microneedle array leading to controlled swelling that permits the diffusion of drug molecules [146-148] applied in an adhesive patch into the skin, or the egress of physiological chemicals such as glucose from the skin to permit diagnostic monitoring [149, 150].

The range of materials, fabrications, manufacturing and engineering principles in the design and development of microneedles is beyond the scope of this article, but excellent reviews are available elsewhere [142, 151, 152].

Human studies have demonstrated that consistent microneedle application/penetration to the skin site of application is essential for reliable drug delivery [153]. This can be achieved either manually by simply applying thumb pressure or by using an applicator (examples are the Zosano Pharma, Vaxxas and Corium International microneedle technologies). Studies have shown that microneedle arrays are well received by consumers and can be administered equally well by a range of consumers and health professionals [154, 155]. At present there are no true microneedle devices on the market. The closest technologies are the Micronjet[®] 600 (NanoPass Technologies Ltd., Israel) and Soluvia[®] (Becton Dickinson, USA) that have both demonstrated better immune response for influenza vaccine compared to IM injection. In both cases these are essentially micronized injection devices that allow intradermal administration from pre-fillable microinjection systems consisting of small needles attached to a conventional syringe [156]. Although there are significant manufacturing and regulatory hurdles that will need to be overcome, there is clear future potential for microneedle based skin delivery in the management of chronic conditions such as diabetes [157] and in global vaccination programs.

6.2.5. High Aspect Ratio Elongated Microparticles

Elongated microparticles (EMP) for enhanced drug delivery developed by Prow's research group in Australia [158, 159], are made of cylindrical shaped solid silica with an average width of $28 \pm 11 \mu\text{m}$ and average length between 120.1 and 483.2 μm . EMP can be applied as a pretreatment (akin to the 'poke and patch') or mixed with existing formulations and massaged onto the skin to facilitate permeation of the simultaneously applied active in the formulation. The EMP/skin surface contact angle is optimized by applying the EMP/formulation with a 3D printed micro-textured applicator that ensures low angular penetration within the epidermis, and penetrate to the dermal-epidermal junction. EMP enhancement ratios of 8.5- and 8.8-fold for Vitamin E (3H-a-tocopherol acetate) and Vitamin B3 (3H-nicotinamide) respectively compared to passive administration in excised human skin were reported [158]. They have recently demonstrated the successful combination of a nanoemulsion and EMP to deliver lipophilic compounds into the skin [160]. Normal transepidermal turnover and desquamation pushes the microparticles out of healthy skin within 3 weeks. An advantage over microneedle and other array-type enhancement technologies is that the EMP can be applied to varied sizes and shapes of application site.

6.3. Combinations of Enhancement Approaches

As with all techniques that act by creating pores in the stratum corneum, an important consideration is how the properties of the drug and vehicle applied within those pores influence passive diffusion within the skin. Many different combi-

nations of penetration enhancement approaches have been explored with the aim of developing low cost, efficient and effective technologies capable of increased and/or targeted delivery to and *via* the skin. Examples are: the combination of indirect physical enhancement energy to improve diffusion within pores or into the tissues in contact with pores generated by direct physical enhancers (microneedles with magnetophoresis [161] and iontophoresis [162]); and nanosystems to increase drug solubility or control release within the skin after access generated by physical enhancer [163].

6.4. Skin Delivery: Technologies Creating the Future?

The application of effective penetration enhancement technologies can offer new opportunities for delivery of a much wider range of therapeutic compounds to and across the skin. Transiently overcoming the skin barrier also offers opportunities for diagnostic technologies applied to the skin. We have focused on a couple of innovative directions utilising the skin: vaccination and smart technologies that combine diagnosis and responsive therapy.

6.5. Skin Delivery for Vaccination

The opportunity to deliver vaccines *via* the skin is an alternative to conventional invasive and painful intramuscular or subcutaneous injections with hypodermic needle and syringe associated with the risk of blood-borne disease transmission needlestick injuries (*i.e.* accidental punctures by contaminated needles) [164]. The skin layers are rich in immune cells such as the epidermal Langerhans cells and the dermal dendritic cells with antigen-presenting capacity [165] with the opportunity to generate a stronger immune response compared to deeper injection [166], and consequently reduce the required dose and associated cost. For example, in our work comparing immunization of mice with the influenza vaccine Fluvax by microprojection array to the buccal mucosa and skin, versus oral and IM delivery, resultant IgA levels and immune response were significantly higher for skin delivery (Fig. 5: [167]). To deliver vaccines, which are typically macromolecules, research and commercialization has focused on the various non-invasive penetration enhancement approaches described above. For example the PharmaJet needle-free jet injector uses a spring-powered, hand-held injector to shoot a precise fluid stream containing the vaccine through the skin and into the muscle [168, 169], with pressure that is reported to feel like the snap of a rubber band [170]. Afluria[®] influenza vaccine was approved by the US FDA in 2014 and initial uptake has been positive. PharmaJet needle-free injector intradermal delivery has also been developed for poliovirus [171] and MMR (measles, mumps, rubella), and is in use in a number of countries (<https://pharmajet.com>).

Alternative minimally invasive vaccination methods have been developed with most focus being on the use of microneedles for the delivery of influenza antigens and DNA vaccines [151, 172-174]. A microneedle patch for the delivery of inactivated influenza vaccine has reached the clinical trial stage (Phase I) in humans (NCT02438423), led by Mark Prausnitz's research team (Georgia Institute of Technology, GA, USA). Administration of a 1 cm² array of 100 dissolvable microneedles (composed of sucrose and polyvinyl alcohol) that detached and dissolved within about 20 mins, was well tolerated and provided titres and seroconversion rates similar to intramuscular injections [168]. The same group

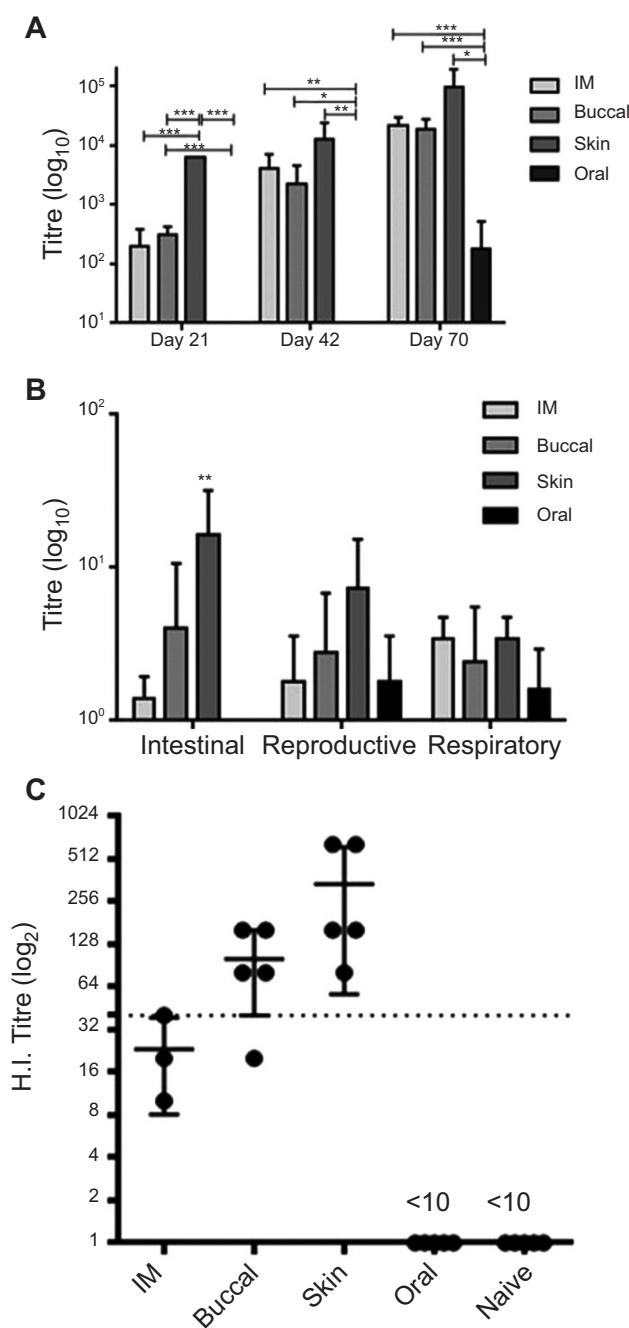


Fig. (5). Anti-influenza immunogenicity of Fluvax[®] 2010 coated Nanopatches applied to the buccal mucosa. (A) Total serum IgG responses at various times after immunisation and boost. (B) IgA responses in samples extracted from faeces, female reproductive tract and nasal passages after the final immunisation (Day 70). (C) Haemagglutination inhibition titres against the A/Perth/16/2009 like influenza strain in serum collected 21 days after the initial immunisation. The black line at a HI titre of 40 represents the accepted minimum that correlates with protection in humans. In all panels bars represent the mean and standard deviation from the mean are shown. Significance of $p \leq 0.01$ (***) , $p = 0.01-0.05$ (**) or $p = 0.05-0.1$ (*) is indicated. In all immune studies $n = 5$ animals were used per group. Reproduced with permission from [167].

has received funding from the Bill and Melinda Gates Foundation for the development of a polio microneedle patch and Phase I clinical trials in humans. The Nanopatch[™] (Vaxxas, Australia) is an extension of the microneedle approach where the array contains 10,000-20,000 projections per cm² (~110-250 μm in length, ~20-40 μm in base width) with the vaccine dry coated onto their surface. It has shown promise during animal clinical trials for vaccination against influenza [175, 176], herpes simplex virus [177], human papillomavirus-associated cervical cancer [178] and poliomyelitis [179, 180]. Self-administration of microneedle patches could improve vaccination coverage [181] and reduce administration costs, and avoiding the need for cold storage has major implications for vaccination in developing countries.

Lademann's group [181] explored the potential to deliver vaccines by topical application of microparticles that penetrate into the hair follicles. The infundibulum within the hair follicle (400-600 μm deep) is lined with a particularly dense network of dendritic cells [182] making it the most immunologically active region, therefore this was specifically targeted for delivery. As they had previously demonstrated that particles of different sizes penetrate follicles to different depths, the vaccine carriers were of 1 mm diameter to target the infundibulum. They showed that their 1 mm silicon dioxide microparticles efficiently penetrated the hair follicles to deliver vaccine constituents to dendritic cells in the infundibulum within 1 hour of topical application. They also showed that particles remained as a depot within the follicle for up to 10 days contrasting with particles on the stratum corneum that were rapidly removed after about 1 day. Follicular delivery of vaccine carried on microparticles provides another potential approach to target the body's largest immune organ.

6.6. Smart Wearable Technologies for Diagnosis and Treatment

The future of skin delivery lies with the technological innovations being developed for enhanced skin penetration and site-specific targeting to extend the range of solutes that can be successfully delivered and thereby the clinical applications of skin delivery. Technological innovations in skin delivery include advanced transdermal patch fabrications, polymeric-based nanoparticles, lipid-based flexible nanovesicles, energy-based enhancers and minimally invasive delivery systems. An in-depth discussion of these technologies is not the focus of this manuscript, but we have highlighted some examples where skin delivery may provide significant advances in healthcare in the future.

There have been considerable advances in the use of microneedle patches to monitor and manage diabetes, one of the major global health challenges. The holy grail of diabetes management is a non-invasive feedback system combining glucose monitoring and responsive drug delivery. Two recent studies have reported the non-invasive management of diabetes using microneedle patches for the monitoring of glucose blood levels and subsequent release of the therapeutic active. In the first study, a 6 mm² array of 121 conical microneedles contained polymer nanoparticles loaded with insulin and glucose oxidase enzyme which access the interstitial fluid when inserted into the skin (Fig. 6A) [183]. A

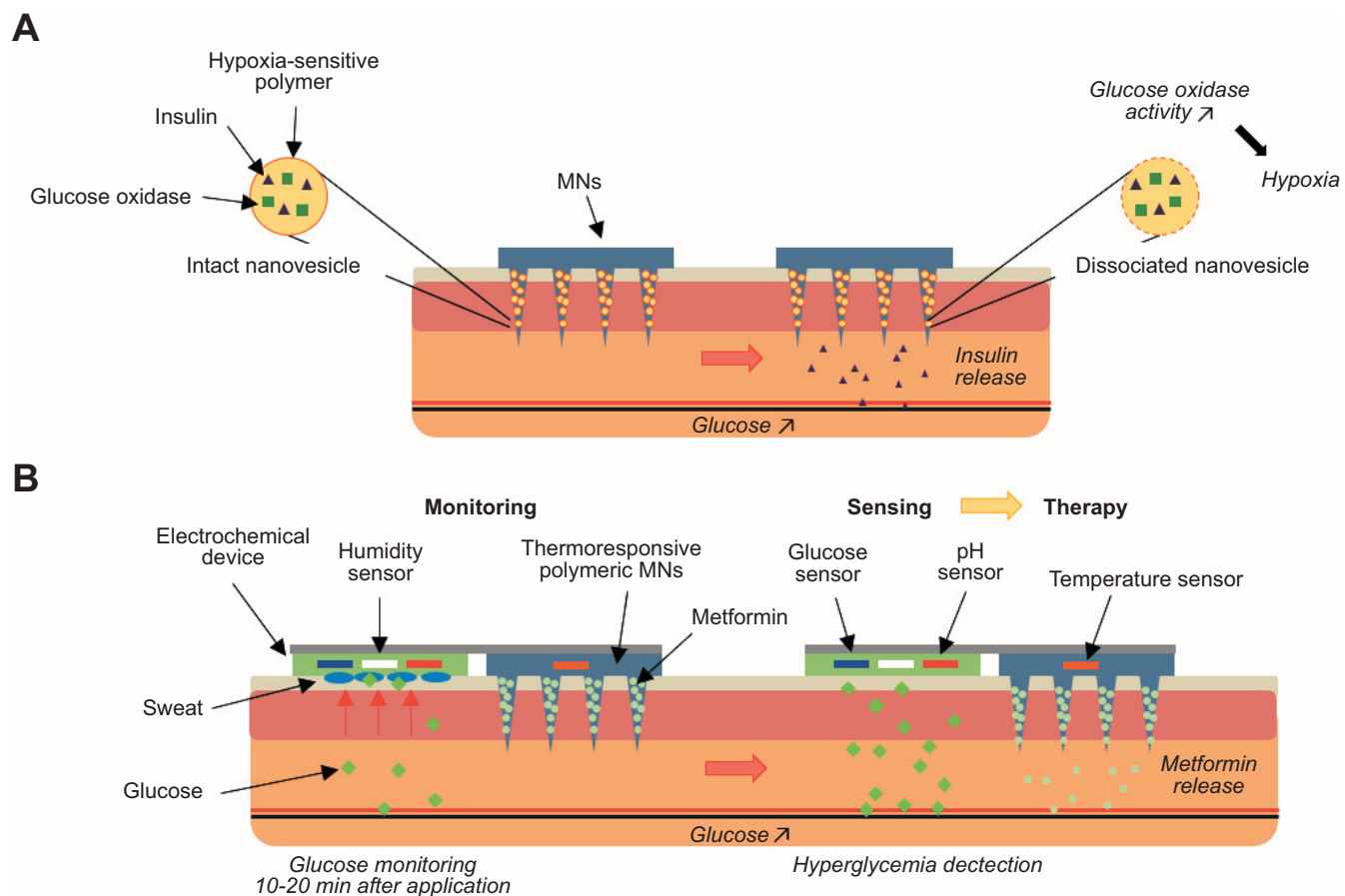


Fig. (6). **a**) The 'smart insulin patch' effectively regulates blood glucose levels by sensing hypoxia in the local microenvironment. **b**) A sophisticated system to sense glucose, pH, humidity and temperature by absorbing sweat and release antidiabetic drug upon trigger.

rise in glucose blood level (hyperglycaemia) causes an increase in enzymatic activity, converting glucose to gluconic acid and consuming oxygen in the process, thereby creating a local hypoxic micro-environment within the microneedle projections. This hypoxic environment triggers dissociation of the polymer nanoparticles with subsequent release of their insulin. The 'smart insulin patch' effectively regulated blood glucose levels in *in vivo* trials with mouse models of chemically induced type 1 diabetes [183]. The second approach utilizes a patch that absorbs sweat from the skin, contains sensors for glucose, pH, humidity and temperature, combined with polymeric microneedles containing the antidiabetic drug metformin (Fig. 6B) [184, 185]. The microneedles are coated with the hydrophobic compound tridecanoic acid to protect them from skin moisture and prevent drug release. Blood glucose is monitored once sufficient sweat is generated, typically after 20-30 min. When elevated glucose level is detected, it triggers the heater embedded in the patch, warming the microneedles above the transition temperature of tridecanoic acid ($\sim 41^\circ\text{C}$), exposing the microneedle polymer to interstitial water and releasing the metformin. The connection of the patch to a portable electrochemical analyser also enabled wireless control while at the same time transmitting collected data for further treatment optimization [184, 185]. In addition to a robust set of *in vivo* data obtained from an *in vivo* genetically diabetic mouse model, the devel-

oped transdermal drug delivery system was shown to be flexible, robust and conforms to the skin. This group have further developed their technology into a wearable/disposable sweat-based glucose monitoring device integrated with a feedback transdermal drug delivery module, with more elegant fabrication providing improvements in wearability and accuracy. This approach has also been applied for monitoring tremor frequencies (seen in movement disorders such as epilepsy and Parkinson's disease) through the recording of muscle activity [186]. The collected data could then be used to activate a microneedle delivered feedback therapy (e.g. anti-Parkinson's disease drug) through thermal stimuli [186]. It is likely that we will see this approach of wearable sensor/delivery patch technology applied to a range of chronic conditions, particularly where potent drug therapies are available, with devices in human trials in the future.

CONCLUSION

The skin is a key site for local and systemic drug delivery. It has the unique qualities of being easily accessible yet relatively impermeable. This allows products from a wide range of formulations to be applied to the skin and then removed as required. Products for skin application have evolved from simple solutions and ointments to multiphase, nanotechnologies and assisted technologies. It is essential that the increasingly sophisticated products being developed

address market, design, safety and sensorial requirements for the range of situations in which they are applied to the skin.

CONSENT FOR PUBLICATION

Not applicable.

FUNDING

Michael Roberts would like to specifically acknowledge the support by grants APP1107356 and APP1055176 from the NHMRC.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

The authors thank the National Health & Medical Research Council of Australia (NHMRC), Australian Research Council (ARC) and the US FDA for their support of our work on topical absorption over many years.

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