Expert Opinion

Ashley Publications
www.ashley-pub.com

Emerging strategies for the transdermal delivery of peptide and protein drugs

Yannic B Schuetz, Aarti Naik, Richard H Guy & Yogeshvar N Kalia†
†School of Pharmaceutical Sciences, University of Geneva, CH-1211 Geneva, Switzerland

Transdermal delivery has been at the forefront of research addressing the development of non-invasive methods for the systemic administration of peptide and protein therapeutics generated by the biotechnology revolution. Numerous approaches have been suggested for overcoming the skin's formidable barrier function; whereas certain strategies simply act on the drug formulation or transiently increase the skin permeability, others are designed to bypass or even remove the outermost skin layer. This article reviews the technologies currently under investigation, ranging from those in their early-stage of development, such as laser-assisted delivery to others, where feasibility has already been demonstrated, such as microneedle systems, and finally more mature techniques that have already led to commercialisation (e.g., velocity-based technologies). The principles, mechanisms involved, potential applications, limitations and safety considerations are discussed for each approach, and the most advanced devices in each field are described.

Keywords: electroporation, encapsulation, iontophoresis, jet-injectors, microneedles, peptide and protein delivery, sonophoresis, stratum corneum ablation, transdermal drug delivery

Expert Opin. Drug Deliv. (2005) 2(3):533-548

1. Peptide and protein delivery

Revolutionary advances in biotechnology have given rise to numerous protein and peptide entities with therapeutic potential. One of the major challenges to the successful clinical use of these 'biotech' molecules is their efficient and targeted delivery to the site of action. Presently, parenteral delivery is the most routinely employed method for administering polypeptide agents, which are otherwise completely destroyed when given orally. These compounds often have short plasma half-lifes, need frequent injections and are, therefore, associated with poor compliance. In recent years, alternative and more patient-friendly modes of drug delivery have been extensively investigated.

2. Transdermal delivery

Over the past few decades the skin has generated a great deal of interest as a portal for the systemic delivery of drugs [1]. The potential advantages of this mode of administration have been well documented [2]. The worldwide transdermal market is currently worth > US$4 billion, yet is based on only 13 drugs. This rather limited number of transdermal drugs is explained by the skin's excellent barrier function, which is accomplished entirely and quite remarkably by the outermost few microns of tissue, the stratum corneum (SC), which is often referred to as a 'brick and mortar' structure [3]. In addition to being pharmacologically potent, a therapeutic agent must possess a balance of physicochemical properties that render it permeable: a relatively low molecular weight (< 500 Da), a moderate octanol–water partition

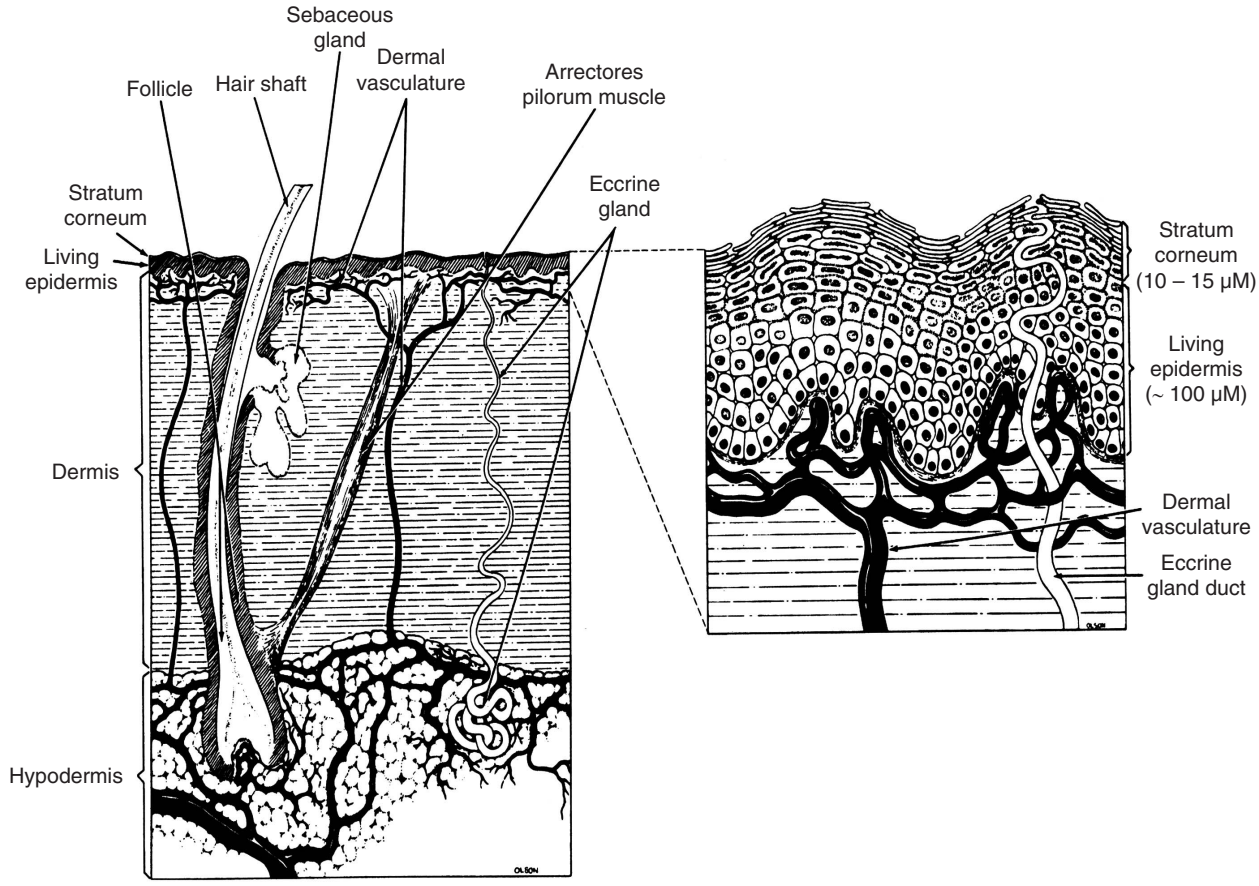

Figure 1. Major skin structures. Reproduced with permission from FLYNN GL: Cutaneous and transdermal delivery: processes and systems of delivery. In: *Modern Pharmaceutics (3rd Edition)*. GS Banker, CT Rhodes (Eds), Marcel Dekker, New York, NY, USA (1996):239-298.

coefficient ($10 < K_{o/w} < 1000$), reasonable aqueous solubility (> 1 mg/ml) and modest melting point (< 200°C) [4].

Most, if not all, peptides and proteins, being large and hydrophilic, do not satisfy these criteria. Yet, the transdermal delivery of these potent therapeutic agents is of particular interest, as percutaneous administration overcomes many of the problems associated with conventional therapy. Hence, to expand the range of drugs that can be delivered transdermally, and to include peptides and proteins, a number of enhancement technologies are under investigation.

3. From skin surface to systemic circulation

Before being taken up by blood vessels in the upper papillary dermis and prior to entering the systemic circulation, substances permeating through the skin must cross the SC and the viable epidermis. There are three possible pathways leading to the capillary network: across the continuous SC, through hair follicles and their associated sebaceous glands, or via sweat ducts. Although, the 'as-the-crow-flies' diffusion distance across the SC is no more than 10 – 15 µm, the actual diffusional pathway may be upto 50-fold greater, depending on the route taken (**Figure 1**). While crossing the viable skin layers, peptidic drugs can undergo extensive enzymatic degradation [5-7]; numerous proteases (including endopeptidases and exopeptidases) have been detected in the skin, both in the dermis and the epidermis [6-8]. However, as a result of the rapid uptake of penetrants into the general circulation on arrival in the highly vascularised dermal papillary layer, peptide/protein metabolism is most likely to occur during passage through the epidermal layer [9]. Although this phenomenon may appear to mitigate one of the benefits of transdermal delivery (avoidance of gastrointestinal and hepatic enzymatic metabolism), the extent of epidermal catabolism is of course dependent on the application area (e.g., patch size) and the rate of epidermal transport: parameters that are optimised in the design of active transdermal systems. In addition, peptide formulations can also be designed to incorporate specific enzyme inhibitors [10,11]. Parenthetically, certain energy driven enhancement techniques may also impact on enzyme activity, as reported for ultrasound, which is suggested to deactivate certain skin enzymes [12,13].

This article reviews the current state of transdermal peptide drug delivery technology. The strategies are classified as a

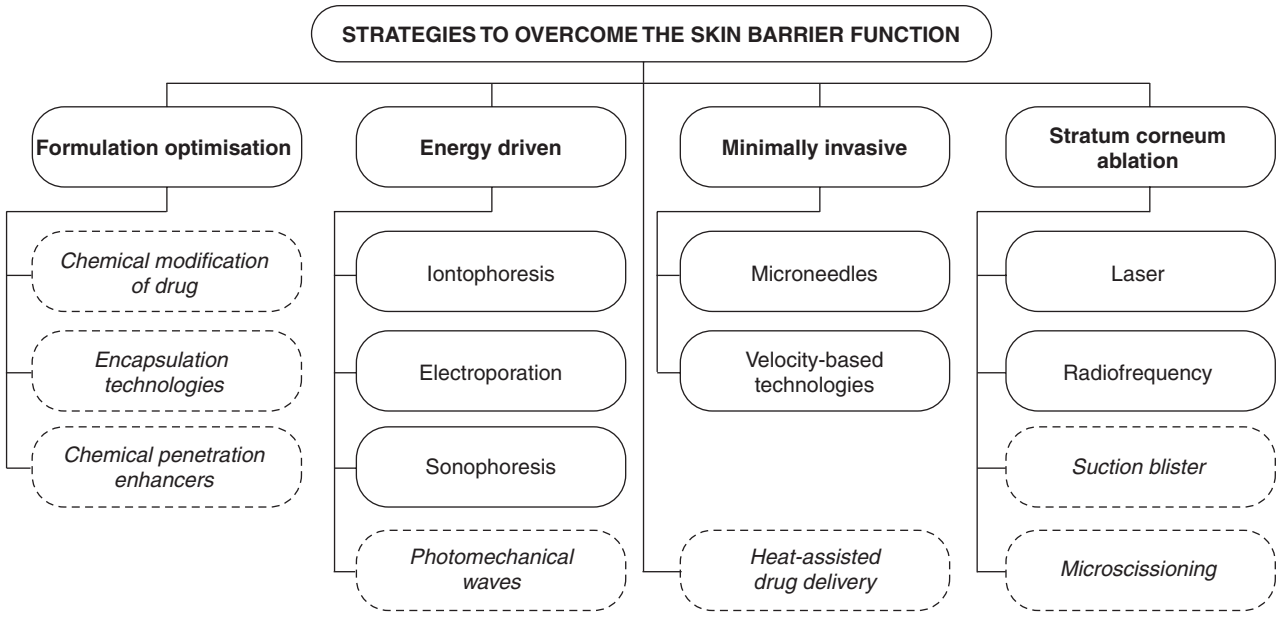

Figure 2. Current strategies for transdermal drug delivery. Italicised legends signify those technologies that are still in their infancy or are unlikely to find an application in peptide and protein delivery in the near future.

function of the manner in which they overcome the skin barrier (**Figure 2**). The approaches range from formulation optimisation, to energy driven techniques including electrically assisted transport, to methods designed to enhance drug delivery by bypassing the barrier or even by removing it.

4. Formulation optimisation

Peptide drugs, which are hydrophilic and often charged molecules, pass through lipophilic membranes such as the SC with some difficulty, if at all. To counteract this there are two main approaches available to the formulation scientist: the use of chemical enhancers to modify the SC permeability transiently (i.e., render the SC more 'leaky' towards hydrophilic molecules), and the modification of the therapeutic molecule to render it more hydrophobic and, therefore, 'acceptable' to the membrane. The latter strategy involves either chemical derivatisation or encapsulation within a lipophilic core.

4.1 Chemical penetration enhancers

There is extensive literature on penetration enhancers, their mechanisms of action and effects on the skin, and their impact on the permeation of applied molecules [14,15]. These compounds appear to act by disrupting or altering the ordered lipid structure of the SC. Whereas numerous studies on the passive delivery of peptides and proteins with chemical enhancement were conducted during the 1980s and 1990s [16], research in this area has somewhat run out of steam, primarily for two reasons. First, whereas low molecular weight therapeutics usually tolerate formulation with these permeation enhancers, proteins are less comfortable in the company of these aggressive chemicals. The second and most important reason concerns safety. Unfortunately, for most enhancers, activity is closely correlated with irritation, thus rendering them clinically unacceptable [17]. Thus, because of the extremely low permeability coefficients of peptide and protein drugs, which are readily predicted from their hydrophilicity and molecular size, the magnitude of enhancement required to ensure delivery of pharmacologically effective concentrations is expected to be beyond the capability of chemical enhancers tolerated by the skin.

4.2 Chemical modification

Whereas improved intestinal absorption through chemical modification with various fatty acids has been reported for some peptide and protein drugs, the application of this approach to transdermal delivery is new. A few research groups have studied the cutaneous delivery of derivatives of peptides such as the vasoactive intestinal peptide [18] and IFN-α [19]. The absorption of palmitoyl derivatives of IFN-α (p-IFN-α) into the viable layers of human breast skin was eightfold greater compared to the parent peptide, suggesting that this approach may find an application in topical delivery. The enhancement potential in transdermal delivery is less obvious, given that only a twofold increase in the percutaneous absorption of p-IFN-α was observed. These results must also be treated with caution given the surprisingly elevated passive permeability of the parent polypeptide (1.47 ng/cm^2/h).

Finally, one must question whether a modest improvement in delivery is sufficient to warrant the additional regulatory complexities concomitant with the prodrug strategy.

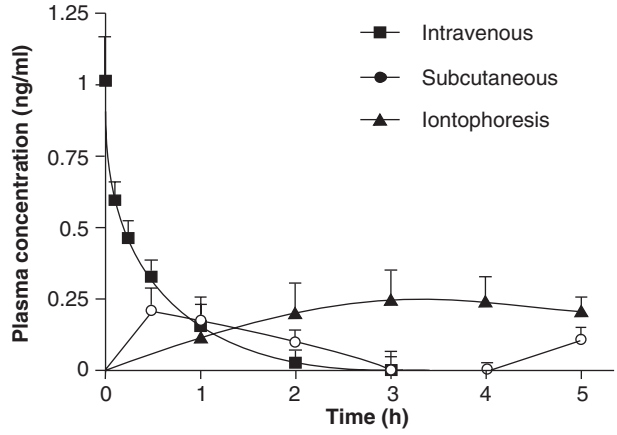

Figure 3. Plasma levels of growth hormone releasing factor in response to transdermal iontophoretic (1 mg/g; 0.17 mA/cm^2; 5 cm^2 patch), intravenous (10 µg/kg; 0.025 mg/ml) and subcutaneous (10 µg/kg; 0.025 mg/ml) GRF administration to the guinea-pig. Reprinted from KUMAR S, CHAR H, PATEL S *et al.*: *In vivo* transdermal iontophoretic delivery of growth hormone releasing factor GRF (1-44) in hairless guinea pigs. *J. Control. Release* (1992) **18**:213-220, with permission from Elsevier.

During drug development, the chemically modified derivative will have to undergo, as for the parent molecule, extensive toxicological testing in addition to the suite of developmental investigations.

4.3 Encapsulation technologies

Encapsulation consists of the entrapment of the drug within delivery systems such as microspheres, liposomes and nanoparticles. Liposomes, typically consisting of phospholipids and cholesterol, are thermodynamically stable vesicles with an aqueous core and at least one surrounding bilayer. Niosomes, which are analogues of liposomes, are non-phospholipid vesicles formed by the self-assembly of nonionic surfactants in an aqueous dispersion. Niosomes and classical liposomal systems have been found to be effective in forming drug reservoirs in the upper layers of the skin, for local therapy. The controlled topical delivery of cyclosporin A [20] and IFN-α [21] has been studied: ≤ 1 µg/cm^2 of liposomally encapsulated IFN-α was found in human skin after a 24 h *in vitro* application. However, the use of liposomes and niosomes for the systemic delivery of macromolecules across the skin has not been successful so far.

Transfersomes are ultradeformable carriers that are claimed to be driven across the skin by the transdermal hydration gradient [22]. They are suggested to be sufficiently flexible to pass through pores appreciably smaller than their own size (200 – 300 nm). Insulin-loaded transfersomes were reported to induce a modest 15% decrease of blood glucose concentration in healthy human volunteers [23]: a response that is unlikely to provoke a therapeutic effect in diabetic patients.

Ethosomes are phospholipid vesicular systems containing ethanol in relatively high concentrations. According to Touitou *et al.*, ethanol fluidises both the ethosomal lipids and the SC lipid mortar; the soft, malleable vesicles then penetrate through the disorganised lipid bilayers [24]. Several studies have been conducted with nonpeptidic drugs such as testosterone [25], trihexyphenidyl hydrochloride (currently used for the treatment of Parkinson's disease) [26] and zidovudine (an antiviral agent) [27]. As is often the case, insulin, because of its huge potential market, was the first peptide studied. The effect of a transdermal insulin formulation on blood glucose levels was investigated *in vivo* in normal and diabetic rats. Despite the significant decrease (≤ 60%) in blood glucose levels achieved in both normal and diabetic rats [28], it is clear that a corresponding effect in humans would require significant scaling-up, which remains to be demonstrated.

Finally, although encapsulation may be considered to offer some protection from cutaneous enzymes, the point at which the drug leaves its 'protective capsule' during its passage through the tissue has yet to be determined. Moreover, any benefits offered are likely to be offset by formulation stability issues.

5. Energy driven methods

5.1 Iontophoresis

Iontophoresis is a century-old technique developed to deliver charged molecules through the skin at an enhanced rate via application of a small electric current (≤ 0.5 mA/cm^2). The drug reservoir on the surface of the skin is in contact with an electrode of the same charge as the solute, connected to a grounding electrode and a power supply. In addition to electromigration (direct effect of the applied electric field on the charged species) and current-induced modification of passive skin permeability, positively charged compounds (present in the anodal compartment) benefit from a third transport mechanism called electro-osmosis: a convective solvent flow, which is a consequence of the skin's net negative charge at physiological pH. This flow also enhances the transport of neutral compounds.

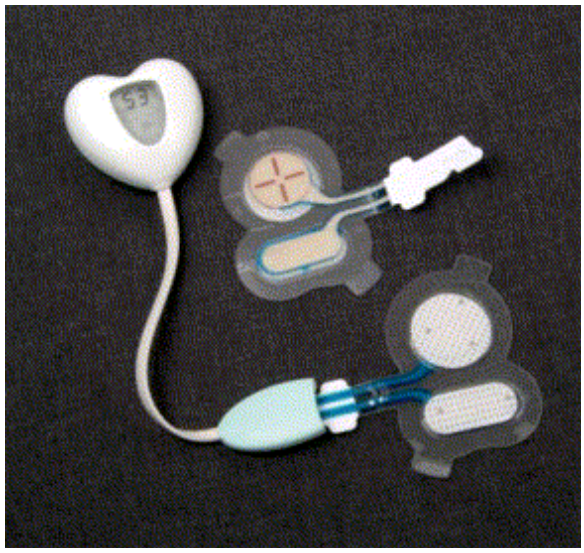

Figure 4. LidoSite™, an iontophoretic delivery system for lidocaine (Vyteris, Inc., NJ, USA). Reproduced with permission from KALIA YN, NAIK A, GARRISON J *et al.*: Iontophoretic drug delivery. *Adv. Drug Deliv. Rev.* (2004) **56**(5):619-658.

A feature of iontophoresis, which distinguishes it from other enhancement technologies, is that it acts primarily on the molecule itself. The technique does not simply involve passive transport of the drug following barrier disruption: the driving force comes from the applied electric field and is not solely dependent on the concentration gradient, as in passive delivery. Hence, by modulating the current applied, iontophoresis allows adaptation of the delivery input rate and profile to the needs of each patient or phase of treatment, and offers the possibility of pulsatile drug delivery. Although the advantages of constant or sustained plasma concentrations have long been endorsed, there are a number of therapies that benefit from the conventional 'peak and trough' plasma profiles. Moreover, certain peptides such as human parathyroid hormone (PTH) and luteinising hormone releasing hormone (LHRH) have distinct, and often opposing, pharmacological effects depending on their delivery profile. LHRH, for example, must be administered as a bolus every 60 – 90 min to treat female infertility, but must be given as a continuous infusion in the treatment of certain cancers. Pulsatile delivery is also desirable when downregulation and tolerance may be a concern, as demonstrated for vasopressin [29]. Considerable interest has been, and continues to be, shown in the iontophoretic delivery of therapeutic peptides and proteins, which, given their often charged character, are good candidates for this technology. Numerous peptides including thyrotropin releasing hormone (TRH) [30], angiotensin [31], octreotide [32], LHRH and analogues [33-37], arginine-vasopressin [38], calcitonin [39], human PTH(1-34) [40] and insulin [41] have been studied. Many of these peptides have also been the subject of mechanistic investigations, and electro-osmosis has been proposed to be the predominant mechanism governing the iontophoretic transdermal delivery of large (molecular weight ≥ 1000 Da) cationic peptides [42]. However, peptides containing closely juxtapositioned cationic and lipophilic residues are able to inhibit this transport mechanism, and hence their own transport, by altering the permselectivity properties of the skin when iontophoresed [43-48]. This inhibition may be considered as a limitation in the iontophoretic transdermal delivery of certain peptide drugs containing this structural motif. Nevertheless, leuprolide, a synthetic nonapeptide analogue of LHRH containing D-Leu-Leu-Arg at position 6 – 8 (corresponding to the mentioned structural signature) has been successfully delivered *in vivo* in humans to produce a peak luteinising hormone (LH) response similar to that by subcutaneous injection [49]. Other *in vivo* studies have also compared the delivery profile obtained after iontophoresis with that measured after conventional needle injections, to demonstrate the potential of this administration route. Such an example is illustrated in **Figure 3** for the delivery of the growth hormone releasing factor (GRF) (1-44), in which steady-state plasma GRF levels after iontophoretic delivery are greater and more sustained, relative to those following intravenous and subcutaneous injections [50]. Not surprisingly, the iontophoretic delivery of insulin has provoked much attention. However, although the delivery of regular insulin by iontophoresis may be sufficient to treat small mammals, the best deliveries achieved, even with monomeric insulin analogues [51], are still 1 – 2 orders of magnitude below those necessary to match the basal secretion level in humans [41].

Owing to the advanced nature of transdermal iontophoretic research and development, these systems are relatively well characterised and understood. For example, the recently launched LidoSite™ device (**Figure 4**; Vyteris, Inc.) [52] for local anaesthesia and the IONSYS™ system for fentanyl delivery (Alza Corporation; currently awaiting final FDA approval) offer iontophoretic platforms, which can be potentially adapted and customised to the local and systemic iontophoretic administration of peptide drugs.

5.2 Electroporation

First used for the introduction of DNA material into cells *in vitro*, the use of electroporation for transdermal delivery was suggested ~ 10 years ago. Unlike iontophoresis, which employs small currents (transdermal voltages ≤ 10 V) for relatively long periods of time (many minutes to hours), electroporation involves exposure of the skin to relatively high voltages (~ 100 – 1000 V) for short periods of time, typically 1 to several hundred milliseconds, which in turn create intense electric fields across the thin SC. Molecular transport through transiently permeabilised skin is thought to result from a variety of mechanisms: enhanced diffusion through the aqueous pathways produced in the lipid bilayers, electrophoretic movement (for charged species) and, to a small extent, electro-osmosis [53].

A study investigating insulin delivery demonstrated that whereas < 0.6 μg/cm^2 insulin was transported across porcine epidermis after electroporation (100 V, 1 Hz, 1 ms pulse for 10 min), around 13 μg/cm^2 (~ 0.33 U/cm^2) reached the receptor

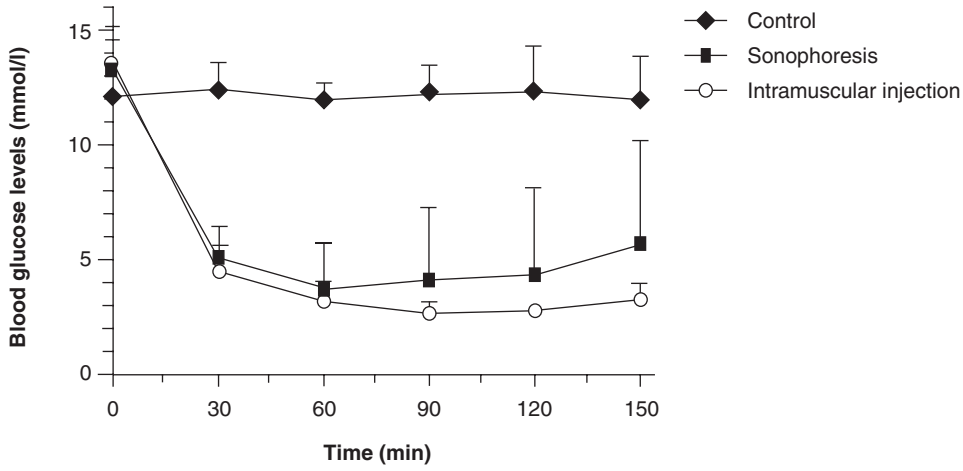

Figure 5. Blood glucose levels in rats after intramuscular injection of insulin 0.5 U and sonophoresis of insulin (I = 2.5 Watts/cm², t_{eff} = 6 min, tUS = 15 min, t_{on} = 3.2 s). Reproduced from BOUCAUD A, GARRIGUE MA, MACHET L *et al.*: Effect of sonication parameters on transdermal delivery of insulin to hairless rats. *J. Control. Release* (2002) **81**:113-119, with permission from Elsevier.
I: Pulse intensity during ultrasound application; t_{eff}: Total duration of ultrasound exposure during treatment time, t_{on}: Duration of each ultrasound pulse; tUS: Total treatment time during which pulsed ultrasound was applied.

compartment when delivered from a formulation containing phospholipids under the same electroporation regimen [54]. The treatment of a diabetic patient (36 U/day) is obviously unfeasible with such an input, but this 20-fold enhancement in transport demonstrates the potential for synergistic combination of electrically assisted and chemical technologies.

Transdermal heparin transport across human skin *in vitro* was possible at therapeutic rates with electroporation, whereas the low-voltage iontophoretic flux with the same time-averaged current was an order of magnitude lower [55]. Similarly, electroporation significantly enhanced the flux of human PTH(1-34) in comparison to iontophoresis [40], suggesting that high-voltage pulsing creates transient changes in skin permeability, which do not occur during iontophoresis.

However, although electrically assisted skin delivery via iontophoresis has been widely investigated in humans and shown to be safe and well tolerated, very few human studies have been conducted with electroporation. In electrochemotherapy investigations, where electric pulses > 1000 V were applied to the skin of melanoma patients to facilitate the chemotherapeutic treatment of tumour nodules, muscle contractions and mild pain were reported during each pulse in addition to a transient erythema [56]. Although these unwanted side effects subsided after the pulse, further investigation is required to determine whether transdermal drug delivery by electroporation is clinically feasible.

5.3 Sonophoresis

Sonophoresis (or phonophoresis) is defined as the movement of drugs through intact skin and into soft tissue under the influence of an ultrasonic perturbation [57]. Low-frequency ultrasound (frequencies < 100 kHz) has been demonstrated to induce the greatest transdermal transport enhancement [58,59]. Numerous studies have been devoted to understanding the mechanisms of sonophoresis [60-63]. Acoustic cavitation, the formation and collapse of gaseous cavities, plays the dominant role in low-frequency sonophoresis [64,65], although significant SC lipid removal has also been reported [60] and may explain the increased skin permeability observed during, and after, low-frequency ultrasound application.

Mitragotri *et al.* reported the delivery of insulin (6000 Da), IFN-γ (17,000 Da) and erythropoietin (48,000 Da) *in vitro* across human epidermis using ultrasound (20 kHz, 100-ms pulses applied every second for 4 h). The insulin flux achieved was shown to be sufficient to treat a diabetic subject, assuming a transdermal patch area of 40 cm^2 containing insulin at a concentration of 100 U/ml [66,67] was used. However, despite this optimistic calculation, a sonophoretic insulin patch has not been developed, thus highlighting the challenge of extrapolating from *in vitro* studies to clinical scenarios. Similarly, a 50% reduction in blood glucose subsequent to low-frequency insulin sonophoresis in rats (**Figure 5** [68]), despite offering a valid proof-of-concept, does not guarantee that a similar approach will work in an adult diabetic patient.

On the whole, research into peptide and protein delivery using this technique has been limited, although a technological platform for potential applications clearly exists. An ultrasonic skin permeation device (SonoPrep®, Sontra Medical), awaiting FDA 510k clearance, enables rapid delivery of lidocaine and local skin anaesthesia within 5 min after a brief skin pretreatment (55 kHz, ~ 10 sec pulses) [69]. Nevertheless, the feasibility of such a system at higher intensities and over longer periods needs to be examined. Singer *et al.* [70] noted minimal urticarial reactions after low-intensity ultrasound, but higher-intensity sonophoresis produced significant thermal injuries similar to second-degree burns.

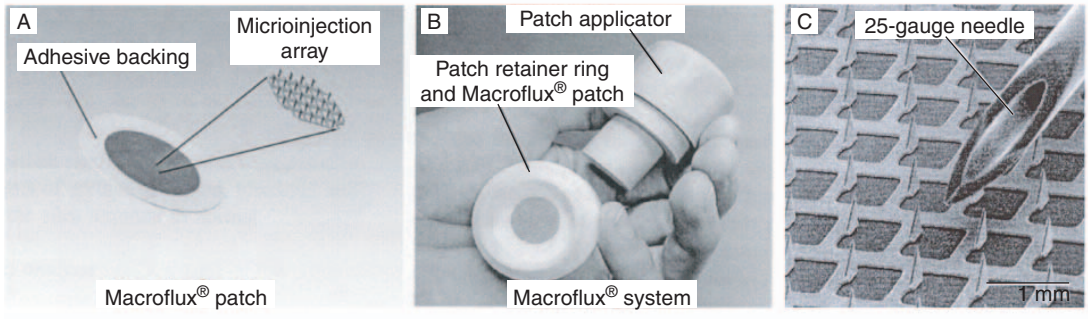

Figure 6. Macroflux® patch technology (Alza Corporation). A. The patch comprising the coated microneedle array affixed to an adhesive backing. **B.** The patch loaded on the disposable retainer ring and the reusable applicator. **C.** Scanning electron photomicrograph of an array of microprojections (L: 330 mm) and a conventional 25-gauge needle. **A** and **B** reprinted from CORMIER M, JOHNSON B, AMERI M *et al*.: Transdermal delivery of desmopressin using a coated microneedle array patch system. *J. Control. Release* (2004) **97**:503-511, with permission from Elsevier. **C** reprinted with permission from MATRIANO JA, CORMIER M, JOHNSON J *et al*.: Macroflux microprojection array patch technology: a new and efficient approach for intracutaneous immunization. *Pharm. Res.* (2002) **19**(1):63-70.

Hence, as with other energy-based technologies, sonophoresis exhibits a window of parameters within which safe application can be practiced. Whether or not this window encompasses macromolecular delivery remains to be seen.

6. Minimally invasive systems

Numerous minimally invasive strategies for transdermal drug delivery have been described, and have recently been reviewed by Down and Harvey [71]. Often, these technologies are the subject of patent claims, but their therapeutic utility remains unsubstantiated. In this section, we have selected systems which have been designed, in effect, to by-pass the skin barrier without blatant SC removal. Microneedles and velocity-based injectors, because of their ability to breach the SC, offer drug delivery platforms that may be suitable for higher molecular weight drugs.

6.1 Microneedles

Over the last few years advances in microelectronics have been innovatively applied to a variety of healthcare-related products, from miniaturised diagnostic tools (e.g., biosensors) to microdevices for therapeutic drug administration. Microneedles (µm dimensions) in various geometries and materials (silicon, metal and polymer) have been produced using recently developed microfabrication techniques. These microneedle arrays are applied to the skin surface such that they pierce the epidermis (devoid of nociceptors), creating microscopic holes through which molecules can be transported to reach the upper dermal layers. The microneedle arrays penetrate to the dermal microcirculation and allow systemic drug delivery, but are short enough to avoid stimulation of dermal nerves.

Solid microneedles either puncture the skin prior to the application of drug contained in a patch system, or are precoated with drug and then inserted into the skin. The first approach implies a two-step application procedure, which may seriously limit its ease-of-use, whereas the second presents the disadvantage of being surface-limited: the total amount of drug that can be loaded, and hence delivered, is limited by the total microneedle surface. Microneedles containing a hollow bore provide an alternative to solid structures and offer the possibility of transporting drugs through the interior of well-defined needles by diffusion or, for more rapid rates of delivery, by pressure-driven flow.

Both solid and hollow microneedles have been used to deliver insulin *in vivo* in diabetic rats. McAllister *et al.* demonstrated a 70% reduction in blood glucose 5 h after a 30-min microinfusion of insulin at a pressure of 14 psi using hollow microneedles [72]. Solid metal microneedles also increased insulin delivery and lowered blood glucose levels by as much as 80% when a 105-microneedle array was inserted into the skin for 10 min and removed before the topical application of an insulin solution for 4 h [73]. Such a reduction of blood glucose in rats corresponds to an insulin dose of 1.6 – 4.1 mU, a dose significantly lower than the ~ 36 U of insulin required by a typical diabetic patient each day (12 U t.i.d.) [67]. Furthermore, the significant lag time between application and therapeutic effect is also of some concern. Recent *in vivo* studies, in hairless guinea-pigs, investigated the transdermal delivery of desmopressin using the Macroflux® system (Alza Corporation) [74,75]. The Macroflux system (**Figure 6**) incorporates a 2-cm^2 array of titanium microneedles, which can be coated with drug for rapid bolus administration, or used in combination with a drug reservoir for continuous passive or iontophoretic applications [75]. When coated with desmopressin, these microneedles allowed pharmacologically relevant amounts of this synthetic peptide hormone to be delivered, with a bioavailability as high as 85% (c.f., oral and nasal bioavailability of 0.1 and 3.4%, respectively) in human volunteers (**Figure 7**) [76]. Average systemic deliveries of desmopressin ranged from 17 to 34 µg after a 5 or 15 min

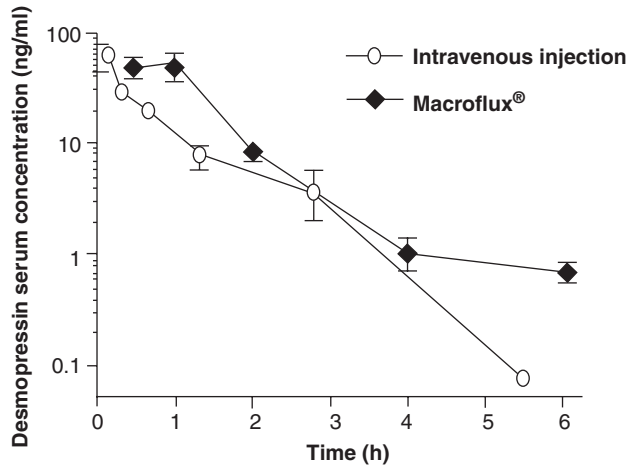

Figure 7. Comparison of serum desmopressin concentrations following administration by intravenous injection (11 µg), or via a coated microneedle array (82 µg) (microneedle array contact time = 5 min). Reprinted from CORMIER M, JOHNSON B, AMERI M *et al.*: Transdermal delivery of desmopressin using a coated microneedle array patch system. *J. Control. Release* (2004) **97**:503-511 with permission from Elsevier.

application time, which is significantly more than the 1 – 4 µg/day recommended by subcutaneous, intramuscular or intravenous injection in the management of primary nocturnal enuresis. This device has also demonstrated promising results with vaccines [77].

Microneedles have been described as painless, inducing neither erythema nor oedema [78,79]. In addition, the µm-scale holes produced are significantly smaller than those created by hypodermic needles [80]. However, the reversibility and the consequences of chronic applications of these arrays remain to be studied. The immediate concern with this technology is the possibility of fractured needle fragments remaining in the skin, although it has been reported that most silicon microneedles remained intact after insertion into skin [78]. Nonetheless, the potential risk of residual material in the skin after treatment needs to be examined. In this respect, metal and polymer microneedles offer significant advantages. In addition to being more robust, less expensive and easily scalable for mass production, many metal and polymer (especially biodegradable) materials have established safety records in medical devices, whereas silicon is a new and relatively untested biomaterial [72]. Microneedles, which are essentially a hybrid of hypodermic needles and transdermal patches, provide an interesting and promising alternative, which is currently being pursued by several companies and has resulted in an impressive number of patents.

6.2 Velocity-based technologies

A jet injector produces a high-velocity jet (> 100 m/s) that penetrates the skin and delivers drugs into the epidermis, intradermally, subcutaneously or intramuscularly by means of a compression spring or compressed air [81]. Most commercial devices produce a single jet for drug delivery through an orifice of ~ 150 µm in diameter. These systems resemble a pen, as illustrated in **Figure 8**.

Interest in these systems as an alternative to the routine use of needles in vaccination has partly stemmed from the increasing incidence of injection-associated, blood-borne pathogen infections (hepatitis B, hepatitis C and human immunodeficiency viruses) in developing countries. An exhaustive, web-based document [201] underlines the attention attracted by this technology.

One category of jet injectors has been developed for the delivery of liquid protein formulations. **Figure 9** illustrates the similarity of a profile obtained with one such system to that measured after a conventional needle injection in a human study. Numerous devices have been on the market for several years. As shown in **Table 1**, most of the marketed devices are dedicated to insulin and human growth hormone (hGH). Today, compounds such as pegylated IFN-α_{2a} (Pegasys®; Hoffmann-La Roche Inc.), erythropoietin and antibodies are also receiving attention for delivery via this system [82].

A second category of jet injectors allows transdermal powdered delivery (TPD), for which the therapeutic compound is formulated as a powder. Fine drug particles (20 – 100 µm in diameter) are thus accelerated in a supersonic flow of helium gas to penetrate the outer layers of the skin. The powder injection delivery of salmon calcitonin (s-CT) was studied in rabbits *in vivo* using the PowderJect® device (PowderJect Pharmaceuticals plc, recently acquired by Chiron Corporation). The administration of 1 mg of powder containing 40 µg of s-CT using a pressure of 60 bar led to an 11% decrease in serum calcium concentration [83]. When the same system was used to deliver human insulin to rats, blood glucose concentration was found to decrease by ~ 40% [83]. A corresponding effect in humans would require significant scaling-up, which remains to be demonstrated. This technology also allows targeting of DNA and protein vaccines to the

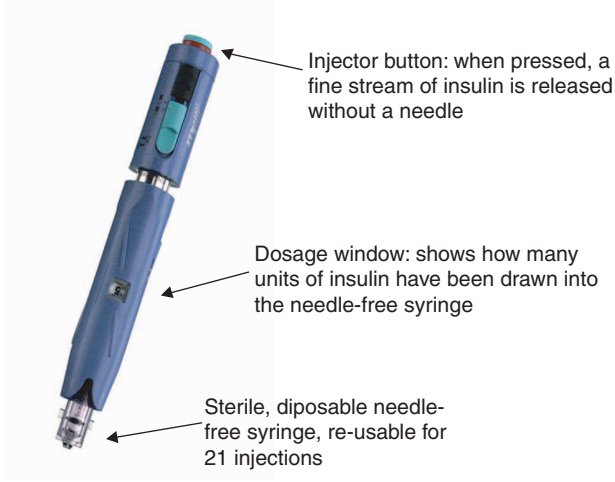

Figure 8. Medi-Jector Vision™ (Antares Pharma, Inc.) needle-free insulin injection system. Reproduced with permission from Antares Pharma, Inc.

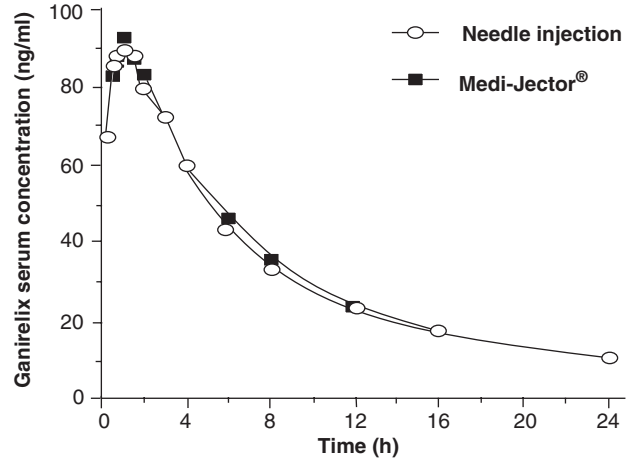

Figure 9. Comparison of serum ganirelix concentrations following administration of Orgalutran® by subcutaneous injection or via the Medi-Jector® device. Adapted from OBERYÉ J, MANNAERTS B, HUISMAN J *et al.*: Local tolerance, pharmacokinetics, and dynamics of ganirelix (orgalutran) administration by Medi-Jector compared to conventional needle injections. *Hum. Reprod.* (2000) **15**(2):245-249. © European Society of Human Reproduction and Embryology. Reproduced with permission of Oxford University Press/Human Reproduction.

Table 1. A representative selection of commercially available needle-free jet injectors.

Device trade name	Marketed by	Drug (proprietary name)	Ref.
Vitajet™	Bioject	Insulin	[81]
Biojector®	Bioject	Vaccines and other liquid medications	
Cool.click™	Serono	hGH (Saizen®)	[117]
Serojet™	Serono	hGH (Serostim®)	
Medi-jector®	Antares Pharma	Insulin, hGH, ganirelix (Orgalutran®)	[116,118]
Zomajet®	Ferring	hGH	[88]
Genotropin ZipTip™	Pfizer	hGH (Genotropin®)	[87]

hGH: Human growth hormone.

epidermis, a skin layer that is populated by numerous antigen-presenting cells, and, therefore, offers the possibility of needle-free immunisation [84].

Although dry powder formulations are better suited to storage than solutions, a significant limitation of the PowderJect technology is the upper limit on the dose that can be delivered, which is ~ 6 mg. Moreover, the powder must survive the high stress of a gas jet within the device and the ballistic impact with the skin at supersonic velocities. Finally, the dispersed fine particles must then dissolve and diffuse into the skin in order to act locally or to reach the systemic circulation.

With the exception of the PowderJect system, which was reported as being painless during the course of Phase I and Phase II clinical studies [83], jet injectors are recognised for needle injection, the superiority of jet injectors in terms of comfort and compliance is, according to several studies, far from obvious [86-91]. Hence, the undisputable advantage of this technology, compared with conventional syringe injection, lies in the absence of a (visible) needle, which may be of benefit to children and needle-phobic patients, and in mass injection programmes in which the risk of contamination may be an important concern.

Since their introduction to the market 8 years ago, jet injectors have not revolutionised the delivery of insulin in diabetic patients, who for the most part continue to use conventional injection systems. This suggests that this innovative technology may not be the choice alternative to the parenteral route for delivering peptides and proteins. However, it seems likely to play a key role in tomorrow's vaccination strategies.

7. Stratum corneum ablation

The simplest method for overcoming the barrier imposed by the SC is to remove it. This can be achieved, for instance, by repeated application of adhesive tape to the skin surface. However, for a number of reasons, including those of convenience, reproducibility and patient compliance, it is difficult to envisage the routine clinical use of such an approach. Laser-assisted ablation and SC ablation by suctioning are perhaps more realistic approaches, but are also likely to be associated with patient compliance issues. Alternatively, more recent technologies currently under investigation include microscissioning (see Section 9.3) and radiofrequency (RF) thermal ablation.

7.1 Suction ablation

Suction ablation uses a vacuum to produce a small blister (5 – 6 mm in diameter), the upper surface of which is excised to reveal a portal for entry of drugs into the dermal circulation [71]. The feasibility of this technique was tested in seven healthy volunteers using the antidiuretic peptide 1-deamino-8-D-arginine vasopressin (DDAVP), in whom the bioavailability was reported to approach 100% [92]. In a separate study using the oxytocin antagonist antocin, therapeutic blood levels were measured in healthy volunteers 1 h after administration [93]. This technology has resulted in a commercial product, Cellpatch® (Epiport Pain Relief AB), which incorporates all the components of the process: suction device, epidermatome (to remove the blister) and a drug reservoir. Clinical studies have tested the feasibility of transepidermal morphine delivery by this methodology in normal healthy volunteers [94] and in postoperative patients [95]. The studies reported an absence of pain (possibly due to the concomitant delivery of morphine), erythema and scar formation. Regeneration of the epidermis occurred 1 week after removal of the system. However, the vacuum removal of the epithelium caused pronounced hyperaemia in the de-epithelialised dermis and the sites showed slight, fading pigmentation 3 months after treatment, thus suggesting that this procedure may not be appropriate for the treatment of chronic disease. In view of this drawback, and given the absence of published work since 1996, suction ablation is unlikely to be a technology of choice in the near future.

7.2 Laser ablation

In this approach, the high energy of the laser creates pores in the skin that permit the transit of drug through the SC from a topically applied patch or gel, for example [71]. There are two optimal wavelengths at which skin ablation can be achieved: a wavelength absorbed by tissue proteins (2940 nm) and one absorbed by tissue water (mid-infrared: 2790 nm). During laser irradiation, the energy is absorbed by the components of the skin in the form of vibrational heating. Water within the irradiated area of the skin rapidly reaches its boiling point, and the resulting vapour pressure elicits a microexplosion that results in ablation as the tissue vaporises. The rapid loss of energy from the ablated site protects the surrounding skin tissues from heat-induced damage. The level of energy imparted to the skin permits removal of the SC in a controllable fashion.

The Erbium:YAG laser (2940 nm), currently used in plastic surgery for the resurfacing of rhytides, scars, photodamage and melasma [96], was demonstrated to greatly enhance 5-fluorouracil permeation across mouse skin *in vitro* [97]. The only study performed with a peptide showed a 2.1-fold increase in IFN-γ transport across pig skin using an Erbium:YSGG laser (2790 nm) [98].

Although laser-assisted drug delivery may be technically feasible, many questions regarding safety remain unanswered [71]. For the moment, the utility of this technology is likely to be limited to niche applications within hospital settings, notably because of the elevated cost of medical lasers.

7.3 Radiofrequency thermal ablation

RF thermal ablation is a well-known and effective technology for electrosurgery and ablation of malignant tissues. A thin electrode is placed directly into the tumour; application of RF energy results in the passage of an alternating frequency current from the tip of the electrode into the surrounding tissue. The movement of ions, which attempt to follow the change in the direction of the alternating current, results in frictional heating of the tissue, to produce coagulative necrosis and cell ablation [99]. This technique has only recently been adapted for use as a physical method to enhance drug transport across the skin. A closely spaced array of tiny electrodes is placed against the skin while an alternating current at radio frequency is applied to each of the microelectrodes. This forms microchannels in the outer layer of the skin through the ablation of cells. The ViaDerm™ RF-microchannel generator (Transpharma Ltd) consists of 140 stainless steel electrodes (length: 100 µm; diameter: 40 µm) spaced 1 mm apart. When a certain pressure is applied to the device in contact with the skin, the RF-generator is activated momentarily, resulting in thermal ablation. Percutaneous penetration studies were performed with granisetron hydrochloride and sodium diclofenac *in vivo* in rats, and *in vitro* using full thickness porcine ear skin. The apparent permeability coefficients obtained for granisetron and diclofenac transport through porcine skin were 7.1- and 3.8-times higher for RF-treated skin compared with intact skin, respectively [99]. Experiments performed on rats *in vivo* with bioactive hGH showed a relative bioavailability of ~ 80% compared with subcutaneous injection [100].

A human safety study on 20 healthy, adult volunteers, reported slight erythema (0.75 out of 8) and negligible pain (5 out of 100) as measured by the Draize irritation index and Visual Analogue Scale score, respectively. An RF thermal ablation device has also been developed by Altea Therapeutics (PassPort™ Patch, GA, USA) and is currently in Phase I human clinical trials in the US.

8. Combination strategies

An exhaustive inventory of all the possible combinations and implicated synergistic mechanisms with respect to transdermal enhancement offers subject matter for several articles and is clearly outside the scope of this review. The reader is referred to a comprehensive summary of these combination strategies with respect to their efficacy and mechanisms [101]. As the enhancement effect can be synergistic, this has added to the interest in the field. Nearly all combinations have been tested, at least *in vitro*, and some of these have been applied to peptide drug delivery. These include the combination of iontophoresis with chemical enhancers [102,103], with electroporation [40,104] and with jet injection pretreatment [105]. The Medipad (Elan Pharmaceutical Technologies) is a hybrid

system coupling iontophoretic delivery with shallow SC puncture [106].

The use of two or more technologies, with different mechanisms of action, permits the same effect to be achieved with safer levels of the 'active driving force' (e.g., lower currents or reduced levels of chemical enhancers). However, paradoxically, this synergy may also result in an increased toxicity: consider the combination of chemical enhancers with iontophoresis or ultrasound. The use of an electrically assisted method could increase the rate and extent of delivery of the chemical enhancer into the skin, or induce a deeper penetration into the tissues, with concomitantly increased skin irritation. Hence, in addition to the evaluation of the practicality of certain dual technologies, which may lead to relatively complex devices, safety must be validated *in vivo* and in human volunteers before combination technologies can 'take off' and be considered as realistic delivery platforms.

9. Novel techniques in early development

The transdermal enhancement technologies presented in this section are relatively novel approaches in early development. Although they have yet to be validated with respect to peptide and protein delivery, they are included here because of their ability to overcome the SC barrier function to a greater extent than many conventional methods.

9.1 Photomechanical waves

Photomechanical waves (PWs) are broadband compressive waves generated by intense laser radiation [107]. A PW delivery device consists of a drug reservoir backed with a laser target material (e.g., polystyrene). This system is placed on the skin and the laser is applied to the target. The energy of the laser is strongly absorbed by the target, resulting in the formation of photomechanical waves, which are hypothesised to transiently permeabilise the SC. Drug diffuses passively through the channels momentarily created. The mechanism of channel formation remains to be elucidated, but it is known that the effects of PWs are due to mechanical forces [108].

Different molecules and even microspheres have been measured in the skin layers *in vivo* after PW application [109-112]; however, interestingly, this technique does not appear to permeabilise the SC *in vitro*. An isolated report describes successful insulin delivery in rats *in vivo* (~ 80% decrease in blood glucose level), but after pretreatment with an anionic surfactant [113]. Clearly, this technique is still very much in its infancy and is unlikely to be a realistic contender for transdermal peptide delivery.

9.2 Heat-assisted drug delivery

Controlled heat-assisted drug delivery (CHADD) is the basis of an innovative patch consisting of a layer containing a heat-generating chemical component and a perforated cover membrane. When the package is opened, air flows at a controlled rate through the holes in the cover membrane into the heating mixture and initiates a chemical reaction that spontaneously produces heat. Heat generated within the patch increases skin temperature and thereby drug penetration rates across the skin. According to the manufacturers, the temperature and duration of the reaction can be controlled by the size and number of holes in the cover membrane and the precise composition and quantity of the chemical components. S-Caine Patch™ (Zars, Inc.) is a new system for anaesthetic delivery [114], which uses the CHADD technology and has successfully completed Phase III trials. This technology has not yet been applied to peptide and proteins although systems containing a benzodiazepine and a 5-hydroxytryptamine receptor (5-HT_3) antagonist are currently in preclinical development. One obvious concern with respect to peptides and proteins is their thermal instability, which could severely limit the application of this technology.

9.3 Microscissioning

Microscissioning entails the use of sharp particles to scize defined areas of the skin. Techniques using a combination of momentum transfer and scizing are well known in cosmetic dermatology. The relatively hard, roughened SC and epidermis resulting from ageing processes can be removed by moderate velocity, sharp particles impinging obliquely against the skin surface. However, only one published study reports the application of this technology to transdermal delivery. Aluminium oxide particles (10 – 70 μm) were accelerated in a nitrogen stream under a pressure of 552 kPa and directed towards the inner wrist of volunteers, after which the site was treated with lidocaine. The experiments, performed on only two adult subjects, demonstrated full anaesthesia around the site within 3 min, whereas topical application without the microconduit required ~ 1.5 h [115]. This technology is very experimental and requires considerable work, notably in terms of safety. The presence of microparticulate debris in the skin as well as blood in the 200 μm diameter microconduit (although suggested to be useful for the purposes of clinical monitoring of analytes) points to the need for further investigation.

10. Conclusions

The proliferation of research activity in the field of transdermal drug delivery serves to highlight the pressing need for alternatives to the conventional invasive administration of peptides and proteins via needles and syringes. However, regardless of how attractive any new drug delivery concept may appear to be, it must not only deliver therapeutically realistic levels of drug to the target site, but must also prove its clinical superiority over conventional injections with respect to long-term safety, patient compliance, ease-of-use, impact on protein quality (physical, chemical and biological stability) and, of course, commercial viability (high manufacturing costs would result in a non-viable product).

It is perhaps this lack of clinical superiority over injectables which prevents the velocity-based approach from being the

technology of choice in transdermal peptide and protein drug delivery. Iontophoresis, which offers the unique advantage of tight control over the delivery kinetics, is expected to gain momentum now that devices are being commercialised. However, one of the largest subclasses of the protein-based therapeutics developed by the biotechnology industry is that of monoclonal antibodies, which have been anticipated to represent 30% of pharmaceutical sales by 2007 [82]. Given their size, iontophoresis, which is more suitable to peptide drugs, is certainly not the method of choice to deliver such large macromolecules. Hence, there is an evident need for developing complementary technologies such as microneedles, which represent an excellent example of interdisciplinary research.

With this large range of technologies currently under development, one can hope that in the not too distant future there will be viable transdermal alternatives for the administration of protein- and peptide-based therapeutics, thus reducing the need to resort to conventional injections.

11. Expert opinion

The oral route is undoubtedly the preferred route of drug administration for most therapeutic agents and ideally this would also be the case for peptide- and protein-based drugs. However, the gastrointestinal tract has evolved to break down macromolecules, including polypeptides, and facilitate the absorption of their constituents. Moreover, upon absorption, the hepatic portal vein takes these products to the liver, which is the primary site of biotransformation and conversion of these molecules and molecular fragments into products that can be easily eliminated. Approaches such as microemulsions, covalent chemical modification and carrier-mediated systems have enabled the oral administration of macromolecules to move forward from the proof-of-concept stage. However, unless a peptide (or protein) can be protected from, or rendered resistant to, the catabolic activity in the gastrointestinal tract, prohibitively large amounts of (expensive) therapeutic agent will have to be delivered to achieve the desired pharmacological effect. One approach is to use peptides that contain D-amino acids and, in general, oral delivery may be more feasible for smaller peptides where there is no significant tertiary structure that must be retained. Based on this logic, it is easier to explain the delivery of desmopressin by the oral route and the recent development of a tablet form for the treatment of nocturnal enuresis. However, with respect to the oral delivery of larger proteins with more complex structures, the likelihood of an oral formulation on the market place is far from the horizon.

Some peptide drugs, for example, nafarelin, are currently available for administration via the nasal route. However, the bioavailability of therapeutic peptides administered by this route is usually < 5%, thus limiting its appeal and subsequent development. Pulmonary delivery has aroused much interest and a great deal of effort has been devoted to developing pulmonary systems for the delivery of insulin. The development of inhalation systems for targeted local drug delivery in the respiratory tract has progressed considerably with the availability of recombinant human deoxyribonuclease for the treatment of cystic fibrosis. Such topical delivery is frequently to be preferred because systemic administration may not achieve the desired drug levels at the disease site, or because high systemic levels of the drugs (e.g., growth factors that are required for a local effect at the target tissue) often cause unwanted systemic toxicity. In addition to low bioavailability, which, as with nasal delivery, can necessitate large and unacceptable drug doses, pulmonary delivery presents paradoxically another dilemma: the large alveolar surface area coupled with the extremely fine nature of the epithelium can result in an extremely rapid absorption with the risk of a large bolus effect. Hence, it may be argued that the pulmonary delivery of proteins may be restricted to therapeutics that do not induce systemic side effects at high peak serum concentrations or local tissue reactions at the site of absorption.

The application of therapeutic agents to the skin was first realised for the treatment of dermatological diseases. As a significant proportion of the population suffers from dermatological disorders, research in this field is expected to steadily continue. Given some of the similarities in formulation strategies, transdermal delivery is expected to benefit from advances in localised dermal delivery. However, the skin is a lipidic barrier membrane and as such, it does not lend itself to the facile delivery of hydrophilic or charged peptides and proteins. Owing to the efficient barrier function of the skin, it is difficult to envisage the delivery of peptides with molecular weights > 5 – 10 kDa at therapeutic input rates by truly non-invasive methods, such as iontophoresis or sonophoresis, which do not set out to puncture the membrane and introduce pores or similar transport channels. It is certainly true that iontophoresis is well suited to peptide delivery owing to the controlled input kinetics that it alone can provide. For the transdermal delivery of larger macromolecules, we will have to resort to the minimally invasive techniques such as microneedles and other related technologies that abrade or remove the SC. These methods certainly open the door to the delivery of a much wider range of macromolecules. The numerous jet injectors also offer a different way into the body and are finding a useful niche for the non-invasive delivery of vaccines and this area, which is also open to exploitation by the microneedle platforms, may prove to be very fruitful. These different delivery technologies provide complementary techniques for the administration of different types of peptides and proteins and there are clearly many opportunities and unmet needs to be addressed. For all of these platforms, the key to success will always be careful selection of the peptide/protein therapeutic agent. Given the advantages of the transdermal route, one can hope that the rational design and synthesis of therapeutics optimised for transdermal delivery with the correct mix of physicochemical properties is not too far off. It is certain that only such close collaboration between medicinal chemists and formulation scientists will enable the successful delivery of tomorrow's drugs.

Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. CEVC G: Drug delivery across the skin. *Expert Opin. Investig. Drugs* (1997) **6**(12):1887-1937.
2. NAIK A, KALIA YN, GUY RH: Transdermal drug delivery: overcoming the skin's barrier function. *Pharm. Sci. Technol. Today* (2000) **3**(9):318-326.
3. SCHEUPLEIN RJ, BLANK IH: Permeability of the skin. *Physiol. Rev.* (1971) **51**(4):702-747.
4. GUY RH: Current status and future prospects of transdermal drug delivery. *Pharm. Res.* (1996) **13**(12):1765-1769.
5. CHOI H-K, FLYNN GL, AMIDON GL: Transdermal delivery of bioactive peptides: The effect of *N*-decylmethyl sulfoxide, pH, and inhibitors on enkephalin metabolism and transport. *Pharm. Res.* (1990) 7(11):1099-1106.
6. BANERJEE PS, RITSCHEL WA: Transdermal permeation of vasopressin. I. Influence of pH, concentration, shaving and surfactant on *in vitro* permeation. *Int. J. Pharm.* (1989) **49**:189-197.
7. SHAH PK, BORCHARDT RT: A comparison of peptidase activities and peptide metabolism in cultured mouse keratinocytes and neonatal mouse epidermis. *Pharm. Res.* (1991) **8**:70-75.
8. FRÄKI JE, LAZARUS GS, HOPSU-HAVU VK: Protein catabolism in the skin. In: *Biochemistry and Physiology of the Skin.* Goldsmith LA (Ed.), Oxford University Press, New York, USA (1983):338-362.
9. MARTIN RJ, DENYER SP, HADGRAFT J: Skin metabolism of topically applied compounds. *Int. J. Pharm.* (1987) **39**:23-32.
10. MORIMOTO K, IWAKURA Y, NAKATANI E *et al.*: Effects of proteolytic enzyme inhibitors as absorption enhancers on the transdermal iontophoretic delivery of calcitonin in rats. *J. Pharm. Pharmacol.* (1992) **44**(3):216-218.
11. MORIMOTO K, IWAKURA Y, MIYAZAKI M *et al.*: Effects of proteolytic enzyme inhibitors of enhancement of transdermal iontophoretic delivery of vasopressin and an analogue in rats. *Int. J. Pharm.* (1992) **81**:119-125.
12. RIESZ P, KONDO T: Free radical formation induced by ultrasound and its biological implications. *Free Radical Biol. Med.* (1992) **13**:247-270.
13. HIKIMA T, HIRAI Y, TOJO K: The effect of ultrasound application on skin metabolism of prednisolone 21-acetate. *Pharm. Res.* (1998) **15**:1680-1683.
14. SMITH EW, MAIBACH HI: *Percutaneous Penetration Enhancers.* CRC Press, Boca Raton, Florida, USA (1995).
15. WILLIAMS AC, BARRY BW: Penetration enhancers. *Adv. Drug Deliv. Rev.* (2004) **56**:603-618.
16. MARRO D, DELGADO-CHARRO MB, GUY RH: Peptides and proteins – transdermal absorption. In: *Encyclopedia of Pharmaceutical Technology.* J Swarbrick, JC Boylan (Eds), Marcel Dekker, New York, USA (2002):2125-2140.
17. LASHMAR UT, HADGRAFT J, THOMAS N: Topical application of penetration enhancers to the skin of nude mice: a histopathological study. *J. Pharm. Pharmacol.* (1989) **41**(2):118-122.
18. GOZES I, RESHEF A, SALAH D *et al.*: Stearyl-norleucine-vasoactive intestinal peptide (VIP): a novel VIP analog for noninvasive impotence treatment. *Endocrinology* (1994) **134**:2121-2125.
19. FOLDVARI M, ATTAH-POKU S, HUGHES H *et al.*: Palmitoyl derivatives of interferon alpha: potential for cutaneous delivery. *J. Pharm. Sci.* (1998) **87**(10):1203-1208.
20. WARANUCH N, RAMACHANDRAN C, WEINER N: Controlled topical delivery of cyclosporin A from nonionic liposomal formulations: mechanistic aspects. *J. Liposome Res.* (1998) **8**(2):225-238.
21. FOLDVARI M, BACA-ESTRADA ME, HE Z *et al.*: Dermal and transdermal delivery of protein pharmaceuticals: lipid-based delivery systems for interferon α. *Biotechnol. Appl. Biochem.* (1999) **30**:129-137.
22. CEVC G: Transferosomes, liposomes and other lipid suspensions on the skin: permeation enhancement, vesicle penetration, and transdermal drug delivery. *Crit. Rev.Therap. Drug Carrier Syst.* (1996) **13**:257-388.
23. CEVC G, GEBAUER D, STIEBER J *et al.*: Ultraflexible vesicles, transfersomes, have an extremely low pore penetration resistance and transport therapeutic amounts of insulin across the intact mammalian skin. *Biochim. Biophys. Acta* (1998) **1368**:201-215.
24. TOUITOU E, GODIN B, WEISS C: Enhanced delivery of drugs into and across the skin by ethosomal carriers. *Drug Dev. Res.* (2000) **50**:406-415.
25. TOUITOU E, DAYAN N, BERGELSON L *et al.*: Ethosomes – novel vesicular carriers for enhanced delivery: characterization and skin penetration properties. *J. Control. Release* (2000) **65**:403-418.
26. DAYAN N, TOUITOU E: Carriers for skin delivery of trihexyphenidyl HCl: ethosomes vs liposomes. *Biomaterials* (2000) **21**:1879-1885.
27. JAIN S, UMAMAHESHWARI RB, BHADRA D *et al.*: Ethosomes: a novel vesicular carrier for enhanced transdermal delivery of an anti-HIV agent. *Ind. J. Pharma. Sci.* (2004) **66**(1):72-81.
28. DKEIDEK I, TOUITOU E: Transdermal absorption of polypeptides. *AAPS Pharm. Sci.* (1999) **1**:S202.
29. BANGA AK, KATAKAM M, MITRA R: Transdermal iontophoretic delivery and degradation of vasopressin across human cadaver skin. *Int. J. Pharm.* (1995) **166**:211-216.
30. SARPOTDAR PPDCR, LIVERSIDGE GG, STERNSON LA: Facilitated iontophoretic delivery of thyrotropin releasing hormone (TRH) across cadaver skin by optimization of formulation variables. *Pharm. Res.* (1989) **6**(Suppl.):S107.
31. CLEMESSY M, COUARRAZE G, BEVAN B *et al.*: Mechanisms involved in iontophoretic transport of angiotensin. *Pharm. Res.* (1995) **12**(7):998-1002.
32. LAU DTW, SHARKEY JW, PETRYK L *et al.*: Effect of current magnitude and drug concentration on iontophoretic delivery of octreotide acetate (sandostatin) in the rabbit. *Pharm. Res.* (1994) **11**(12):1742-1746.
33. HEIT MC, MONTEIRO-RIVIERE NA, JAYES FL *et al.*: Transdermal iontophoretic delivery of luteinzing hormone releasing hormone (LHRH): effect of repeated administration. *Pharm. Res.* (1994) **11**(7):1000-1003.
34. MILLER LL, KOLASKIE CJ, SMITH GA *et al.*: Transdermal iontophoresis of gonadotropin releasing hormone (LHRH) and two analogues. *J. Pharm. Sci.* (1990) **79**(6):490-493.

35. KNOBLAUCH P, MOLL F: *In vitro* pulsatile and continuous transdermal delivery of buserelin by iontophoresis. *J. Control. Release* (1993) **26**:203-212.
36. RODRIGUEZ BAYON AM, GUY RH: Iontophoresis of nafarelin across human skin *in vitro*. *Pharm. Res.* (1996) **13**(5):798-800.
37. MEYER BR, KREIS W, ESCHBACHE J *et al.*: Successful transdermal administration of therapeutic doses of a polypeptide to normal human volunteers. *Clin. Pharmacol. Ther.* (1988) **44**(6):607-612.
38. CRAAN-VAN HINSBERG WH, BAX L, FLINTERMAN NH *et al.*: Iontophoresis of a model peptide across human skin *in vitro*: effects of iontophoresis protocol, pH, and ionic strength on peptide flux and skin impedance. *Pharm. Res.* (1994) **11**(9):1296-1300.
39. THYSMAN S, HANCHARD C, PRÉAT V: Human calcitonin delivery in rats by iontophoresis. *J. Pharm. Pharmacol.* (1994) **46**:725-730.
40. MEDI BM, SINGH J: Electronically facilitated transdermal delivery of human parathyroid hormone (1-34). *Int. J. Pharm.* (2003) **263**:25-33.
41. SAGE BH: Insulin iontophoresis. In: *Protein Delivery – Physical Systems.* LM Sanders, RW Hendren (Eds), Plenum Publishing Corporation, New York, USA (1997):319-341.
42. GUY RH, KALIA YN, DELGADO-CHARRO MB *et al.*: Iontophoresis: electrorepulsion and electroosmosis. *J. Control. Release* (2000) **64**(1-3):129-132.
43. HIRVONEN J, GUY RH: Iontophoretic delivery across the skin: electroosmosis and its modulation by drug substances. *Pharm. Res.* (1997) **14**(9):1258-1263.
44. DELGADO-CHARRO MB, GUY RH: Characterisation of convective solvent flow during iontophoresis. *Pharm. Res.* (1994) **11**(7):929-935.
45. DELGADO-CHARRO MB, GUY RH: Iontophoretic delivery of nafarelin across the skin. *Int. J. Pharm.* (1995) **117**:165-172.
46. HIRVONEN J, KALIA YN, GUY RH: Transdermal delivery of peptides by iontophoresis. *Nat. Biotechnol.* (1996) **14**(13):1710-1713.
47. HIRVONEN J, GUY RH: Transdermal iontophoresis: modulation of electroosmosis by polypeptides. *J. Control. Release* (1998) **50**:283-289.
48. HOOGSTRAATE AJ, SRINIVASAN V, SIMS SM *et al.*: Iontophoretic enhancement of peptides: behaviour of leuprolide versus model permeants. *J. Control. Release* (1994) **31**:41-47.
49. ROBERTS MS, KREIS W, ESCHBACHE J *et al.*: Transdermal versus subcutaneous leuprolide: A comparison of acute pharmacodynamic effect. *Clin. Pharmacol. Ther.* (1990) **48**(4):340-345.
50. KUMAR S, CHAR H, PATEL S *et al.*: *In vivo* transdermal iontophoretic delivery of growth hormone releasing factor GRF (1-44) in hairless guinea pigs. *J. Control. Release* (1992) **18**:213-220.
51. LANGKJAER L, BRANGE J, GRODSKY GM *et al.*: Iontophoresis of monomeric insulin analogues *in vitro*: effects of insulin charge and skin pretreatment. *J. Control. Release* (1998) **51**:47-56.
52. KALIA YN, NAIK A, GARRISON J *et al.*: Iontophoretic drug delivery. *Adv. Drug Deliv. Rev.* (2004) **56**(5):619-658.

• **A comprehensive review of iontophoretic mechanisms and therapeutic applications. Part of a special issue of *Advanced Drug Delivery Reviews* (2004) 56(5) devoted to transdermal drug delivery technologies (e.g.** [53,81]).

53. DENET A-R, VANBEVER R, PRÉAT V: Skin electroporation for transdermal and topical delivery. *Adv. Drug Deliv. Rev.* (2004) **56**:659-674.
54. SEN A, DALY ME, HUI SW: Transdermal insulin delivery using lipid enhanced electroporation. *Biochim. Biophys. Acta* (2002) **1564**:5-8.
55. PRAUSNITZ MR, EDELMAN ER, ELAZER R *et al.*: Transdermal delivery of heparin by skin electroporation. *Biotechnology* (1995) **13**(11):1205-1209.
56. SERSA G, CEMAZAR M, RUDOLF Z: Electrochemotherapy: advantages and drawbacks in treatment of cancer patients. *Cancer Ther.* (2003) **1**:133-142.
57. KOST J: Phonophoresis. In: *Electronically Controlled Drug Delivery.* B Berner, SM Dinh (Eds), CRC Press, Boca Raton, Florida, USA (1998):215-228.
58. TEZEL A, SENS A, TUSCHERER J *et al.*: Frequency dependence of sonophoresis. *Pharm. Res.* (2001) **18**:1694-1700.
59. CHIEN YW, SIDDIQUI O, SHI W-M *et al.*: Direct current iontophoretic transdermal delivery of peptide and protein drugs. *J. Pharm. Sci.* (1989) **78**(5):376-383.
60. ALVAREZ-ROMAN R, MERINO G, KALIA YN *et al.*: Skin permeability enhancement by low frequency sonophoresis: lipid extraction and transport pathways. *J. Pharm. Sci.* (2003) **92**(6):1138-1146.
61. MERINO G, KALIA YN, GUY RH: Ultrasound-enhanced transdermal transport. *J. Pharm. Sci.* (2003) **92**(6):1125-1137.
62. BOMMANNAN D, MENON GK, OKUYAMA H *et al.*: Sonophoresis: II. Examination of the mechanism(s) of ultrasound-enhanced transdermal drug delivery. *Pharm. Res.* (1992) **9**:1043-1047.
63. MITRAGOTRI S, EDWARDS D, BLANKSCHTEIN D *et al.*: A mechanistic study of ultrasonically enhanced transdermal drug delivery. *J. Pharm. Sci.* (1995) **84**(6):697-706.
64. TEZEL A, SENS A, MITRAGOTRI S: Investigations of the role of cavitation in low-frequency sonophoresis using acoustic spectroscopy. *J. Pharm. Sci.* (2002) **91**:444-453.
65. TEZEL A, MITRAGOTRI S: Interactions of inertial cavitation colapses with stratum corneum lipid bilayers. *Biophys. J.* (2003) **85**:1-11.
66. MITRAGOTRI S, BLANKSCHTEIN D, LANGER R: Ultrasound-mediated transdermal protein delivery. *Science* (1995) **269**:850-853.
67. KRALL LP: *World Book of Diabetes in Practice.* Elsevier, New York, USA (1988).
68. BOUCAUD A, GARRIGUE MA, MACHET L *et al.*: Effect of sonication parameters on transdermal delivery of insulin to hairless rats. *J. Control. Release* (2002) **81**:113-119.
69. KATZ NP, SHAPIRO DE, HERRMANN TE *et al.*: Rapid onset of cutaneous anesthesia with EMLA cream after pretreatment with a new ultrasound-emitting device. *Anesth. Analg.* (2004) **98**:371-376.
70. SINGER AJ, HOMAN CS, CHURCH AL *et al.*: Low-frequency sonophoresis: pathologic and thermal effects in dogs. *Acad. Emerg. Med.* (1998) **5**(1):35-40.
71. DOWN JA, HARVEY NG: Minimally invasive systems for transdermal drug delivery. In: *Transdermal Drug Delivery.* RH Guy, J Hadgraft (Eds), Marcel Dekker, New York, USA (2003):327-359.

•• **A thorough review of minimally invasive technologies covering the devices, potential applications and limitations.**

72. MCALLISTER DV, WANG PM, DAVIS SP *et al.*: Microfabricated needles for transdermal delivery of macromolecules and nanoparticles: Fabrication methods and transport studies. *Proc. Natl. Acad. Sci. USA* (2003) **100**(24):13755-13760.
73. MARTANTO W, DAVIS SP, HOLIDAY NR *et al.*: Transdermal delivery of insulin using microneedles *in vivo*. *Pharm. Res.* (2004) **21**(6):947-952.
74. CORMIER M, JOHNSON B, AMERI M *et al.*: Transdermal delivery of desmopressin using a coated microneedle array patch system. *J. Control. Release* (2004) **97**:503-511.
75. CORMIER M, DADDONA P: Macroflux® technology for transdermal delivery of therapeutic proteins and vaccines. In: *Modified Release Drug Delivery Systems.* MJ Rathbone, J Hadgraft, MS Roberts MS (Eds), Marcel Dekker, New York, USA (2002):589-598.
76. FJELLESTAD-PAULSEN A, HOGLUND P, LUNDIN S *et al.*: Pharmacokinetics of 1-deamino-8-D-arginine vasopressin after various routes of administration in healthy volunteers. *Clin. Endocrinol.* (1993) **38**(2):177-182.
77. MATRIANO JA, CORMIER M, JOHNSON J *et al.*: Macroflux microprojection array patch technology: a new and efficient approach for intracutaneous immunization. *Pharm. Res.* (2002) **19**(1):63-70.
78. HENRY S, MCALLISTER DV, ALLEN MG *et al.*: Microfabricated microneedles: a novel approach to transdermal drug delivery. *J. Pharm. Sci.* (1998) **87**(8):922-925.
79. KAUSHIK S, HORD AH, DENSON DD *et al.*: Lack of pain associated with microfabricated microneedles. *Anesth. Analg.* (2001) **92**:502-504.
80. PRAUSNITZ MR: Microneedles for transdermal drug delivery. *Adv. Drug Deliv. Rev.* (2004) **56**:581-587.
81. SCHRAMM J, MITRAGOTRI S: Transdermal drug delivery by jet injectors: energetics of jet formation and penetration. *Pharm. Res.* (2002) **19**(11):1673-1679.
82. WENIGER BG: Needle-free jet injection bibliography, device & manufacturer roster and patent list. (2004).
83. KING T: Protein delivery via pre-filled needle-free liquid injection. *Drug Delivery Technology* (2003) **3**(7):52-57.

• **A concise summary of the state-of-the-art in the technology.**

84. BURKOTH TL, BELLHOUSE BJ, HEWSON G *et al.*: Transdermal and transmucosal powdered drug delivery. *Crit. Rev. Therap. Drug Carrier Syst.* (1999) **16**(4):331-384.
85. DEAN HJ, FULLER D, OSORIO JE: Powder and particle-mediated approaches for delivery of DNA and protein vaccines into the epidermis. *Comp. Immunol. Microbiol. Infect. Dis.* (2003) **26**:373-388.
86. SCHNEIDER U, BIRNBACHER R, SCHOBER E: Painfulness of needle and jet injection in children with diabetes mellitus. *Eur. J. Pediatr.* (1994) **153**(6):409-410.
87. DORR HG, ZABRANSKY S, KELLER E *et al.*: Are needle-free injections a useful alternative for growth hormone therapy in children? Safety and pharmacokinetics of growth hormone delivered by a new needle-free injection device compared to a fine gauge needle. *J. Pediatr. Endocrinol. Metab.* (2003) **16**(3):383-392.
88. AGERSO H, MOLLER-PEDERSEN J, CAPPI S *et al.*: Pharmacokinetics and pharmacodynamics of a new formulation of recombinant human growth hormone administered by ZomaJet 2 Vision, a new needle-free device, compared to subcutaneous administration using a conventional syringe. *J. Clin. Pharma.* (2002) **42**:1262-1268.
89. VERRIPS GH, HIRASING RA, FEKKES M *et al.*: Psychological responses to the needle-free Medi-Jector or the multidose Disetronic injection pen in human growth hormone therapy. *Acta Paediatrica* (1998) **87**(2):154-158.
90. BENNETT J, NICHOLS F, ROSENBLUM M *et al.*: Subcutaneous administration of midazolam: a comparison of the Bioject jet injector with the conventional syringe and needle. *J. Oral Maxillofac. Surg.* (1998) **56**(11):1249-1254.
91. MURRAY FT: HIV patient acceptance of a needle-free device (SeroJet™) for administering recombinant human growth hormone in the treatment of HIV infection-associated cachexia. *Today's Therapeutic Trends* (2001) **19**(4):283-295.
92. MURRAY FT, WRIGHT GJ, LANDY H *et al.*: Comparative bioequivalence and patient acceptance of recombinant human growth hormone administered by a needle-free jet injector device or standard syringe injection. *Today's Therapeutic Trends* (2001) **19**:137-155.
93. SVEDMAN P, LUNDI S, SVEDMAN C: Administration of antidiuretic peptide (DDAVP) by way of suction de-epithelialised skin. *Lancet* (1991) **337**:1506-1509.
94. LUNDIN S, SVEDMAN P, HÖGLUND P *et al.*: Absorption of an oxytocin antagonist (Antocin) and a vasopressin analogue (dDAVP) through a standardized skin erosion in volunteers. *Pharm. Res.* (1995) **12**(12):2024-2029.
95. WESTERLING D, HOGLUND P, LUNDIN S *et al.*: Transdermal administration of morphine to healthy subjects. *Br. J. Clin. Pharmacol.* (1994) **37**(6):571-576.
96. SVEDMAN P, LUNDI S, HÖGLUND P *et al.*: Passive drug diffusion via standardized skin mini-erosion; methodological aspects and clinical findings with new device. *Pharm. Res.* (1996) **13**(9):1354-1359.
97. MANALOTO RMP, ALSTER T: Erbium:YAG laser resurfacing for refractory melasma. *Dermatol. Surg.* (1999) **25**(2):121-123.
98. LEE W-R, SHEN S-C, WANG K-H *et al.*: The effect of laser treatment on skin to enhance and control transdermal deliver of 5-fluorouracil. *J. Pharm. Sci.* (2002) **91**(7):1613-1626.
99. NELSON JS, MCCULLOUGH JL, GLENN TC *et al.*: Mid-infrared laser ablation of stratum corneum enhances in vitro percutaneous transport of drugs. *J. Invest. Dermatol.* (1991) **97**(5):874-879.
100. SINTOV AC, KRYMBERK I, DANIEL D *et al.*: Radiofrequency-driven skin microchanneling as a new way for electrically assisted transdermal delivery of hydrophilic drugs. *J. Control. Release* (2003) **89**:311-320.
101. LEVIN G, GERSHONOWITZ A, SHERMAN A *et al.*: Viaderm™ – novel microelectronic device enhances skin permeability of human growth hormone. (2003).
102. MITRAGOTRI S: Synergistic effect of enhancers for transdermal drug delivery. *Pharm. Res.* (2000) **17**(11):1354-1359.
103. CHOI EH, LEE SH, AHN SK *et al.*: The pretreatment effect of chemical skin penetration enhancers in transdermal drug delivery using iontophoresis. *Skin*

Pharmacol. Appl. Skin Physiol. (1999) **12**:326-335.

104. PILLAI O, NAIR V, PANCHAGNULA R: Transdermal iontophoresis of insulin: IV. Influence of chemical enhancers. *Int. J. Pharm.* (2004) **269**(1):109-120.
105. BOMMANNAN DB: Effect of electroporation on transdermal iontophoretic delivery of luteinizing hormone releasing hormone (LHRH) *in vitro*. *Pharm. Res.* (1994) **11**(12):1809-1814.
106. SUGIBAYASHI K, KAGINO M, NUMAJIRI S *et al.*: Synergistic effects of iontophoresis and jet injector pretreatment on the in-vitro skin permeation of diclofenac and angiotensin. *J. Pharm. Pharmacol.* (2000) **52**:1179-1186.
107. MEEHAN E, GROSS Y, DAVIDSON D *et al.*: A microinfusor device for the delivery of therapeutic levels of peptides and macromolecules. *J. Control. Release* (1996) **46**:107-116.
108. DOUKAS AG, FLOTTE TJ: Physical characteristics and biological effects of laser-induced stress waves. *Ultrasound Med. Biol.* (1996) **22**:151-164.
109. DOUKAS AG, MCAULIFFE DJ, LEE S *et al.*: Physical factors involved in stress-wave-induced cell injury: The effect of stress gradient. *Ultrasound Med. Biol.* (1995) **21**:961-967.
110. LEE S, KOLLIAS N, MCAULIFFE DJ *et al.*: Topical drug delivery in humans with a single photomechanical wave. *Pharm. Res.* (1999) **16**(11):1717-1721.
111. LEE S, MCAULIFFE DJ, FLOTTE TJ *et al.*: Photomechanical transcutaneous delivery of macromolecules. *J. Invest. Dermatol.* (1998) **111**:925-929.
112. LEE S, MCAULIFFE DJ, FLOTTE TJ *et al.*: Photomechanical transdermal delivery: the effect of laser confinement. *Lasers Surg. Med.* (2001) **28**(4):344-347.
113. LEE S, MCAULIFFE DJ, KOLLIAS N *et al.*: Photomechanical delivery of 100-nm microspheres through the stratum corneum: implications for transdermal drug delivery. *Lasers Surg. Med.* (2002) **31**(3):207-210.
114. LEE S, MCAULIFFE DJ, MULHOLLAND SE *et al.*: Photomechanical transdermal delivery of insulin *in vivo*. *Lasers Surg. Med.* (2001) **28**(3):282-285.
115. SHOMAKER TS, ZHANG J, LOVE G *et al.*: Evaluating skin anesthesia after administration of local anesthetic system consisting of an S-Caine patch and a controlled heat-aided drug delivery (CHADD) patch in volunteers. *Clin. J. Pain* (2000) **16**(3):200-204.
116. HERNDON TO, GONZALEZ S, GOWRISHANKAR TR *et al.*: Transdermal microconduits by microscission for drug delivery and sample acquisition. *BMC Medicine* (2004) **2**(1):12.
117. OBERYÉ J, MANNAERTS B, HUISMAN J *et al.*: Local tolerance, pharmacokinetics, and dynamics of ganirelix (orgalutran) administration by Medi-Jector compared to conventional needle injections. *Hum. Reprod.* (2000) **15**(2):245-249.
118. Cool.click: a needle-free device for growth hormone delivery. *Med. Lett. Drugs Therap.* (2001) **43**:2-3.
119. HOUDIJK E, HERDES E, DELEMARRE-VAN DE WAAL H: Pharmacokinetics and pharmacodynamics of recombinant human growth hormone by subcutaneous jet- or needle-injection in patients with growth hormone deficiency. *Acta Paediatrica* (1997) **86**:1301-1307.

Website

201. http://www.cdc.gov/nip/dev/Jetinject-Bib.pdf
Needle-free injection technology: bibliographic references, device & manufacturer roster and patents list.

Affiliation

Yannic B Schuetz[1,2], Aarti Naik[1,2], Richard H Guy[2,3] & Yogeshvar N Kalia[1,2†]
[†]Author for correspondence
[1]Centre interuniversitaire de Recherche et d'Enseignement, Site d'Archamps, F-74160 Archamps, France
[2]School of Pharmaceutical Sciences, University of Geneva, CH-1211 Geneva, Switzerland
Tel: +33 450 315 024; Fax: +33 450 952 832;
E-mail: yogi.kalia@pharm.unige.ch
[3]Present address: University of Bath, Department of Pharmacy & Pharmacology, Claverton Down, Bath, BA2 7AY, UK