REVIEW ARTICLE

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Ultradeformable Liposomes: A Recent Tool for Effective Transdermal Drug Delivery

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Over the last two decades attempts have been made repeatedly and sometimes successfully to carry agents into the body through the intact skin by using lipid suspension. Use of composite lipidic agent carrier (liposomes, niosomes) was not successful to date due to the inability of such vesicles to pass through the narrow (<30 nm) intercellular passage in the outer skin layers. A solution to this problem is the use of orders of magnitude more deformable supramolecular aggregates, transfersomes. Such innovative drug carriers are driven across the skin by the naturally occurring transdermal gradients and promote transfer of various agents very efficiently and reproducibly. Transfersomes were successfully used in animal and humans also for the transcutaneous and protein delivery. In this review we discuss the theoretical prospect, basic principle behind the development, mechanism of penetration and applications of transfersomes.

Skin is the most extensive and readily accessible organ in the body. Advantages claimed for transdermal drug delivery systems (TDDS) include avoidance of GI incompatibility and variable GI absorption, avoidance of first pass metabolism, reduced frequency of administration, improved patient compliance and rapid termination of drug input by removal of the system from the skin surface. One of the major limitations in transdermal delivery is the low penetration rate of substance through the skin. The diffusion barrier for most substances is localized in the upper layer of the skin, the stratum corneum, which consists of corneocytes embedded in a lipid matrix. Several techniques are used to increase the drug penetration rate across the skin e.g. iontophoresis, sonophoresis and use of colloidal carriers such as lipid vesicles; liposomes and nonionic surfactant vesicles, niosomes. But all above mentioned approaches are not much successful for effective transdermal delivery of drugs[1,2].

The vesicular approach e.g. liposomes, niosomes in transdermal drug delivery system has been studied for many purposes but limited skin permeability limits their use for topical delivery of drugs at clinical and Industrial level. To overcome the difficulties of poor skin permeation recently two vesicular carrier systems, transfersomes and ethosomes have been reported to enhance transdermal delivery of drugs when applied onto the skin nonocclusively[3-8]. A transfersome, in the widest sense of the word, is any supramolecular entity that can pass spontaneously through a permeability barrier and thereby transport material from the application to the destination site. In order to meet the goal, however, transfersome must adjust its properties, most notably its deformability, to the shape and the size of the pores in the barrier and be able to penetrate the mammalian skin intact. Each transfersomes consists of at least one inner aqueous compartment, which is surrounded by a lipid bilayer with specially tailored properties. These novel carriers are applied in the form of semi-dilute suspension, without occlusion, and offer the efficient dermal and transcutaneous drug delivery of high and low molecular weight substances[9,10].

The concept of transfersomes as a carrier for transdermal drug delivery was first developed by Cevc and coworkers, in 1992. Since then, many investigations have been carried out on transfersomes and their possible appli-

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cation as drug carriers. Transfersomes are basically modified liposomes developed to increase the transdermal permeation of drugs. These vesicular transfersomes are several orders of magnitudes, more deformable than the standard liposomes and thus well suited for skin penetration. Deformability of transfersomes is achieved by using surface-active agent in the proper ratio. The concentration of surface-active agent is very crucial in the formulation of transfersomes because at sublytic concentration these agents provide flexibility to vesicles membrane and at higher concentration cause a destruction of vesicles[11,12]. The resulting flexibility of transfersomal membrane minimizes the risk of complete vesicle rupture in the skin and allows the ultradeformable transfersomes to change its membrane composition locally and reversibly, when it is pressed against or attracted into a narrow pore. This dramatically lowers the energetic cost of membrane deformation and permits the resulting highly flexible particles first to enter and then to pass through the pores rapidly and efficiently. Different approaches for transdermal drug delivery are summarized in Table 1.

Salient features of transfersomes:

Transfersomes posses an infrastructure, consisting of hydrophobic and hydrophilic moieties together and, as a result, can accommodate drug molecules with a wide range of solubility. These have higher entrapment efficiency that protects the encapsulated drug from metabolic degradation. Thus transfersomes act as depot, releasing their content slowly and gradually and act as a carrier for low as well as high molecular weight drugs. These are biocompatible and biodegradable as they are made from natural phospholipids. The most important characteristic of transfersomes is deformability of vesicle membrane due to this they can pass easily through narrow constrictions without measurable loss. The deformability of vesicle membrane is responsible for its better skin penetration, resulting in higher transdermal flux of encapsulated drug. Preparation method for transfersomes is also simple that can be easily scaled up.

Basic principle behind development of transfersomes:

Transfersomes when applied under suitable condition shows high permeation ability across the skin. The reason for this high flux rate is naturally occurring transdermal osmotic gradients i.e. another much more prominent gradient is available across the skin[19]. This osmotic gradient is developed due to the skin penetration barrier, prevents water loss through the skin and maintains a water activity difference in

TABLE 1: COMPARISON OF DIFFERENT APPROACHES FOR TRANSDERMAL DRUG DELIVERY.

Method	Advantage	Disadvantage
Penetration enhancer[13,14]	Increase penetration through skin and give both local and systemic effect	Skin irritation Immunogenicity Only for low molecular weight drugs
Physical methods e.g. Iontophoresis[15]	Increase penetration of intermediate size charged molecule	Only for charged drugs Transfer efficiency is low
Liposomes[16]	Phospholipid vesicle, Provide sustained release, biocompatible, biodegradable	Less skin penetration, Less stable Suitable for topical delivery
Proliposome[17]	More stable than liposomes	Less skin penetration Suitable for topical delivery
Niosomes[18]	Non-ionic surfactants vesicles, greater stability, easy handling	Less skin penetration
Transfersomes[3-8]	More stable, high penetration due to high deformability, biocompatible and biodegradable, suitable for both low and high molecular weight and also for lipophilic as well as hydrophilic drugs and reaches up to the deeper skin layers	None, but for some limitations like the higher cost and stability of the formulations

the viable part of the epidermis (75% water content) and nearly completely dry stratum corneum, near to the skin surface (15% water content)[20]. This gradient is very stable because ambient air is perfect sink for the water molecules even when the transdermal water loss is unphysiologically high. All polar lipids attract some water, this is due to the energetically favorable interaction between the hydrophilic lipid residues and their proximal water. Most lipid bilayers thus spontaneously resist an induced dehydration[21,22]. Consequently all lipid vesicles made from the polar lipid vesicles move from the rather dry location to the sites with a sufficiently high water concentration. So when a lipid suspension (transfersomes) is placed on the skin surface, transfersomes are partly dehydrated by the water evaporation loss and then the lipid vesicles feel this osmotic gradient and try to escape complete drying by moving along this gradient. They can only achieve this if they are sufficiently deformable to pass through the narrow pores in the skin, because transfersomes composed of surfactant have more suitable rheologic and hydration properties that are responsible for their greater deformability. Less deformable vesicles including standard liposomes are confined to the skin surface where they dehydrate completely and fuse and hence they have less penetration capability than transfersomes (fig. 1). Transfersomes are optimized in this respect and thus attain maximum flexibility and can take full advantages of the transepidermal osmotic gradient (water concentration gradient)[23-28].

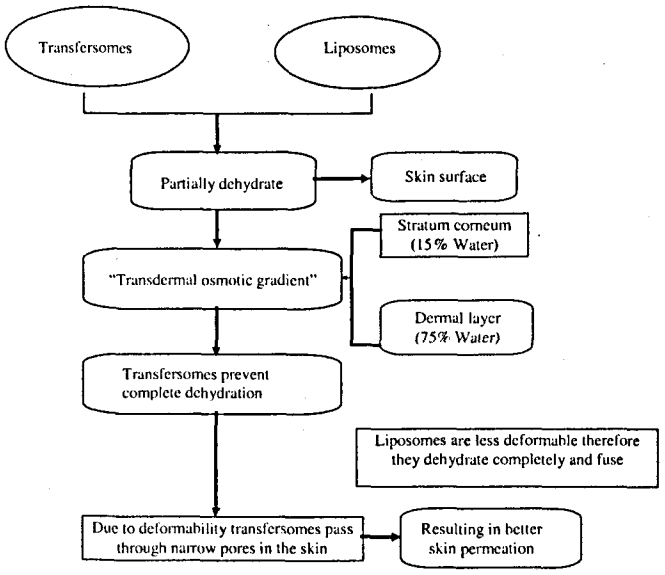

Fig. 1: Basic principle behind development of transfersomes.

Proposed mechanism of transfersomes penetration:

When a suspension of transfersome vesicles is placed on the surface of the skin, the water evaporates from the relatively dried skin surface and the vesicles start to dry out. Due to the strong polarity of major transfersomes ingredients assisted by the softness of the membrane, the vesicles are attracted to the areas of higher water content in the narrow gaps between adjoining cells in the skin barrier. This, together with the vesicles, extreme ability to deform, enables transfersomes aggregates to open temporarily the tiny pore through which water normally evaporates out of the skin. Such newly activated passages can accommodate sufficiently deformable vesicles, which maintain their integrity but change their shape reversibly, reach in the regions of high water content in the deeper skin layers, where the vesicles distribute. Since they are too large to enter into the blood vessels locally, they bypass the capillary bed and get to subcutaneous tissue. Ultimately transfersomes vesicles arrive into the systemic blood circulation via the lymphatic system. The presence of surface-active agent in the transfersomes enhances the rheological properties and sensitivity to the driving force, which results from water concentration gradient across the skin. This enhances the propensity of sufficiently large but deformable penetrants, transfersomes to move across the skin barrier. Such capability combined with inclination to deform into elongated shapes while maintaining the vesicles intregity can explain the unusually high efficiency of transfersomes transport across the skin[29-32] (fig. 2).

Safety considerations:

Phospholipid suspensions comprising liposomes were reported to be harmless and non-irritating to the skin after repeated epicutaneous administration, they may even have additional advantageous cosmetic effect. Macroscopic observations made with transfersomes indicate in the same direction, such ultradeformable vesicles tested on the skin in a microscopic toxicity assay revealed no difference between the saline, as a negative control and various transfersomal formulations[33]. From the point of view of systemic toxicity similarly favorable situation is expected. Main component of transfersomes is typically soya phosphatidylcholine of greater than 95% purity, which is generally regarded as safe because it is already used as emulsifiers in microemulsion for the parenteral nutrition and also used in injectable drug formulation. In light of these data one can expect the transfersomes product to be very safe from the carrier point of view[34].

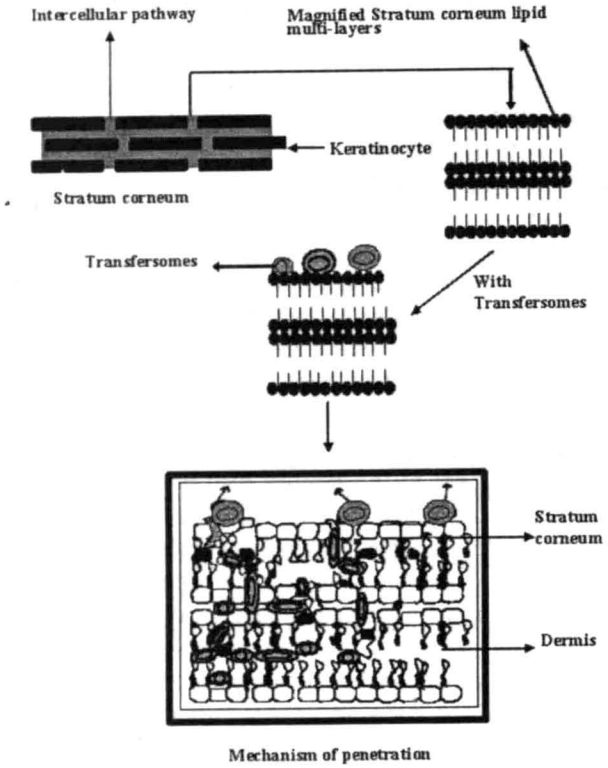

Fig. 2: Proposed mechanisms for penetration of transfersomes across the lipid domain of stratum corneum.

Method of preparation:

The most commonly used method reported for the preparation of transfersomes is conventional rotary evaporation sonication method. This method of preparation of transfersomes comprises of two steps. First, a thin film is prepared, hydrated and then brought to the desired size by sonication and secondly, sonicated vesicles are homogenized by extrusion through a polycarbonate membrane. The mixture of vesicle forming ingredients (phospholipid and surfactant) is dissolved in volatile organic solvent (chloroform-methanol) and organic solvent is evaporated above the lipid transition temperature using a rotary evaporator. Final traces of solvent are removed under vacuum overnight. The deposited lipid films are hydrated with buffer (pH 6.5) by rotation at 60 rpm for 1 h at the room temperature. The resulting vesicles are swollen for 2 h at room temperature. To prepare small vesicles, resulting LMVs are sonicated at room temperature. The sonicated vesicles are homogenized by manual extrusion 10 times through a sandwich of 200 and 100 nm polycarbonate membranes. Drugs are incorporated in the formulation according to their nature, lipophilic drugs are incorporated initially during thin film preparation step and hydrophilic ones are incorporated during the hydration step. Most of the authors reported the same method of preparation of transfersomes because this method is simple, reproducible and cost effective[4,11,32]. Various chemical substances used in the preparation and characterizations of transfersomes are listed in Table 2 and the various commonly used methods for the characterization of transfersomes are listed in Table 3.

APPLICATION OF TRANSFERSOMES AS DRUG CARRIER SYSTEM

Transfersomes as a carrier for proteins[32]:

The delivery of large biogenic molecules such as peptides or proteins into the body is difficult. When given orally they are completely degraded in the GI tract, when used in a degradation preventing formulation, their uptake in the gut becomes problematic and extremely insufficient, even with the best formulation currently available. These are the reasons why nearly all-therapeutic peptides still have to be introduced into the body through an injection needle in spite of the inconvenience of this method. Numerous attempts have therefore been made for the delivery of peptides and proteins across the skin. All recent approaches either chemical (penetration enhancers, lipid vesicles) or physical (iontophoresis, sonophoresis) have some limitations and improve this situation somewhat. These proteins when incorporated in transfersomes efficiently transfer across the skin e.g. insulin is most common and regularly used drug and is generally administered by subcutaneous route that is inconvenient. Cevc *et al.*[32] studied insulin-loaded transfersomes (Transfersulin™) and concluded that transfersomes-associated insulin is carried across the skin with an efficacy of >50%. After application of Transfersulin™ formulation on the intact skin, the first signs of systemic hypoglycemia are observed after 90 to 180 min, depending on the specific carrier composition. This result, which is nearly the same in mice, pigs, or humans, implies a delay of 45 to 145 min relative to the onset of the subcutaneous insulin action. Maximum transfersomes-mediated decrease in the blood glucose concentration is estimated to be approximately 35±10% of the effect of similar subcutaneously injected insulin dose but the cumulative effect is much higher (>70%) and often approaches 100%.

Transfersomes as a means of transdermal immunization[44-48]:

Many environmental pathogens attempt to enter the body through the skin. Skin therefore, has evolved into an excellent protective barrier, which is also immunologically

TABLE 2: DIFFERENT ADDITIVES EMPLOYED IN FORMULATION OF ANSFERSOMES.

Class	Example	Uses
Phospholipid[4,5]	Soya phosphatidyl choline Egg phosphatidyl choline Dipalmityl phosphatidyl choline Distearyl phosphatidyl choline	Vesicles forming component
Surfactant[4,11,12]	Sod. Cholate Sod. Deoxycholate Tween-80 Span-80	For providing flexibility
Alcohol [8,11,12]	Ethanol Methanol	As a solvent
Dye[4,5,9]	Rhodamine-123 Rhodamine-DHPE Fluorescein-DHPE Nile-Red 6 Carboxy fluorescence	For CSLM study
Buffering agent[4,9,11,12]	Saline phosphate buffer (pH 6.5) 7 % v/v Ethanol Tris buffer ((pH 6.5)	As a hydrating medium

active. On the basis of above fact another most important application of transfersomes is transdermal immunization using transfersomes loaded with soluble protein like integral membrane protein, gap junction protein. This approach offers at least two advantages first they are applicable without injection and second, they give rise to rather high antibody titer value.

Hofer *et al.*[48] studied transfersomes as carrier for albumin and reported 50% blood concentration of albumin after 24 h post application, the reported value is very similar to that resulting from a subcutaneous injection of the albumin suspension. The transfersomal formulation of this protein also induced a strong immune response after the repeated epicutaneous application and measured antibody titer value reached 70% of that resulting from commercial anti-BSA solution.

Transfersomes as a carrier for delivery of immunomodulators[49]:

Leukocytic derived interferon-α (IFN-α) is a naturally occurring protein having antiviral, antiproliferative and some immunomodulatry effects. Because of these properties, INF-α has found its place in therapy of several viral diseases and exhibits encouraging anticancer activities. Interferon-α is chemically protein and hence its delivery is very difficult. Transfersomes as drug delivery system have the potential to provide controlled release of the administered drug and increase the stability of labile drugs. Hofer *et al.*[49] studied the formulation of interleukin-2 and interferon-α containing transfersomes for potential transdermal application. They reported delivery of IL-2 and INF-α trapped by transfersomes in sufficient concentration for immunotherapy.

Cyclosporine-A is a potent immunosuppressant that is recommended in long-term therapy for prevention of graft rejection after kidney, liver, heart, lung transplantation. Cyclosporine-A is a better candidate for transdermal drug delivery due to problems in its oral administration and recommended for long term therapy. Transdermal delivery of cyclosporine-A has never been a simple task due to its highly lipophilic nature, large molecular weight and a ring structure. Guo *et al.*[36] incorporated cyclosporine-A in the transfersomes and reported significant increase in transdermal flux and also sustained zero order release that increases the therapeutic efficacy and provides the maximum patient compliance.

TABLE 3: METHODS FOR THE CHARACTERIZATION OF TRANSFERSOMES.

Parameter	Method
Vesicle shape (morphology)[36]	Transmission electron microscopy
Entrapment efficiency[37,38]	Mini column centrifugation method
Vesicle size and size distribution[11]	Dynamic light scattering method
Skin permeation potential[39,40]	Confocal laser scanning microscopy Fluorescence microscopy Transmission electron microscopy Thin layer chromatography
Phospholipid surfactant interaction[6,12]	^{31}P NMR Differential scanning calorimeter
Degree of deformability[41,42,45]	Extrusion method
Surface charge and charge density[35]	Zeta meter
Turbidity[17,36]	Nephalometer
In vitro drug release study[11,12]	Side by side diffusion cell with artificial or biological membrane, Dialysis bag diffusion
Effect on the skin structure[32]	Histological studies Transmission electron microscopy
Stability study[40]	Dynamic light scattering method Transmission electron microscopy

Transfersomes as a carrier for corticosteroids[4,43]:

Corticosteroids are wide category of drug, they benefit by virtue of their antiinflammatory, immunosuppressive, vasoconstrictor and antiproliferative action, and are used in atopic eczema, allergic contact dermatitis, arthritis, rash, sunburn, neurodermatitis and psoriasis. The available marketed formulations for topical use fulfill only a few therapeutic goals and have problems such as local irritation, requirement for higher amounts of drug and local and systemic toxicities of penetration enhancers. Cevc *et al.*[4] and Jain *et al.*[43] studied transfersomes as carrier for delivery of these corticosteroids and reported that transfersomes based formulations efficiently deliver the drug to the target site, deeper layer of skin and also sustain the release of drug. They also compare the biological anti-oedema activity of transfersomes formulation with corresponding commercial product and found them superior, probably owing to the superior drug targeting potential of the drug.

Transfersomes as a carrier for NSAIDS[8,50,51]:

Cevc and Blume[50] studied the transfersomes as a carrier for site-specific delivery of diclofenac and reported Transfenac, a lotion-like formulation of diclofenac based on the transfersomes. They also compared the biological activity of Transfenac formulation with conventional hydrogel preparation. Diclofenac association with ultradeformable carrier permits it to have a longer effect and to reach 10 times higher concentration in the tissue under the skin in comparison with the drug from a commercial hydrogel. Transfenac achieves intramuscular agent concentration between 2 and 20 μg/g at t=2 h. depending on the tissue depth, when it is administered in the dose range 0.25-2 mg/kg of rat body weight.

The main advantage of transfersomal formulation of diclofenac is lowering of the drug dose because the gel formulation only permits this drug to penetrate to a depth of 3 to 4 mm. In contrast to this, the transfersomal formulation of diclofenac always makes sure that the drug concentration in the soft tissues under the application site is at least 10 times more than hydrogel preparation. In all species investigated so far (mice, rats, pigs) diclofenac was found to penetrate deep into the soft tissues under the drug application

site from the transfersomes. Similar observation was also made for ibuprofen delivery by means of transfersomes. Thus, transfersomes not only dramatically improve the efficacy of diclofenac penetration through the intact skin permeability barrier but also carry most of their associated agents directly into the depth of the soft tissues under the application site. The probable reason for this is the (transient) drug confinement to the carrier that prevents rapid agent elimination after the passage through the skin, being too big to disappear in the blood capillaries.

Transfersomes as a carrier for anticancer drugs[8]:

Tamoxifen is the most common agent for the treatment of all stages of breast cancer. Despite the widespread use of this anti-estrogen, it would be very desirable to develop a regio-selective and convenient to use formulation of the drug to lower the incident of side effects such as depressions or thrombosis.

Cevc,[8] developed tamoxifen formulation based on ultradeformable vesicles and applied on the shaved murine back, most of the epidermally applied transfersomes penetrated the skin, leaving less than 5% of the drug-derived radioactivity on the body surface and the integrated dose of tamoxifen in the uterus, an organ with targetable estrogens receptors, is by the factor of 2 times higher than that achieved by an oral application of the same amount of drug. Experiments with the radioactively labeled tamoxifen in transfersomes in mice revealed significantly higher drug concentration in the body and better site-specificity after epicutaneous application, when compared with the orally administered tamoxifen. The biological activity of tamoxifen administration on the skin by means of transfersomes is equally impressive. Such treatment accelerates the growth of murine uteri even at doses as low as 0.1 to 0.2 mg/kg/d. Tenfold higher amount of tamoxifen in soya oil must be injected subcutaneously in mice to achieve comparable biological effects by the nontransfersomal formulation.

Transfersomes as a carrier for topical analgesic and anesthetic agents[8,52,53]:

The most preferable route for the introduction of analgesics into body is per oral. The effects of oral pain treatment are relatively diffuse and weak. Transdermal delivery of topical analgesic agents is a better choice for increasing the therapeutic efficacy of these agents. Transfersomes can circumvent this problem and provide formulation of unprecedented quality for the induction of local anesthesia and newly developed analgesics transfersomal formulation for the management of local pain in rats and humans. Planas *et al.*[52] studied transfersomes as a carrier for tetracaine and lidocaine for their local analgesic and anesthetic purpose. These transfersomal formulations depending on their composition penetrate rapidly through the intact dermis and bring appreciable amount of drugs into the deeper layer of the skin and suppressed peripheral pains at their very root. They also evaluated therapeutic potential of analgesic transfersomes in Sprague-Dawley rats subjected to heat stimulus reaction to >70s, 130% longer than in controls that received a placebo or a standard lidocaine solution. In human, they tested for pain suppression activity assessed by the pinprick method and concluded that the effectiveness of dermally applied anesthetic transfersomes is similar to that of the corresponding subcutaneous injection of similar drug quantities and that optimally designed transfersomes offer a suitable and promising means for the noninvasive treatment of local pain with wide direct topical application. A summary of drug candidates applied as transfersomes for transdermal drug delivery is presented in Table 4.

CONCLUSIONS

Transfersomes are specially optimized vesicles, which can respond to an external stress by rapid and energetically inexpensive, shape transformations. Such highly deformable particles can thus be used to bring drugs across the biological permeability barriers, such as skin. Transfersomes thus offer a singularly good opportunity for the non-invasive delivery of small, medium and large sized drug molecule. The results of the first human trials with the epicutaneously applied transfersomal insulin support this conclusion. Multiliter quantities of sterile, well-defined transfersomes containing agent can be and have been prepared relatively easily. It therefore should be not before long that the corresponding drug formulation would have found their way into clinics to be tested for the widespread usages. Thus it can be a logical conclusion that transfersomes hold a promising future in effective transdermal delivery of bioactive agents.

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TABLE 4: APPLICATION OF TRANSFERSOMES AS A DRUG CARRIER.

Drug	Results
Insulin[32,53]	High encapsulation efficacy Transfer across the skin with an efficacy of >50% Provide noninvasive means of therapeutic use
Interferon-α[49, 54] Interleukin-2	Efficient delivery means (because delivery by other route is difficult) Controlled release Overcome stability problem
Soluble proteins[44-48] Gap junction protein Human serum albumin Integral membrane protein	Permits non-invasive immunization through normal skin Antibody titer is similar or even slightly higher than subcutaneous injection
Corticosteroids[4,43] Hydrocortisone Triamcinolone acetonide	Improve site specificity and overall drug safety Biologically active at dose several times lower than the currently used formulation Used both for local and systemic delivery
Topical analgesic and anesthetic agent [8, 50, 51] Diclofenac, Tetracaine, Lidocaine	Suitable means for the noninvasive treatment of local pain on direct topical drug application. Prolonging drug action
Oestradiol[55, 56]	Improved transdermal flux Provide controlled release Prolonging drug action
Tomoxifen[8] Norgesterol[41]	Improved transdermal flux Reduce drug toxicity Improved transdermal permeation Reduce side effects
Cyclosporine[36]	Improved therapeutic efficacy Prolonging drug action
Dexamethasone[57,58]	Improved transdermal flux Prolonging drug action

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