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Ultradeformable vesicles: concepts and applications relating to the delivery of skin cosmetics

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Skin aging is a phenomenon resulting in reduced self-confidence, thus becoming a major factor in social determinants of health. The use of active cosmetic ingredients can help prevent skin aging. Transfersomes are well known to be capable of deeply penetrating the dermis. This scoping review provides an insight into transfersomes and their prospective use in anti-aging cosmetics. Numerous reports exist highlighting the successful skin delivery of therapeutic agents such as high-molecular-weight, poorly water soluble and poorly permeable active ingredients by means of transfersomes. Moreover, *in vitro* and *in vivo* studies have indicated that transfersomes increase the deposition, penetration and efficacy of active ingredients. However, the use of transfersomes in the delivery of active cosmetic ingredients is limited. Considering their similar physicochemical properties, transfersomes should possess considerable potential as a delivery system for anti-aging cosmetics.

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Skin aging is a process of changing physical appearance that can reduce an individual's self-confidence. These skin changes are closely related to ones in the balance of the production and decomposition of collagen, elastin and glycosaminoglycans, which constitute quality parameters of the dermis layer [1,2]. There are several triggers, which can be internal physical factors (e.g., DNA damage due to reactive oxygen species, the development of chronic diseases, and metabolic disorders connected with aging) or external factors, including exposure to sunlight and oxidant materials which result in the skin losing elasticity and firmness and the appearance of wrinkles [1–3]. Wrinkles are a sign of aging skin caused by collagen degradation, and these visible skin folds can have an impact on quality of life and physical appearance [4].

Anti-aging strategies that have been implemented include protection against UV rays, invasive procedures and skincare products or cosmetics (e.g., sunblocks). Meanwhile, the use of cosmetics in improving skin biological function and skin care has involved the addition of local biologically active cosmetic ingredients. Anti-aging ingredients have become a popular means of improving intrinsic skin biological function. Therefore such compounds must be able to penetrate the barrier of the stratum corneum (SC) in order to reach the dermis layer and rejuvenate and repair skin wrinkles.

Active anti-aging cosmetic ingredients include coenzyme Q10, which demonstrates low water solubility [5,6], growth factors such as EGF and TGF-β contained in amniotic membrane stem cell metabolite products (AMSC-MPs) that have a significant molecular weight [7,8], vitamins and other herbal and biological products [9]. These compounds should possess different physicochemical characteristics. However, only small molecules less than 500 Da in size and lipophilic molecules with logP values between -1 and 4 can penetrate the SC, which constitutes

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the skin barrier [10,11]. Therefore the use of nanoparticulate carriers in skin delivery has the potential for anti-aging cosmetics to improve the decreased quality of the dermis layer in aged skin. The presence of active cosmetic ingredients within a nanocarrier system in the epidermis and dermis layers indicates that they can promote collagen and elastin repair activity which enhances skin firmness [12].

Certain nanocarriers (e.g., liposomes, transfersomes, glycerosomes, ufosomes and hybrid vesicles) have been developed to improve skin drug delivery. Liposomes are a lipid-based vesicular carrier consisting of an inner water phase surrounded by lipid bilayer membranes [13]. The addition of softening bilayers such as surfactants to liposomes can produce a transfersome [14], while the use of glycerol as the edge activator (EA) of the liposomal bilayer membrane generates glycerosomes [15].

Transfersomes represent the first generation of elastic liposomes which demonstrate liposome-like characteristics with the ability to deform and reform their shapes. Liposomes are known to provide three different environments for substances entrapped inside them: the lipid–water interface, the hydrophobic nucleus and the aqueous interior. Thus, liposomes can entrap hydrophobic, hydrophilic or amphiphilic active ingredients within their structures, in addition to improving their stability. With the presence of EAs, the vesicles can become elastic, resulting in their ability to enhance the penetration by active cosmetic ingredients, thus enabling them to reach deeper skin layers [16]. Under the mechanical stress resulting from transepidermal osmotic gradient force, EAs will be transferred to areas of higher curvature or pressure in the lipid bilayer. This process causes changes in the shape and volume of transfersome vesicles with minimal energy requirements. Moreover, the addition of EAs can disrupt the ordered arrangement of phospholipid molecules within spaces in the lipid bilayers, significantly reducing the transition temperature of the transfersome bilayer membrane [17].

Transfersomes can shrink, thereby facilitating penetration of the skin via intercellular routes and pores of the SC that are much smaller than their own vesicles' diameters. These vesicles are ten-times more deformable than conventional liposomes [18]. However, because the transfersome vesicle has limited entrapment capacity, and content leakage of active ingredients still tends to occur due to water diffusion from dispersing media, a provesicular carrier has been developed, namely protransfersomes. Protransfersomes are lipid provesicles in the form of crystalline liquid which will turn into very flexible transfersome vesicles *in situ* by absorbing water from the skin [19]. These characteristics enable the protransfersomes to protect the encapsulated materials as well as vesicular lipids from any unwanted chemical reactions, such as hydrolysis and oxidation associated with degradation, and physical reactions such as sedimentation, aggregation, fusion or leakage of trapped substances or hydrolysis of encapsulated active ingredients [20–22]. They extend shelf life and are capable of targeting materials encapsulated in the deeper layers of the skin [23–25].

Transfersomes are widely employed in the topical and transdermal delivery of various active pharmaceutical ingredients. However, their applications to cosmetic delivery are limited. Moreover, the recent development of cosmetics is largely intended to improve the appearance of skin by having local biological effects on its tissues. The similar physicochemical properties of the active ingredients provides direct analogies for successful skin delivery using transfersomes, thereby also rendering them prospective active cosmetic ingredients. This review will demonstrate the potential use of transfersomes in enhancing active ingredient penetration, which promotes optimal anti-aging activity within cosmetic delivery systems. This, in turn, increases their effectiveness in impeding skin aging.

This review analyzes the potential use of transfersomes as a carrier in the delivery of anti-aging cosmetics. The existing research into the use of transfersomes in cosmetics is limited. Consideration of the similar physicochemical properties of active pharmaceutical ingredients, such as insulin and hormones, is intended to identify analogies of these substances' successful delivery through ultradeformable vesicles which could also be applied to active cosmetic ingredients.

The method employed in writing this review consisted of an electronic literature study involving the accessing of national and international journal search sites related to the keywords 'ultradeformable vesicles', 'transfersomes', 'protransfersomes', 'anti-aging active compound', 'protein for anti-aging', 'topical delivery', 'skin delivery', 'deformability', 'skin penetration' and 'topical drug classification system'.

The eligibility criteria applied when selecting journal articles comprised original research, short case studies, experimental research design and the year of publication falling within the period 1992–2020. The sites accessed for the purposes of conducting the search included PubMed (Scopus & Scimago) and Google Scholar articles which contained search keywords. Articles published by predatory journals or publishers which include review articles were excluded from the study. Identification, data correlation analysis and paper selection were conducted on the basis of the CONSORT diagram shown in Figure 1.

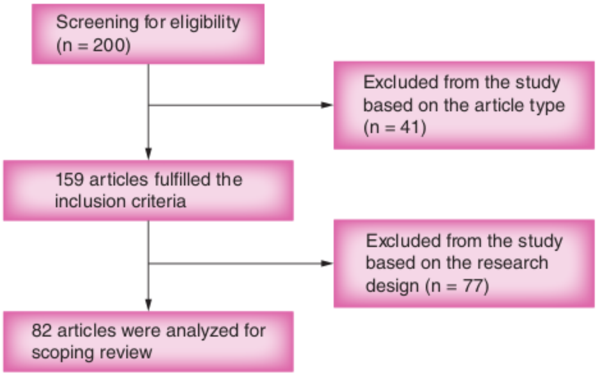

Figure 1. CONSORT diagram of article screening and selection.

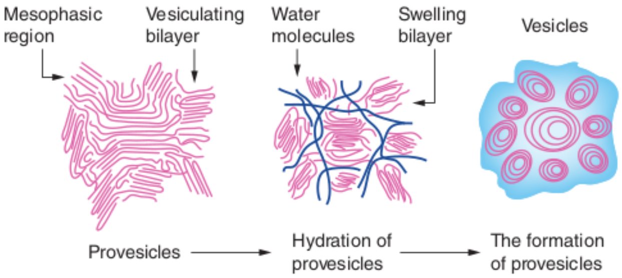

Figure 2. The physical formation of transfersomes from protransfersome gel.

Characteristics of protransfersomes & transfersomes as vesicular carriers for skin delivery

For topical delivery, transfersomes have many advantages relating to their high membrane elasticity and deformability. These can be achieved by combining two lipophilic or amphiphilic components, namely phospholipids and biosurfactants, at the appropriate ratio or formula to form bilayer vesicles [23]. In transfersomes, the surfactant as the EA is in the form of a single-chain surfactant capable of destabilizing the lipid bilayer and causing an increase in the fluidity and elasticity of the vesicular membrane, with the result that the vesicles can change shape and pass through the pore intact by shrinking in size to five- to ten-times smaller than the original, thus increasing the penetration of the active cosmetic ingredients [26]. Protransfersomes, extremely flexible liquid lipid provesicles, provide benefits for improving the stability of transfersomes [19].

During application, the active ingredient interacts with the skin, which is both attached and adheres to the SC. Due to the osmotic gradient resulting from the difference in water content of skin tissue, the active ingredient will be transported to the deeper layers of the skin by passing through the SC. Under light microscopy, the protransfersome, which is originally crystalline and lamellar-shaped, will turn into transfersome vesicles after hydration. This is due to the difference in the degree of hydration of the surfactant and phospholipid molecules, together with the change in shape of the hydrated molecules. Because of its limited solvent content, the resulting protransfersome will form a compact palisade and vesiculation lamellae. The addition of water will cause further swelling of the bilayer and vesicles due to the interaction of the air with the surfactant head groups and will tend to produce random spherical vesicles of transfersomes, as presented in Figure 2 [27].

The deformability of the vesicles is influenced by the chemical structure of the surfactants, with surfactants that have a low hydrophilic–lipophilic balance value generally forming smaller vesicles. The surfactant concentration must also be proper, otherwise the vesicles will harden and be damaged [27]. Another role of surfactants is to increase the hydration properties of transfersome vesicles, with the result that they tend to seek moisture in deeper skin layers after application to the skin hydrotaxis (i.e., xerophobia) [26].

Transfersomes can penetrate the skin layers by means of different mechanisms depending on their composition (Figure 3): either the vesicles maintain their intact shape (deformability) via the intercellular pathway; or the vesicles fuse and mix with skin lipids (transcellular) due to destabilization of the membrane by surfactants; or the vesicles go directly to deeper skin tissues via appendageal routes. Transfersomes can easily shrink to one-tenth of their original

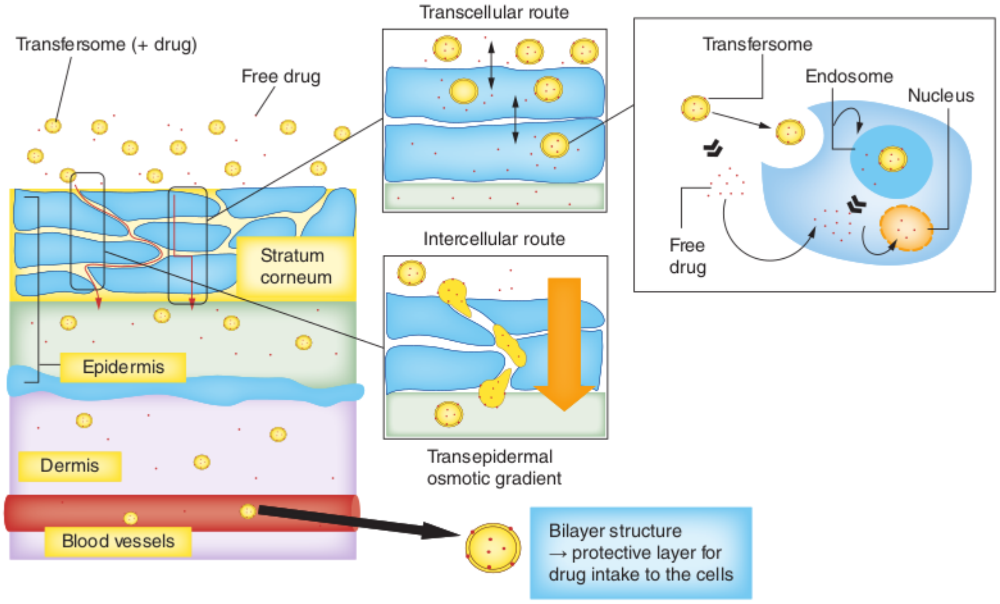

Figure 3. The presence of a transdermal osmotic gradient leads to skin penetration of transfersomes via transcellular and intercellular routes into deeper skin layers.

size in order to pass through the pore by means of a transdermal osmotic gradient due to differences in the water content of the skin surface, which is about 15%, and the dermis, which has a high water content of 75% [28]. This osmotic gradient helps the active ingredients pass through the skin passively via the hydrophilic ducts of the SC [17]. When the transfersomes are applied to skin in non-occlusive conditions, they will dehydrate due to water evaporation, the hydrophilic nature of which causes the vesicles to be attracted to a layer with a high water content, allowing the intact vesicles to penetrate via the intercellular spaces [29,30].

The osmotic gradient is high in the SC and decreases with skin depth to the stratum spinosum layer (0.042 mm) [31]. This osmotic gradient acts as a propulsion for most transfersome vesicles with a total lipid mass of more than 0.1 mg/cm^2 within 1. The occlusive means of application can cause at least 90% of the drug to be retained in the SC due to excess hydration, with the result that only small levels of the drug can enter the bloodstream [21]. The SC is composed of corneocytes embedded in hydrophobic lipids that form a crystalline lamellar phase. The corneocytes are coated with cross-linked soft keratin [23]. The water content in the SC is not evenly distributed according to its thickness. A thin layer of water is in equilibrium with the surrounding water content, while the moisture content of a thicker layer of the epidermis is close to saturation level. Viable skin contains 70–80% water, whereas the surface of the SC is drier than this viable dermis [24]. The electronic diffraction results of biopsy specimens show that the water content regularly drops from 70–80% in the stratum granulosum layer to 15–25% in the upper layer, which means that the water is completely bound, even for fairly high water content values, in these skin layers [25].

Transfersomes act as encapsulating carriers for various active ingredients

Transfersomes as trapping carriers represent the first generation of elastic liposomes that can deliver various active ingredients with different lipophilicities and are able to encapsulate active substances with high molecular weights. Transfersomes are widely reported as being used for topical and transdermal delivery of various active pharmaceutical ingredients. However, the recent development of cosmetics is primarily intended to improve the appearance of the skin through localized biological effects on its tissues. Identifying the similar physicochemical properties of the active ingredients provides direct analogies for successful transfersome-based skin treatment, rendering them prospective active cosmetic ingredients. Active ingredients with hydrophilic properties will be encapsulated in the aqueous core, while lipophilic substances are trapped within the lipid membrane layer. The process of encapsulating

Table 1. The utilization of transfersomes and protransfersomes as delivery carriers for various active cosmeceutical agents.

Therapeutic class	Drug or therapeutic agents	Properties			Type of drug carriers	Ref.
		Water solubility	Permeability	Stability		
Antioxidant	Curcumin	Low	Low (high MW)		Transfersomes	[36,37]
	Resveratrol	Low	High	Low	Transfersomes	[25]
	Psoralen (+ resveratrol)	Low	Low		Transfersomes	[38]
	Epigallocatechin-3-gallate (EGCG)	High	Low	Low	Transfersomes	[39,40]
Antidiabetic agent	Glimepiride	Low	High		Protransfersomes	[41,42]
Anticancer agent	Cisplatin	Low	Low		Protransfersomes	[14,19,43]
	Cisplatin	Low	Low		Transfersomes and protransfersomes	[14]
	Cisplatin + imiquimod	Low	Low		Protransfersomes + carbopol	[44]
	5-Fluorouracil (+ resveratrol)	High	Low		Transfersomes	[10,45]
	Methotrexate	Low	Low		Transfersomes	[46–48]
Analgesic	Ketoprofen	Low	High		Protransfersomes	[23,49,50]
	Ketorolac	High	Low		Protransfersomes	[51]
					Transfersomes	[12,52,53]
	Diclofenac	Low	High		Protransfersomes	[54,55]
	Diflunisal	Low	High		Transfersomes	[56,57]
Hormone and protein	Levonorgestrel	Low	Low		Protransfersomes	[22,58]
	Norgestrel	Low	Low		Protransfersomes	[59]
	Insulin		Low	Low	Transfersomes	[29,35]
	siRNA		Low	Low	Transfersomes	[60]
	Sulforaphane		Low	Low	Transfersomes	[61]
	Phytoestrogen quercetin	Low	High	Low	Transfersomes	[28,62]
Antihypertensive agent	Timolol	Low	Low		Protransfersomes	[24,27,63]
	Nifedipine	Low	High		Protransfersomes	[64,65]
	Minoxidil + caffeine	Low	Low		Transfersomes	[66–68]
Anti-infection	Azaleic acid	Low	Low		Transfersomes and protransfersomes	[20]
	Amphotericin B	Low	Low		Transfersomes	[69–71]
	Rifampicin	Low	High		Transfersomes	[72,73]
Selective estrogen receptor modulator	Raloxifene hydrochloride	Low	High		Transfersomes	[74,75]
Vitamins	Retinoyl palmitate	Low	High	Low	Transfersomes	[76]
	Tocopherol	Low	High	Low	Transfersomes	[77]
Herbal products	Emu oil from *Dromaius novaehollandiae*	Low	Low		Transfersomes	[78]

large molecules for subsequent penetration of deeper skin layers is that of forming a reservoir for slow and sustained release of the encapsulated substances, allowing for a reduction in the frequency of administration [32].

The encapsulation of active cosmetic ingredients with high molecular weights can be based on several studies of transfersome formulation for delivery of proteins, such as growth hormones contained in AMSC-MPs, stem cells and RNAs [7,29,32–34]. The reverse-phase evaporation method for transfersome formulation has been used in the encapsulation of hydrophilic polypeptide molecules (e.g., insulin) by using sodium cholate as the EA, with an entrapment efficiency of up to 81% [35].

Table 1 shows the use of transfersomes for loading various types of active ingredients, which has been proven to improve stability, penetration and effectiveness while reducing the toxicity; this enables their use as references for ultradeformable vesicle formulations of cosmetic active ingredients with similar physicochemical properties. The encapsulation methods of active ingredients in transfersomes depend on the ingredients' solubility and permeability, as discussed in the following sections.

Protein molecules

Proteins are known as active ingredients with a high molecular weight that have been used in skin care. An appropriate delivery system is required to ensure that this active ingredient is stable and can penetrate the skin to produce therapeutic effects. A number of proteins have been formulated into transfersomes, one being insulin, whose particle size can be reduced to 100 nm and which can easily penetrate deeper skin layers, producing a hypoglycemic effect when compared with conventional insulin vesicles via the transdermal route [29]. This is because insulin, which is composed of large molecules and demonstrates high affinity, is distributed in the skin by interstitial fluid flow through the lymphatic system in the skin dermis layer in the presence of lymphatic vessels and capillaries [35]. These anatomical characteristics can be utilized in transdermal delivery of such proteins [79].

Apart from insulin, the progestin hormone used for the purposes of oral birth control or contraception has also been formulated as transfersomes: norgestrel, which is composed of soya phosphatidylcholine and sodium cholate at a weight ratio of 90:10, and levonogestrel, which contains the same elements at a weight ratio of 85:15. Transfersomes have been shown to increase transdermal penetration, double the contraceptive's effectiveness, enhance active ingredient stability, augment entrapment efficiency and facilitate greater reproducibility [22,80].

The current use of siRNA can represent an alternative anti-aging therapy because it is known to be capable of regulating the expression of certain genes that are intimately involved in the skin aging process [81]. This is expected to enhance the ability of skin cells to repair themselves, given that during aging the skin layer tends to become thin and easily damaged due to reduced skin matrix production and lipid synthesis, lower antioxidant capacity and hyperpigmentation [82]. However, several obstacles exist to skin penetration by this oligonucleotide, including its large molecular size, which renders passing through the skin layer difficult, and its negative charge, which hinders internalization by the cells [83,84]. The use of nanocarriers such as transfersomes in encapsulating and modifying the natural properties of siRNA could enhance the biological efficacy and stability of cosmetic delivery.

The use of siRNA and mRNA formulated as transfersomes in the treatment of atopic dermatitis has been shown to increase effectiveness and reduce side effects. RNA which is enzymatically degraded and has low membrane permeability can be delivered to the deeper layers of the skin using a gene carrier with the addition of penetration enhancers containing cysteine, arginine and histidine. Such enhancers work through different mechanisms. The arginine residue forms a complex with siRNA, the histidine portion allows the complex to escape the endosome and the cysteine constituents stabilize and release siRNA in a reducing environment. Peptide modification with stearic acid further stabilizes the complex through hydrophobic interactions. Formulated with the small unilamellar vesicles fusion method, the siRNA particle size can reach 70 nm and is protected from enzymatic degradation. The increased effectiveness of siRNA as a regulator of cytokine production, leading to a reduction in inflammatory cytokines in mice, indicated that transfersomes successfully deliver siRNA transdermally [32]. Phospholipon® 90G and Brij® O20 combined with sponge *Haliclona* sp. *spicula* (SHS) for siRNA delivery, which acts as an enhancer by making many microchannels that are approximately 800 micropores per mm^2, applied at a dose of 10 mg SHS/1.77 cm^2 for 48 h into the SC successfully facilitated protein penetration to the deeper layers of the skin [85].

Cristiano *et al.* [61] have also formulated the enzyme product of sulforaphane (1-isothiocyanate-(4R)-(methylsulfinyl)-butane) encapsulated within transfersomes consisting of Phospholipon 90G and sodium cholate at a weight ratio of 88:12, using the thin-layer evaporation method, for melanoma therapy. The use of transfersomes has been shown to increase penetration into the deeper layers of the skin, thereby increasing the agent's anti-cancer activity.

The new paradigm emphasizes stem cells as an attractive biotechnology product to be formulated as anti-aging cosmetics. The effectiveness of stem cells in regenerating damaged cells due to oxidant-induced shortening of chromosome telomeres has also been studied [86,87]. Stem cells possess the unique characteristic of being unspecialized and, as such, are able to reproduce themselves repeatedly through asymmetric division [88]. As well as those derived from animals, stem cells from plants are also used as cosmetics after being made into standardized stem cell extracts [89]. The characteristics of stem cell products or extracts, which have high molecular weights and are unstable for transdermal preparations, have prompted researchers to utilize nano-sized delivery systems, one of which is elastic liposomes which can reduce particle size to <100 nm as a means of facilitating penetration into deeper skin layers [33].

It has been reported that AMSC-MPs contain numerous cytokines and growth factors, including EGF, TGF-β, bFGF and KGF [7,84,90]. These growth factors and cytokines play important roles in modulating cell behavior in tissues, increasing the proliferation of epidermal keratinocytes and dermal fibroblasts, thereby stimulating the production of extracellular matrix such as collagen [91]. Recently, microneedle- and laser-assisted drug delivery have been used to deliver AMSC-MPs to the skin dermis layer because these hydrophilic macromolecules have a molecular weight >25 kDa [8], which hinders their penetration of the deep skin layers [7,34]. The ability of transfersomes to encapsulate hydrophilic substances inside the vesicles is dependent on their high deformability, which enables them to pass through intercellular space and enable deep penetration of AMSC-MPs into the dermis.

Active substances with low solubility & high permeability

Transfersomes, which can load active ingredients characterized by low solubility and high permeability, can have an effect by means of several methods, namely: high pressure homogenation, modified coacervation phase separation and conventional thin film hydration. Active ingredients belonging to this group include resveratrol, quercetin, glimepiride, diclofenac, ketoprofen, rifampicin, nifedipine, raloxifene and retinyl palmitate [23,25,28,41,64,72,75,76].

In cosmetics, resveratrol has been shown to promote the proliferation of fibroblasts, in turn increasing the production of collagen matrix, which renders it a potential anti-aging therapy [92]. Moreover, its high antioxidant capacity plays an important role in preventing oxidative damage to skin tissue cells caused by exposure to UV and retarding the photo-aging process [92,93]. Despite demonstrating high levels of permeability in topical delivery [93], resveratrol has low water solubility, and significant issues exist with regard to its stability [94,95]. Resveratrol has been combined with various active ingredients, including psoralen which, in combination with UV-A, can stimulate melanin production and tyrosinase activity in melanocytes. The resulting transfersome vesicles have a homogeneous particle size, are stable and demonstrate high trapping efficiency, with the result that the use of transfersomes both enhances the effect of the combination of active ingredients and is able to inhibit the increase of free radicals for vitiligo therapy [38]. Arora *et al.* [25] prepared transfersomes using central composite design and found that transfersomes comprised of cholesterol hydrochloride, cholesterol and sodium deoxycholate could increase the depot effect on the skin. The addition of a cosmetic base cream and gel has no effect on the physical characteristics of the vesicles; on the contrary, it can increase acceptability during use.

Quercetin, a polyphenol compound, has been reported as having an antifibrotic effect capable of reducing scar formation and accelerating wound healing [96]. The use of Quercetin has also been reported as effective in protecting human skin tissues from photo-aging through inhibition of MMP-1 expression, which prevents collagen degradation [97,98]. However, the use of quercetin is highly restricted by its low water solubility with a partition coefficient value of 1.82 [98,99]. In a previous report, quercetin as a phytoestrogen employed in osteoporosis therapy, had low bioavailability when taken orally [28]. Therefore Pandit *et al.* [28] formulated quercetin in transfersomes using a fractional factorial design optimized by a complete factorial design. The results showed that quercetin loaded in transfersomes, prepared with phosphatidylcholine and Tween® 80 at a weight ratio of 2:1, has a homogeneous and stable particle size and can increase therapeutic effectiveness by topical administration of its transfersomal system, as indicated by femoral thickness, length and density and also by serum biochemical parameters such as calcium, phosphorus, alkaline phosphatase and tartrate-resistant alkaline phosphatase. Thus the use of transfersomes successfully improved topical delivery of quercetin.

Low or reduced levels of retinoids, which affect the maturation of skin epithelial cells, can cause skin disease. Therefore an external supplement in the form of retinyl palmitate can be applied. In the presence of enzymes in the skin, retinyl palmitate will be converted to retinol and oxidized to form tretinoin, which induces thickening of the epidermal layer and collagen production [100]. Retinyl palmitate has low water solubility, therefore in order to improve it for the purposes of dermal delivery, transfersomes were prepared with a weight ratio of phosphatidylcholine:Tween 80 of 18:1. This successfully promoted skin penetration, as evidenced by the discovery of retinyl palmitate in various layers of the skin, suggesting that the transfersomes can be used as carriers for active ingredients with similar characteristics [76].

Interestingly, the topical use of 3% diclofenac sodium with hyaluronic acid repairs, to a great extent, signs of skin damage due to chronic UV exposure, including irregular pigmentation and coarseness. This is probably due to its promotion of cyclooxygenase inhibition, which reduces melanin transfer to the epidermal keratinocytes [101,102]. Diclofenac itself has come to be regarded as a poorly water-soluble substance with good permeability [103]. El Zaafarany *et al.* [104] compared the characteristics of diclofenac topical transfersomes prepared by means of two manufacturing methods, with differing active ingredient contents and phospholipid:EA ratios, and using five variations of surfactants as EAs. The preparation methods used were vortex sonication and rotary evaporator sonication. The manufacturing method has a significant effect, with the transfersome prepared by the rotary evaporator (thin film) and sonication method producing higher trapping efficiency than the vortex and sonication method due to perfect hydration of the vesicles. In the vortex method, visual observation shows that lipids tend to collect and adhere to the vial walls, rendering difficult hydration of the vesicles. The vortex method is unable to disperse lipids completely, resulting in a clumpy dispersion, difficult homogenization and susceptibility to rapid sedimentation and aggregation [104].

Adding a specific amount of the active substance to the transfersomes affects the loading capacities. Consequently, if it exceeds the optimal capacity of the vesicles, precipitation of active ingredients will occur. The phospholipids:EA ratio also greatly affects transfersome vesicles' characteristics. Optimum deformability is obtained from a phospholipids:EA ratio of 85:15. If the amount of phospholipids is excessive, vesicles will form with low deformability due to a lack of surfactant. A similar phenomenon will occur if too great a quantity of the surfactant is added due to the formation of a rigid micelle mixture [104].

The use of various types of surfactants possessing different chemical structures also results in contrasting vesicle characteristics. Comparing the effect of surfactant types with the optimal phospholipid:surfactant ratio, it was found that the vesicles containing Tween 80 had the highest deformability. This is due to the fact that Tween 80 is composed of flexible, non-bulky hydrocarbon chains. In contrast, the sodium cholate confers lower deformability due to its steroid-like structure, which is larger than the hydrocarbon chain of Tween 80. However, in terms of the entrapment efficiency values, the order is systems containing Span® 85 > Span 80 > Na cholate > Na deoxycholate > Tween 80. The use of Tween 80 in transfersomes loading diclofenac sodium effectively improved deformability of the vesicles, thus increasing skin delivery in non-occlusive topical application [104].

Nifedipine, an antihypertensive drug, has been reported to effectively repair wrinkles as well as promote skin elasticity and hydration when delivered as a 0.5% topical preparation [105]. It blocks muscular contraction and relaxes facial muscular fibers, thus reducing the depth of wrinkles [106]. On the other hand, nifedipine demonstrates very low solubility in water with a high partition coefficient, which limits its use in dermal delivery [107]. Nifedipine constitutes a transdermal protransfersome preparation produced by a coacervation phase separation method. The protransfersome consists of phospholipid and sodium deoxycholate at a weight ratio of 85:15 and produces a bioavailability 6.5-times greater than that of oral administration. This is supported by a high entrapment efficiency of up to 97% and an increase in penetration ability up to three-times greater than the drug suspension, triggering an increase in the drug's antihypertensive effectiveness [64].

Raloxifene hydrochloride is an active therapeutic compound used in the treatment of breast cancer and osteoporosis, but it has low bioavailability. It has been claimed in recent reports that raloxifene is able to improve both collagen synthesis by fibroblasts in human skin tissue and skin elasticity due to its effects on selective estrogen receptor modulators [108,109]. Mahmood *et al.* [26] succeeded in increasing its bioavailability by formulating it into transfersomes for transdermal delivery. The formula was designed with a Box–Behnken design composed of Phospholipon® 90G and sodium deoxycholate at a weight ratio of 300:35, resulting in vesicles with high entrapment efficiency, good stability and high penetration rates.

Transfersomes are also used for delivery of glimepiride, which is an oral antidiabetic drug. The side effects of hypoglycemia, as well as digestive and hepatic disorders that often occur, can be reduced by the ability to release it gradually, thereby increasing patient compliance. The Box–Benkhen design was used in a transdermal transfersome formulation which has a weight ratio of phospholipids:sodium deoxycholate:glimepiride = 200:45:1. Positive vesicle characteristics were obtained, thereby increasing effectiveness due to high penetration into the skin's deeper layers, and showed a higher penetration flux than glimepiride suspension [41]. Increased drug bioavailability, which reduced both the side effects on the gastrointestinal tract and the long-term therapeutic effects due to lower quantities of drugs being used during the therapy, was superior to the oral administration of glimepiride.

The antituberculosis drug rifampicin can be prepared as a transfersome to improve its transdermal bioavailability and patient compliance due to continuous drug release. A comparison of the base of the gel and the suspension confirmed the particle sizes to be similar, but the ζ-potential of the gel was more negative because of the acidity of carbopol as the gelling agent. In addition, the permeation value, depot effect and bioavailability of gel preparations were greater due to the composition of the formula containing Phospholipon 90G and Tween 80 at a weight ratio of 15:7 between ethanol and D-limonene [72].

The capability of transfersomes to encapsulate hydrophobic molecules with physicochemical properties similar to those of glimepiride and rifampicin – such as coenzyme Q10 [110], α-tocopherol [111], idebenone [112], α-lipoic acid [112,113], ferulic acid [114] and tretinoin [115] – within the lipid bilayer would enable modification of physicochemical properties of active ingredients encapsulated in carriers which are nano-sized particles, amphiphilic self-assembling phospholipids with surfactant presence, thus affecting their dispersibility, solubilization and release into aqueous media at the intended sites, especially dermis, for anti-aging therapy [116].

Active substances with high solubility & low permeability

There are several active substances within this category, including epigallocatechin-3-gallate (EGCG), 5-fluorouracil and methotrexate. The study of the use of transfersomes for delivery of a combination of EGCG and hyaluronic acid as an antioxidant for topical application has been reported. The transfersome was prepared by a combination of thin-layer hydration and high pressure homogenization methods. The formula optimization was performed using a Box–Behnken design prepared with phosphatidylcholine:sodium cholate at a weight ratio of 85:15, resulting in increased UV protection and promoting EGCG's antioxidant and anti-aging effects [39].

It has previously been reported that 5-fluorouracil can be used to manage actinic keratosis and is able to induce collagen synthesis during matrix remodeling and wound healing through a 5% topical administration, reversing photo-aging [117]. The use of transfersomes dispersed in a carbopol-based gel has been observed to successfully enhance penetration through hypertrophic scar tissue to the dermal layer, even penetrating deeper skin layers without physical changes or allergic reaction [118]. Another report suggested that using Tween 80 as the EA in transfersome-loaded carbopol gel significantly improves skin deposition and penetration of 5-fluorouracil [119].

As a potent analgesic, ketorolac can be formulated for transdermal delivery, which has the advantage of gradual release, thus reducing the gastrointestinal side effects that often accompany it. To overcome the low permeability of ketorolac [120], Nava *et al.* [52] succeeded in formulating it into transfersomes consisting of Epikuron™ 200 and Tween 80 at a respective weight ratio of 86:14. The transfersome has a particle size of approximately 127.8 nm, with a low polydispersity index, a relatively neutral charge with a ζ-potential of -12 mV and high entrapment efficiency of 73.11%. Moreover, its release is delayed, causing it to remain in the skin for a long period, thus producing local therapeutic effects [52].

Transfersome vesicles possess the ability to modify the permeability of active ingredients due to encapsulation within the carrier which can change passive diffusion into active transport, allowing low permeable ketorolac-like active cosmetic ingredients, such as ascorbic acid [121], to permeate biological membranes. The use of biomimetic phospholipid as a component of transfersomes would enable vesicles to carry active ingredients via the paracellular or transcellular routes, among others, or through fusion with the cell membrane. This underpins the potential of transfersomes to deliver active ingredients promoting dermal repair and rejuvenation [122].

Active substances with low solubility & low permeability

In transdermal delivery, the active ingredients should be dissolved to maximally penetrate the skin. To overcome the problem of low solubility and low permeability of active ingredients, transfersomes are used as the carriers as they have been shown to successfully deliver active ingredients including curcumin, psoralen, cisplatin, paclitaxel and ketorolac to the deeper layers of the skin [36,38,44,52,123]. Numerous reports have demonstrated that curcumin can be a potential agent for reversing aging. Its high antioxidant capacity offers protection against the negative effects of free radicals, as well as anti-inflammatory effects which potentially stimulate the production of TGF-β and fibroblasts, while also inducing extracellular matrix production and angiogenesis, which both play a significant role in repairing skin and maintaining its health [124–127]. Curcumin has been seen to demonstrate low water solubility and poor permeability for oral and topical delivery [128]. The low bioavailability of curcumin can be increased by transfersomes prepared with purified phosphatidylcholine (Epikuron 200) as the phospholipid and sodium cholate as the surfactant, at a weight ratio of 85:15 using a thin-layer hydration method followed by extrusion. These nanovesicles' characteristics, including small and homogeneous particle size with high entrapment efficiency (up to 93.91%) and loading amount of 7.04% with improved skin permeability, proved useful in increasing antitumor activity [36].

Cisplatin is a platinum-based chemotherapeutic agent with extremely low skin penetration through the main route of skin appendages [129]. Moreover, it also demonstrates limited solubility in water and, consequently, often requires solubilizing agents as well as absorption enhancers to improve its effects [130]. Transfersomes composed of soya lecithin and sodium cholate at a weight ratio of 17:3 produced a gradual release of cisplatin, thereby reducing its side effects on healthy cells. Cisplatin, either alone or together with a stabilizer such as a combination of soya lecithin:Pluronic:sodium cholate at respective weight ratios of 17:1.5:1.5 or other antioxidants produced positive nanovesicle characteristics with small and homogenous particle size and high entrapment efficiency (up to 97.97%), thus increasing anticancer effectiveness in skin melanoma therapy [19,43,131]. The use of protransfersomes and transfersomes also improved cisplatin levels in plasma during transdermal application, which proves these ultradeformable vesicles successfully enhance penetration of poorly soluble and poorly permeable active ingredients such as cisplatin [14].

The use of transfersomes for the transdermal delivery of methotrexate can increase the effect of drug deposition in the skin and can release the drug efficiently. Transfersomes prepared at a phosphatidylcholine:Tween 80 weight ratio of 7:3 were shown to be superior to conventional liposomes in delivering drugs into the deeper skin layers [132]. In combination with resveratrol, they can increase the anticancer activity of methotrexate against skin melanoma and some squamous cell carcinomas such as actinic keratosis, Bowen's disease and keratoacanthoma [45].

Methotrexate was formulated by an extrusion method using phosphatidylcholine and with two types of EAs (Tween 80 and sodium cholate) to compare its physicochemical characteristics and penetration abilities across skin [47]. From the study, it was clear that the resulting transfersome had a homogeneous and stable unilamellar structure and could increase the penetration of methotrexate into the skin layer by up to five-times. As the EA, Tween 80 was more effective at increasing vesicle deformability than sodium cholate [47].

According to these results, transfersomes and protransfersomes are able to improve the solubility and permeability of active cosmetic ingredients with low water solubility and poor permeability, such as kinetin [133,134] and superoxide dismutase, which also has a high molecular weight (30 kDa) [135]. Their ability to entrap hydrophobic molecules within the lipid domain of the bilayer membrane, as well as the amphiphilic properties of the phospholipids used in transfersomes, significantly improves the solubility and permeability of such compounds, rendering them useful in delivering active cosmetic ingredients.

In vitro evaluation of transfersomes & protransfersomes

Several nanocarrier lipids, both conventional and elastic liposomes, have different characteristics of vesicle shapes depending on their constituent components, namely surfactant for transfersomes and ethanol for ethosomes. From microscopic observation, it is clear that all of them are spherical vesicles, but have different vesicle sizes, as can be seen in transmission electron micrographs [61].

During hydration in the presence of water, the protransfersome gel with lamellar appearance transforms into transfersomes due to the hydrating fluid being absorbed by the gel system [136]. This hydrated gel forms spherical vesicular structures due to the different degrees of hydration between surfactants and phospholipids. Starting from the protransfersome with a limited amount of solvent, a mixture of lamellar liquid crystals is formed which resembles the interrelated palisade and vesiculated lamellae. The addition of excess water will cause swelling of the lipid bilayer due to the interaction of water with the surfactant hydrophilic groups above the solvent threshold concentration, with the result that the bilayer randomly forms a spherical structure which resembles a vesicle [22] and can be described as presented in Figure 2.

The increase in vesicle deformability is also evidenced by the increasing amount of active ingredients penetrating the skin, which is the important factor in efficient skin permeation. This deformability is highly influenced by the presence of an EA in the form of a single-chain surfactant with a high radius of curvature, which renders the vesicles unstable and enables the double layers of vesicles to change shape easily [137]. EAs reduce the energy required to deform the vesicles, with the result that transfersome vesicles can flex to pass through tiny pores in the skin or through intercellular gaps [26]. However, this deformability can be reduced when the amount of surfactant increases [73].

The lipid lamellae in the SC have a high proportion of negatively charged lipids [138]. Consequently, the ionically charged surfactant affects the penetration of the active substances. Vesicles with cationic surfactants can increase the penetration of active substances to a greater extent than anionic or non-ionic surfactants, as revealed by the considerable fluorescent intensity of labeling agents entrapped in transfersomes. This result is due to electrostatic attraction to the negative charge in the SC. This difference in charge can strengthen the interaction between cationic transfersomes and intracellular lipids [137].

Release studies of active substances from the carrier can be used to predict how the carrier can deliver active ingredients and produce therapeutic effects before being tested *in vivo*, which is an expensive process. In the *in vitro* release test using a Franz diffusion cell, the active substances' release from transfersomes is limited by two barriers, namely the phospholipid and the dialysis membrane. The concentration of EA has an effect on the release of active ingredients which is directly proportional. If the concentration is low, then the release of the active substances is similarly low. This is because the lipid membrane becomes regular and does not leak easily. Meanwhile, if the concentration of EA is excessive, the vesicles will be stiffer, with the result that they leak easily and are less sensitive to osmotic gradients [27]. Pena-Rodríguez *et al.* [76] studied the penetration of retinyl palmitate by comparing transfersomes composed of phosphatidylcholine and Tween 80 with free active ingredients. They found that about 69% of conventional liposome-loaded retinyl palmitate could not penetrate the skin and that only 2% reached the epidermis to be retained in the SC. Lipid vesicles can act as a reservoir system for the continuous delivery of active cosmetic ingredients. However, the vesicles of the anionic surfactant deviate from the first-order kinetics of drug release following the diffusion flow of the skin [137].

El-Alim *et al.* [56] compared the release rate of diflunisal in solution with those of liposomes, ethosomes and transfersomes. The results showed that within 2 h the amount of diflunisal released from the solution was 84.52%, higher than that released from liposomes (68.10%) ethosomes (58.21%) or transfersomes (65.88%). The peak level of diflunisal release in solution was reached within 3 h, whereas diflunisal in vesicles continues for up to 5 h before reaching peak levels.

In vivo evaluation of transfersomes & protransfersomes

From several studies it is known that skin penetration by drugs can be via intercellular or transcellular routes. Transfersomes can pass through these routes due to their elastic properties and the water concentration gradient in the skin layer. The nature of this tendency to attract water triggers the vesicles' ability to penetrate the deeper layers of the skin because of their higher water content. After entering the dermis, the active substances will circulate through the blood vessels to the systemic blood circulation. Due to the higher drug penetration, effectiveness also increases.

The pharmacokinetic study in mice conducted by Jain *et al.* [22] indicated that levonorgestrel levels in blood plasma were very low for free active ingredient (0.015 ± 0.005 µg/ml), in contrast to the transfersome-loaded levonorgestrel, which reached levels of 0.139 ± 0.050 µg/ml after topical aplication. The level rose to approximately eight-times higher within 4 h and was maintained for up to 48 h. Therefore it can be proved that by using transfersomes, levonorgestrel can be gradually released over a protracted period.

A similar study was performed by Hussain *et al.* [72], who compared the plasma levels after oral administration of rifampicin and transdermal application of transfersome-loaded rifampicin. The comparative data for C_{max} and T_{max} indicated levels of 10.5 ± 1.4 µg/ml after 2.0 h and 6.9 ± 0.80 µg/ml after 10.6 h, respectively, for oral administration. Meanwhile, the AUC value of rifamipicin after 24 h for oral administration was 41.71 ± 5.2 µg/ml, while for transdermal application it was 56.23 ± 2.7 µg/ml. This suggests that the use of transfersomes for transdermal administration can increase the systemic availability of rifampicin by reducing the dose-related side effects and toxicity of the orally administered rifampicin.

An *in vivo* test using tape stripping was used by Fernández-García *et al.* [70] to compare amphotericin B levels in the SC and dermis after the application of amphotericin B transfersomes to undamaged skin and by microneedle. This study proved that amphotericin B transfersomes can penetrate to the deeper layers of the skin, while using a microneedle before the application of amphotericin B transfersomes resulted in increased penetration of the active ingredient during the first hour, especially in deeper skin areas. The use of microneedles produces temporary skin micropores that aid drug delivery throughout the skin. However, these micropores close within 2 h and scar tissue is formed which can reduce the surface area for the active ingredient [29]. In this study, there was no significant difference in the degree of skin penetration between transfersome-loaded amphotericin B and amphotericin B added to dimethyl sulfoxide as a skin penetration enhancer. This study proved that the transfersome is capable of acting as an enhancer in itself.

Transfersomes are largely evaluated *in vivo* through the use of both human and animal subjects. In human subjects, the transfersomes can be assessed for their transepidermal water loss value both before and after application. From the results of the tape strip, it is known that there is no significant difference in this value, therefore confirming that the transfersomes do not affect skin integrity [76]. Although transfersomes can act as a depot for epidermal absorption, the SC is desquamated, with the result that the active ingredient can be lost. In one study, by using transfersomes, about 63% of the retinyl palmitate successfully penetrated the epidermis [76]. Fluorescent photomicrographs showed that the transfersomes contained Nile red, indicating that transfersomes can deliver active ingredients penetrating the deeper layers of the skin [76]. Moreover, the fluorescence correlated with transfersomes was extensively observed in the space between the corneocytes in the epidermis [19].

Arora *et al.* [25] studied penetration of the antioxidant resveratrol by transfersome carriers composed of soya phospholipids and sodium cholate at a weight ratio of 85:15. At an appropriately high phospholipid content level, the lipophilic resveratrol can be trapped within the lamellar lipids of vesicles. The use of transfersomes successfully increased the penetration of resveratrol, thus improving the *ex vivo* antioxidant activity as determined by the 2,2-diphenyl-1-picrylhydrazyl test. This improved effectiveness is due to an increased flux of active ingredients caused by disrupting the SC barrier through an amalgam effect of a combination of phospholipids and surfactants. In addition, the skin-penetrating amount of vesicle-entrapped active ingredients was increased due to the longer residence time in the skin.

In albino Wistar rat subjects, the application of timolol-loaded transfersomes composed of phosphatidylcholine:Span 80 and Tween 80 at a weight ratio of 3:1 to the shaved back skin was observed to reduce the occurrence of erythema and edema compared with conventional liposomes. Neither erythema nor edema occurred after this *in vivo* application [24].

Discussion

The formulation of ultradeformable vesicles (transfersomes and protransfersomes) can be seen to increase the effectiveness of active ingredients due to improvements in their physicochemical characteristics and skin penetration. With the combination of phospholipids that resemble skin membranes and the addition of surfactants as EAs, the formation of vesicles can reduce the particle size, enabling them to easily penetrate intercellular gaps and skin pores. The ability to deliver active ingredients with various characteristics of lipophilicity, solubility, permeability and high molecular weight – including proteins, RNA and hormones – also constitutes an advantage of this delivery system. Transfersomes can be applied in the cosmetics industry because the research conducted indicates that the use of a base preparation including gel and cream neither changes the skin penetration profile nor reduces the effectiveness of the active ingredient. Rather, it can increase the length of time the drug remains in the skin and product acceptability [8,94].

It is expected that transfersomes and protransfersomes can potentially be used in the cosmetic field with local biological effects, especially in anti-aging products. Skin aging is known to be caused by the presence of reactive oxygen species that induce oxidative stress in cells, reduce cell proliferation and disrupt the dermal extracellular matrix [139,140]. However, active cosmetic ingredients used in anti-aging therapy, such as CoQ10 and AMSC-MP, suffer from skin penetration-related drawbacks including low water solubility and high molecular weight. The use of transfersomes and protransfersomes may facilitate the penetration by active cosmetic ingredients of the deep skin layers, in particular the dermis, which is composed of almost 70% collagen [141]. With increased skin penetration, the effectiveness and stability of cosmetic products will be improved, providing potential use for beauty and health.

Conclusion & future perspective

Transfersomes and protransfersomes demonstrate encouraging potential for use in cosmetics, especially anti-aging products. The use of phospholipids and EAs in these carriers has benefits for producing nanovesicles with desirable characteristics supporting high skin penetration, thus increasing the effectiveness of active cosmetic ingredients.

Delivering cosmetic active ingredients to target sites, especially for agents affecting biological functions, can be ultimately supported by appropriate delivery carriers. This review represents the underlying researches in topical or transdermal delivery of active ingredients to the development of therapeutic products for esthetic medicines and cosmetics. A positive approach of the use of ultradeformable carriers (transfersomes) and their provesicular states (protransfersomes) has been largely explored to improve skin penetration by utilizing the natural characteristics of phospholipids and EAs to form intact flexible vesicles which pass through intercellular gaps. As delivery carriers, these deformable vesicles show great potential for transporting either hydrophobic or hydrophilic molecules with low or even high molecular weight (such as proteins) to penetrate into deeper skin tissues, which become the main target sites of most cosmetics, especially for anti-aging therapy. Further explorations and investigations are definitely required to comprehensively evaluate the potential use of ultradeformable vesicles in improving the efficacy of cosmeceuticals, which is currently still limited.

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Executive summary

Transfersomes encapsulate various active ingredients

- Transfersomes are a vesicular drug carrier that consists of a bilayer membrane composed of phospholipids and biosurfactants at the appropriate ratio surrounding an inner aqueous phase.
- Active ingredients with hydrophilic properties will be encapsulated in the aqueous core, while lipophilic active ingredients can be trapped within the lipid membrane layer.
- Transfersomes also provide the possibility of encapsulating active ingredients with high molecular weight.

Ultradeformable liposomes as vesicular drug carriers for skin cosmetics

- Ultradeformable vesicles can change shape and pass through the pores intact by shrinking to five- to ten-times smaller than the original, due to the transepidermal osmotic gradient.
- Transfersomes can penetrate the skin layers by means of different routes: the intercellular pathway, transcellular route and appendageal route.

Ultradeformable liposomes improve skin delivery of active cosmetic ingredients

- The lipid vesicles of transfersomes can act as a reservoir system for the continuous delivery of active cosmetic ingredients.
- The transfersome is capable of acting as an enhancer in itself.
- The skin-penetrating amount of transfersome-entrapped active ingredients is increased due to the longer residence time in the skin.
- Neither erythema nor edema occurred after the *in vivo* application of transfersomes.

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