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Review Article

A REVIEW ON TRANSFEROSOME: IS A BOON TO HUMAN LIFE

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Abstract: *Transfersome* is a highly adaptable and stress-responsive, complex aggregate. Its preferred form is an ultradeformable vesicle possessing an aqueous core surrounded by the complex lipid bilayer. Interdependency of local composition and shape of the bilayer makes the vesicle both self-regulating and self-optimising. This enables the *Transfersome* to cross various transport barriers efficiently, and then act as a Drug carrier for non-invasive targeted drug delivery and sustained release of therapeutic agents. Large biogenic or biotechnologic molecules are normally delivered into the body by means of an injection needle. However, numerous and ingenious attempts were made to improve on this.
Key**words:** Transferosome, liposomes, lipid barriers, permeability

INTRODUCTION

Transfersome is a term registered as a trademark by the German company IDEA AG, and used by it to refer to its proprietary drug delivery technology. The name means "carrying body", and is derived from the Latin word 'transferre', meaning 'to carry across', and the Greek word 'soma', for a 'body'. A *Transfersome* carrier is an artificial vesicle designed to be like a cell vesicle or a cell engaged in exocytosis, and thus suitable for controlled and, potentially targeted, drug delivery.

Discovery

The term *Transfersome* and the underlying concept were introduced in 1991 by Gregor Cevc. Numerous groups have since been working with similar carriers, frequently under different names (elastic vesicle, flexible vesicle, Ethosome, etc.) to describe them.

In broadest sense, a *Transfersome* is a highly adaptable and stress-responsive, complex aggregate. Its preferred form is an ultradeformable vesicle possessing an aqueous core surrounded by the complex lipid bilayer. Interdependency of local composition and shape of the bilayer makes the vesicle both self-regulating and self-optimising. This enables the *Transfersome* to cross various transport barriers efficiently, and then act as a Drug carrier for non-invasive targeted drug delivery and sustained release of therapeutic agents.

Large biogenic or biotechnologic molecules are normally delivered into the body by means of an injection needle. However, numerous and ingenious attempts were made to improve on this[1,2]. They were based on inventive galenic formulations, including oral polymer[3], liposome[4] or microemulsion[5,6] suspensions, on the technical innovations, such as subcutaneous reservoirs [7] and pumps [8], or on the unusual, e.g. rectal [9], periocular[10], intranasal[11] or dermal[6] applications. None of these approaches to date gave completely satisfactory results due to various reasons. Adverse side effects, drug metabolism by first pass effect in the liver, poor patient compliance, or rejection of an invasive medication often hamper the success and efficacy of therapeutic treatment. To overcome these problems many drug carriers were developed such as Liposomes, dendrimers, and other complex polymers system. Transport of the drug through skin is best route of drug delivery because of the skin is largest organ human organ with total weight 3 kg and a surface of 1.5 -2.0 m2. Drug carries used in transdermal drug delivery such as liposomes, noisomes, or microemulsions has problem that they remains mostly confined to the skin surface and therefore do not transport drugs efficiently through the skin[12]. By using the concept of rational membrane design[13] we have recently devised special composite bodies, so-called Transfersomes[14], which overcome the filtration problem and penetrate the skin barrier along the transcutaneous moisture gradient. Transfersomes are sufficiently flexible to pass even through the pores appreciably smalle than their own size. By optimally matching the penetrant adaptability to the transportinduced stress the size-exclusion principle is evaded nearly completely. The resulting highly deformable vesicles then pass through the narrow, otherwise confining, pores with the efficacy of water, 1000 times smaller. This leads the carrier through the "virtual" pores between the cells in the organ without affecting its biological and general barrier properties. Owing to this unusual barrier penetration mechanism, transfersome carriers can create a highly concentrated drug depot in skin[15], deliver material into deep subcutaneous tissue[16],or even deliver the drug into the systemic circulation[17]. Transfersome® vesicles are mixed lipid aggregates with an ultra-adaptable bilayer membrane and are suitable for transdermal drug delivery. The carriers are composed of at least one bilayer-forming lipid and one or more bilayer softening amphiphiles, e.g. a phospholipid and a surfactant .The mixed lipid vesicle is able to penetrate fine skin pores smaller than its average size, driven by hydration gradient in the mammalian skin barrier, i.e. the epidermis, and more specifically spoken the

stratum corneum In contrast to diffusion based, more conventional, transdermal drug delivery systems, TDDS, such as gels, crèmes, ointments and patches, the carrier based delivery systems can transport drug substances more or less independent of their molecular weight and lipophilicity/hydrophilicity through the natural skin barrier, and into deeper, peripheral tissues.

Simple, stiff soybean phosphatidylcholine (SPC) vesicles, i.e. the conventional liposomes, can be transformed into flexible Transfersome® by addition of membrane softening surfactants, such as polyoxyethylene (20) oleyl ether (C18:1EO20), as is described prior in this work. In this study, the amphiphilic molecules ethanol, bupivacaine, and ketoprofen were combined with C18:1EO20 saturated SPC mixed lipid bilayer vesicles having an effective surfactant to lipid molar ratio of *Re* = 0.25 mol/mol. The combination served the major objective of this study: development of different polyoxyethylene (20) oleyl ether based Transfersome® formulations for optimum transdermal drug delivery. Bupivacaine is a cationic, long acting local anaesthetic of the anilide type, which to date is mainly applied by infiltration, peripheral, epidural, and spinal injection. The two former methods are predestined for drug delivery from a topically applied formulation to the site of local anaesthesia and analgesia. Unfortunately, the current, medicinal products comprising mainly lidocaine have only have a short and weak analgetic effect, let alone an anaesthetic one. The development of locally acting, bupivacaine containing, ultra-adaptable lipid carrier formulations could lead to stronger and longer acting anaesthesia, due to the four times higher potency of bupivacaine compared to lidocaine (Ruetsch et al. 2001). The expectation is also supported by the published data of Grant et al. (2001; 2004) who reported prolonged bupivacaine action after subcutaneously injection of the drug in combination with liposomes.

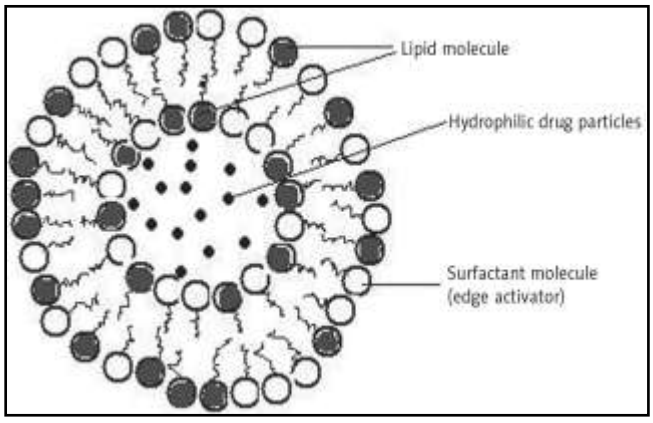

Fig. Structural representation of one transfersome unit

Silent features of Transfersomes

- Transfersomes possess an infrastructure consisting of hydrophobic and hydrophilic moieties
- together and as a result can accommodate drug molecules with wide range of solubility.
- Transfersomes can deform and pass through narrow constriction (from 5 to
- 10 times less than their own diameter) without measurable loss.
- This high deformability gives better penetration of intact vesicles.
- They can act as a carrier for low as well as high molecular weight drugs e.g. analgesic, anesthetic, corticosteroids, sex hormone, anticancer, insulin, gap junction protein, and albumin.
- They are biocompatible and biodegradable as they are made from natural phospholipids similar to liposomes.
- They have high entrapment efficiency, in case of lipophilic drug near to 90%.
- They protect the encapsulated drug from metabolic degradation.
- They act as depot, releasing their contents slowly and gradually.
- They can be used for both systemic as well as topical delivery of drug.

Product development of transfersomes

Scope of transfersomes is mainly intended for topical application although other routes may be considered for further investigations. Drug should be selected in such a way that it fi ts in the criteria of topical delivery. It should have ideal limits for aqueous solubility, lipophilicity, molecular size, melting point and pH of the aqueous saturated solution.

Limitations of transfersomes[1,2]

- Transfersomes are chemically
- unstable because of their
- predisposition to oxidative
- degradation.
- Purity of natural phospholipids is
- another criteria militating against
- adoption of transfersomes as drug
- delivery vehicles.
- Transfersomes formulations are

Scope of transfersomes[5,7]

Transfersome technology is best suited for non-invasive delivery of therapeutic molecules across open biological barriers. The Transfersome vesicles can transport across the skin, for example, molecules that are too big to diffuse through the barrier. Examples include systemic delivery of therapeutically meaningful amounts of macromolecules, such as insulin or interferon, across intact mammalian skin. Other applications include the transport of small molecule drugs which have certain physicochemical properties which would otherwise prevent them from diffusing across the barrier. Another attraction of the Transfersome technolog is the carrier's ability to target peripheral, subcutaneous tissue. This ability relies on minimisation of the carrier associated drug clearance through cutaneous blood vessels plexus: the non-fenestrated blood capillary walls in the skin together with the tight junctions between endothelial cells preclude vesicles getting directly into blood, thus maximizing local drug retention and propensity to reach the peripheral tissue targets.

Mechanism of Penetration of Transfersomes

Transfersomes when applied under suitable condition can transfer 0.1 mg of lipid per hour and cm2 area across the intact skin. This value is substantially higher than that which is typically driven by the transdermal concentration gradients. 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 [18]. This osmotic gradient is developed due to the skin penetration barrier, prevents water loss through the skin and maintains a water activity difference in the viable part of the epidermis (75% water content) and nearly completely dry stratum corneum, near to the skin surface (15% water content)[19]. This gradient is very stable because ambient air is a perfect sink for the water molecule 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 [20,21]. 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 [22, 23], So when lipid suspension (transfersomes) is placed on the skin surface, that is 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[19]. 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 than that responsible for their greater deformability[23] less deformable vesicles including standard liposomes are confined to the skin surface, where they dehydrate completely and fuse, so they have less penetration power than transfersomes. Transfersomes are optimized in this respect and thus attain maximum flexibility, so they can take full advantages of the transepidermal osmotic gradient (water concentration gradient)[24]. Transfersome vesicle can therefore adapt its shape to ambient easily and rapidly, by adjusting local concentration of each bilayer component to the local stress experienced by the bilayer.

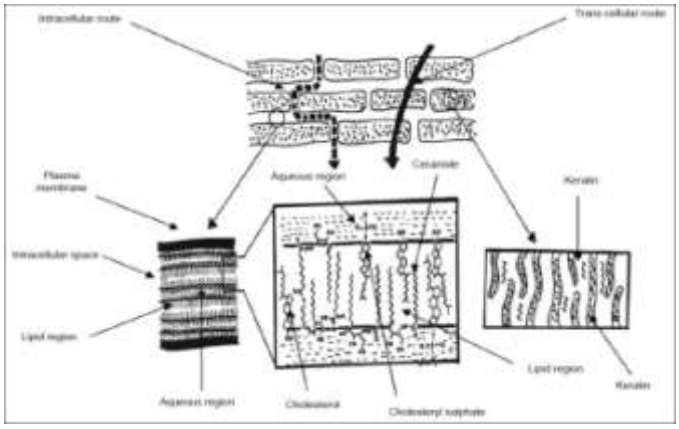

Fig. Diagrammatic Representation of the Stratum Corneum and the Intercellular and Transcellular Routes of Penetration

Characterization

The mechanical properties and transport ability of a vesicle can be studied by measuring stress- or deformation-dependent vesicle bilayer elasticity and permeability changes. In a single experiment the objective may be reached by determining the pressure dependent area density of the *Transfersome* suspension flux through a nano-porous filter, with pores at least 50% smaller than the average vesicle size. For the proper *Transfersome* vesicles, the experiment derived proportionality function, so-called "Penetrability", increases non-linearly with the flux driving force (head pressure), often sigmoidally). The bulk suspension viscosity governs the highest achievable penetrability; a suspension of ideal *Transfersome* vesicles, experiencing no friction in the barrier, therefore yields similar maximum penetrability value as the comparably tested vesicles-suspending fluid. On the other hand, the characteristic pressure needed to enforce a significant transport rate with the vesicles suspension mainly depends on the explored bilayer adaptability. Analysis of experimental Penetrability vs. Driving pressure curves can therefore yield the characteristic bilayer elasticity and permeability values, based on theoretical description of material flow as an activated transport process.

Preparation of Transfersomes

Various published and patented procedure are available for the preparation of transfersome. Generally phosphatidylcholine is mixed in ethanol with sodium cholate or some other biocompatible surfactant. Subsequently a suitable buffer is added to yield a total lipid concentration of 10% w/w. the suspension is then sonicated, frozen, and thawed 2-3 times to catalyze vesicle growth and is finally brought to the preferred vesicle size by pressure homogenization, ultrasonication, or some other mechanical method. Final vesicle size, as determined dynamic light scattering, is approximately 120 nm for a typical transfersome preparation containing 8.7% by weight SPC, 1.3% by weight sodium cholate, and up to 8.5% by volume ethanol. The best carrier composition has to be found experimentally and for each drug separately to obtain appropriate transfersome carriers with maximum deformability and stability[15].

Different Additives Used in Formulation of Transfersome

Class	Example	Uses
Phospholipids	Soya phosphatidyl choline Dipalmitoyl phosphatidyl choline Distearoyl phoshatidyl choline	Vesicles forming component
Surfactant	Sod. cholate Sod.deoxycholate tween-80 Span-80	For Providing flexibility
Alcohol	Sod. cholate Sod.deoxycholate tween-80 Span-80	As a solvent
Dye	Rhodamine-123 Rhodamine-DHPE	For CSLM study

	Fluorescein-DHPE Nilered	
For CSLM study	Saline phosphate buffer (pH 6.4)	As a Hydrating medium

Characterization of Transfersomes

The characterization of transfersomes is generally similar to liposomes, noisomes and micelles.

1. Entrapment Efficiency[31,32]

The entrapment efficiency is expressed as the percentage entrapment of the drug added. Entrapment efficiency was determined by first separation of the unentrapped drug by use of mini-column centrifugation method. After centrifugation, the vesicles were disrupted using 0.1% Triton X-100 or 50% n-propanol. The entrapment efficiency is expressed as:

Entrapment efficiency= (amount entrapped/ total amount added)*100.

2. Vesicles Size and Size Distribution

T he vesicles size and size distribution were determined by dynamic light scattering method (DLS), using a computerized inspection system (Malvern Zetamaster, ZEM 5002, Malvern, U.K.). For vesicles size measurement, vesicular suspension was mixed with the appropriate medium (7% v/v ethanol) and the measurements were conducted in triplicate[26].

3. Degree of Deformability or Permeability Measurement[32,34]

Degree of deformability is an important and unique parameter of transfersomal formulations because it differentiates transfersomes from other vesicular carriers like liposomes that are unable to cross the stratum corneum intact. The deformability study is done against the pure water as standard. Transfersomes preparation is passed through a large number of pores of known size (through a sandwich of different microporous filters, with pore diameter between 50 nm and 400 nm, depending on the starting transfersomes suspension). Particle size and size distributions are noted after each pass by dynamic light scattering (DLS) measurements.

4. Propensity of penetration[34]

The magnitude of the transport driving force plays an important role:

Flow = Area x (Barrier) Permeability x (Trans-barrier) force

Therefore, the chemically driven lipid flow across the skin always decreases dramatically when lipid solution is replaced by the some amount of lipids in a suspension.

5. Confocal Scanning Laser Microscopy (CSLM) study[35]

Conventional light microscopy and electron microscopy both face problem of fixation, sectioning and staining of the skin samples. Often the structures to be examined are actually incompatible with the corresponding processing techniques; these give rise to misinterpretation, but can be minimized by Confocal Scanning Laser Microscopy (CSLM). In this technique lipophilic fluorescence markers are incorporated into the transfersomes and the light emitted by these markers used for following purpose:

(a) For investigating the mechanism of penetration of transfersomes across the skin,

(b) For determining histological organization of the skin (epidermal columns, interdigitation), shapes and architecture of the skin penetration pathways. For comparison and differentiation of the mechanism of penetration of transfersomes with liposomes, niosomes and micelles. Different fluorescence markers used in CSLM study are,

(c) Fluorescein-DHPE(1,2- dihexadecanoyl-sn-glycero-3- phosphoethanolamine-N-(5- fluores denthiocarbamoyl), triethylammonium salt)

(d) Rhodamine-DHPE (1,2- dihexadecanoyl-sn-glycero- 3ogisogietgabikanube-N LissamineTmrhodamine B sulfonyl), triethanolamine salt)

(e) NBD-PE (1, 2-dihexadecanoylsn- glycero-3-phosphoethanolamine-N-(7- nitro-Benz-2- oxa-1, 3-diazol- 4-yl) triethanolamine salt)

(f) Nile red.

6. Number of Vesicles per Cubic mm[33,34]

This is the most important parameter for optimizing the composition and other process variables. Transfersomal formulation (without sonication) was diluted five times with 0.9% of NaCl solution, and the number of transfersomes per cubic mm was counted by optical microscopy by using a haemocytometer.

7. In vitro Drug Release

In vitro drug release study is performed for determining the permeation rate. Time needed to attain steady state permeation and the permeation flux at steady state and the information from in-vitro studies are used to optimize the formulation before more expensive in vivo studies are performed. For determining drug release, transfersomes suspension is incubated at 32°C and samples are taken at different times and the free drug is separated by mini column centrifugation[28]. The amount of drug released is then calculated indirectly from the amount of drug entrapped at zero times as the initial amount (100% entrapped and 0% released).

8. Stability Studies[35]

Transfersomes stability was determined at 4°C and 37°C by TEM visualization and DLS size measurement at different time intervals (30, 45, and 60 days), following vesicles reparation. phospholipids applied in the form of transfersomes after 24 hr is essentially the same after an epicutaneous application or subcutaneous injection of the preparations. When used under different application conditions, transfersomes can also positioned nearly exclusively and essentially quantitatively into the viable skin region.

Usage

Transfersome technology is best suited for non-invasive delivery of therapeutic molecules across open biological barriers. The *Transfersome* vesicles can transport

across the skin, for example, molecules that are too big to diffuse through the barrier. Examples include systemic delivery of therapeutically meaningful amounts of macromolecules, such as insulin or interferon, across intact mammalian skin. Other applications include the transport of small molecule drugs which have certain physicochemical properties which would otherwise prevent them from diffusing across the barrier.

Peripheral tissue targeting

Another attraction of the *Transfersome* technology is the carriers ability to target peripheral, subcutaneous tissue. This ability relies on minimisation of the carrier-associated drug clearance through cutaneous blood vessels plexus: the non-fenestrated blood capillary walls in the skin together with the tight junctions between endothelial cells preclude vesicles getting directly into blood, thus maximising local drug retention and propensity to reach the peripheral tissue targets. The Non-steroidal anti-inflammatory drug (NSAID) ketoprofen in a *Transfersome* formulation gained marketing approval by the Swiss regulatory agency (SwissMedic) in 2007; the product is expected to be marketed under the trademark *Diractin*. Further therapeutic products based on the *Transfersome* technology, according to IDEA AG, are in clinical development.

Manufacturing

Transfersome vesicles are prepared in a similar manner as liposomes, except that no separation of the vesicle-associated and free drug is required. Examples include sonicating[1], extrusion, low shear rates mixing (multilamellar liposomes), or high high-shear homogenisation unilamellar liposomes) of the crude vesicle suspension.

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