



Transfersome vs liposomes as drug delivery vehicle for the treatment of skin cancers

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Article History:

Received on: 02.03.2019

Revised on: 07.06.2019

Accepted on: 11.06.2019

Keywords:

Skin cancer,
Melanoma,
Transfersomes,
Edge activator,
Topical,
Transdermal

ABSTRACT

Skin cancer is one of the leading causes of cancer-related deaths worldwide. It is estimated that by 2030, there will be around 13.1 million deaths can occur due to skin cancer. Current treatment of skin cancer includes surgery, radiation and chemotherapy. Chemo-resistance, off-target effects and poor bioavailability are the major challenges for currently available drugs for the treatment of skin cancer. Transfersomes is a novel, elastic vesicular drug carrier composed of phospholipids, surfactants and ethanol for enhanced transdermal delivery. This article we have reviewed about merits/demerits, liposomes vs transfersomes, preparation methods, characterization, mechanism of skin delivery, and application and regulatory aspects of transfersomes in treating various skin cancers.



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ISSN: 0975-7538

DOI: <https://doi.org/10.26452/ijrps.v10i3.1375>

Production and Hosted by

IJRPS | <https://ijrps.com>

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INTRODUCTION

Skin cancer is one of the leading causes of cancer-related deaths worldwide. According to WHO it was reported that by Geneva on 21 June 2017 estimated number of non-melanomas was 4, 50,000 and 10,000 melanomas in each year in USA, Europe, and Australia in 2030 it will be around 13.1 million deaths can occur due to skin cancer ([American Cancer Society, 2017](#)). The skin cancer can be of following types, (a) Basal cell carcinoma (BCC), (b) Malignant melanoma (MM), (c) squamous cell carcinoma (SCC) together said to be the

non-melanocyte skin cancer (NMSC) ([Simões et al., 2015](#)). Apart from this, there are some prevailing cancer like Kaposi's sarcoma (KS), Merkel cell carcinoma (MCS) Table 1. Chemo-resistance, off-target effects, poor penetration and poor bioavailability, are the major challenges for currently available drugs for the treatment of skin cancer Table 2. Transfersomes is a novel, elastic vesicular drug carrier composed of phospholipids, surfactants and ethanol for enhanced transdermal delivery. Transfersomes offer various advantages like (a) capacity to carry high and low molecular weight drugs (b) prevent drug metabolic degradation, (c) capacity to carry both hydrophilic and hydrophobic drugs (d) They act as the reservoir which releases the drug slowly, and equally into the skin. Transfersomes, therefore, are gaining importance as drug delivery carriers to skin cancer cells. In this review, we have discussed the applications of transfersomes as delivery systems for targeting skin cancer.

Skin Anatomy

The skin is wide spread and comprises 10% of human body weight. The human skin is composed of three layers (a) Epidermis, (b) Dermis, (c) Subcutaneous tissue ([McGrath and Uitto, 2010](#)).

Table 1: Summary of various skin cancers

Types of cancer	Causes	Affected parts in systemic circulation	Occurrence of tumour	Reference
Melanoma skin cancer (Malignant)	UV light causes DNA damage /mutation of BRAF and PTEN	Active melanocyte, common place affected: Male: chest Female: leg	<ul style="list-style-type: none"> • Black/brown melanoma. • Sometimes itches and bleeds. 	(Sulaimon and Kitchell, 2003)
Non-melanoma skin cancer				
Basal cell carcinoma (BCC)	Over exposure to sun leads to modification of Hedgehog ligand.	Mainly in dermis and grows slowly in neck region.	Painless but small glossy blood vessels are seen.	(Jerant et al., 2000)
Squamous cell carcinoma (SCC)	It does immune suppression and exposure to sun leads to loss of genomic integrity.	Squamous layer, grown area like lips, neck, ear, common to fair skin people.	<ul style="list-style-type: none"> • Erythematous. • Appears like crusted. • Forms huge lump. 	(Glass, 1989)
Merkel cell carcinoma (MCC)	MCV which produces change in DNA of lymphocytes which converts completely into lymphomas.	<ul style="list-style-type: none"> • Merkel cell over growth leads to huge tumour. • Occurs in immune system (parts of body) 	Purple lumps.	(Taubenberger, 1918)
Kaposi sarcoma (AIDS-related)	Occurs by KSHV (Kaposi's sarcoma-associated herpesvirus)	Mainly grows on face, lymph nodes are affected.	Huge lymph nodes forms bluish-black patch.	(Mitsuyasu, 1988)

Epidermis

Epidermis is about 0.8-0.06mm thickness and subdivided into five different layers like (a) stratum corneum, (b) stratum granulosum, (c) stratum lucidum, (d) stratum spinosum, (e) stratum basal (Boer et al., 2016). The stratum corneum contains dead cells filled with keratin (70-80%), stratum spinosum contains keratinocyte and stratum basal contains melanocyte, Langerhans and merkel cell. The melanin pigments like eumelanin and Phaeo-melanin. The melanin protects the skin from the UV-induced DNA-damaged by direct immersion of UV photon (Armstrong and Krickler, 2001).

Dermis

The thickness of the dermis is about 3-5mm than the epidermis. They contain elastic connective tissue and collagen fibrils. Collagen is a major strength of the tissue and holds tissue together.

Dermis cell contains mast cell, fibroblasts, lymphocytes and melanocytes. The dermis does not show the same resistance to drug penetration as SC, where the lipophilic drug observed in this layer is reduced (Bruls et al., 1984).

Subcutaneous tissue

Hypodermis is the other name of the subcutaneous tissue, which contains a large number of fats. These are interconnected fatty layers by collagen and elastin fibres. They also have nerve endings, sebaceous glands and hair follicle roots.

Skin Cancer Pathology and Liposomes Vs Transfersomes in Treating Skin Cancers

Causative factors for the development of skin cancer are mainly due to increased exposure to UV-radiation (200-400nm) (Tornaletti and Pfeifer, 1996). It was reported that epidermal keratinocytes are more prone to UV-exposure, and this results in

Table 2: USFDA approved drugs for the treatment of skin cancers

Chemotherapeutic agents	Mechanism of action	Reference
Imiquimod	Stimulation of innate and acquired immune response.it also modifier acts as toll-like receptor 7 agonist	(Sulaimon and Kitchell, 2003)
5-FU (Fluorouracil-Topical)	By inhibition of thymidylate synthase (TS), the rate limiting enzyme in pyrimidine nucleotide synthesis.	(Longley, 2003)
Erivedge(Vismodegib)	Target Hedgehog pathway signalling	(Cirrone and Harris, 2012)
Efudex (Fluorouracil –Topical)	Binding of the DRNA of the drug (FDUMP) and folate cofactor, N5-10-Methylenetetrahydrofolate, to TS to form covalent bound ternary complex.	(Luqmani, 2005)
Melanoma		
Cobimetinib Intron A (Recombinant Interferon Alfa-2b)	MEK inhibitor which binds and inhibit the activity of MEK1, which inhibition of ERK2 phosphorylation, activation and reduction of the tumour cell replication.	(Samatar and Poulikakos, 2014)
Intron A (Recombinant Interferon Alfa-2b)	Inhibition of the cell proliferation effect and reversion of tumour cells to a normal phenotype.	(Mandelli et al., 2010)
Nivolumab	Acts by blocking negative regulated T-cell activation and response which allows the immune system to attack tumour.	(Topalian, 2014)
Opdivo (Nivolumab)	IgG4 binds to PD-1 receptor; blocks its interaction with PD-L1 and PD-L2, releasing PD-1 pathway which inhibit the immune response and anti-tumour immune response.	(Nguyen and Ohashi, 2015)
Vemurafenib	harbouring Activation of activation BRAF (V600E)	(Sosman, 2012)
Merkel cell carcinoma		
Avelumab	IgG1 that's binds to the programmed death-ligand 1(PD-L1) and inhibition binding to its receptor programmed cell death 1 (PD-1)	(Tsang, 2019)
Bavencio	Programmed death ligand 1 (PD-l) blocking antibody	(Bavencio, 2017)

the activation of the carcinogenic signalling pathway. In addition, UV-exposure results in DNA damage by formatting of cyclo-butane, pyrimidine dimers, immune-suppression, oxidative stress and genetic mutation.

As mentioned earlier, the skin acts as the barrier and protection of the human body from various harmful chemicals and radiations. It is significantly divided into epidermis, dermis and hypodermis. The epidermis has the three types of cells; squamous cell, basal cell and between them melanocytes are present. Depending on the location of cancer in these cells, the skin cancer is divided into squamous cell carcinoma,

basal cell carcinoma, and melanoma (Diepgen and Mahler, 2002).

Among these cancers, melanoma is one of the most dangerous skin cancer; these cancers grow when unrecovered DNA damage to cells of the skin. The melanoma is caused by the ultraviolet radiation and tanning beds on the skin layer, which activates mutation leads to rapid replication of cells and formation of the malignant tumours (Banerjee and Harris, 2000). In humans, especially in men, the melanoma mostly found on trunk, neck, head and arms, whereas in women, it is mainly found on legs and arms (Brenn, 2011). The melanocyte starts

to spread around the skin surface usually projects dark spot or unusual growth of mole on the skin. If melanoma is not treated in an early stage, it can grow deeper into the lymphatic vessels and blood vessel which carries into various parts of the body.

The major limitation in skin cancer chemotherapy is delivering the chemotherapeutic agent to the tumour site, especially in melanoma, which is well located beneath the stratum corneum (J.Buster and You, 2012). Among the various strategies developed (microneedles, iontophoresis, sonophoresis, and nanoparticles) to overcome the stratum corneum barrier, nanotechnology based approaches are gaining much attention as they offer advantages like increased permeability, target specificity, a drug-controlled release which reduces unwanted side effects and increase the efficacy of chemotherapy. There are different types of pharmaceutical carrier like liposomes, transfersomes, ethosomes, polymeric micelles, lipid nanoparticles, micro-emulsions, dendrimers and inorganic nanoparticles have been explored in the treatment of skin cancer.

Liposomes

The Nano-technology has been drastically evolved from liposome first generation (Mezei and Gulasekharan, 1980) to neutral and cationic liposome encapsulation of siRNA (Villares *et al.*, 2008). Liposomal drug delivery acts as a solubilising matrix for poorly soluble drugs, penetration enhancement and local drug depot (prolonged control release property) in stratum corneum. The liposomes are generally colloidal, concentric bilayer vesicle where either natural or synthetic lipids completely enclose the aqueous compartment. The necessary components of the liposome drug delivery include phospholipids and cholesterol (fluidity buffer). The cholesterol can be added to phospho-tidyl-choline up to 1:1 or 2:1 molar ratio (Templeton *et al.*, 1997). The detailed description of the composition of liposomes is illustrated in the figure composition Figure 1.

The skin permeation of liposomes works on two basic mechanisms Figure 2 (a). Trans-epidermal: Where liposomes will penetrate through stratum corneum following trans-cellular and trans-appendageal route (penetration through sweat gland and across the hair follicles). (b) Vesicle burst: (Cevc and Blume, 2005) suggested that most of the liposomes generally burst on the skin surface, and they enhance the delivery of drug through stratum corneum (Nguyen and Bouwstra, 2005). However, it is a well-known fact and accepted that liposomes are limited to outer layers of the skin, i.e., stratum corneum due to less amount of

vesicle material found in the deeper layer of the skin (Dreier *et al.*, 2016). Hence, the development of new vesicular systems such as transfersomes and ethosome came into the picture where these intact vesicles pass through deeper layers of skin due to their flexible structure.

Transfersomes

Since, the majority of the studies show that the classical liposomes have no value as carrier for transdermal drug delivery, because they do not penetrate deeper layers in skin, and has the capacity only to remain strong on the upper layer of the stratum corneum, invention and formulation of a new class of highly deformable liposomes which is said to be transfersomes are developed (Cevc and Blume, 2005). The Transfersomes are generally considered as first generation of large deformable vesicles, which was invented by Cevc and Blume in 1992. These vesicles mainly consist of phospholipids, solvent and edge activator (Figure 1). The various phospholipids like Soya-phospho-tidyl-choline, Egg-phospho-tidyl-choline dipalmityl, and Phospho-tidyl-choline-distearyl are used as vehicle forming agents. In transfersomes, the edge activator (Surfactants) plays an important role provides flexibility; these are single-chain surfactants (e.g., Span-80/60 and Tween-80/60). They destabilize the lipid bilayer of the vesicle and enhance the higher deformability by lowering the interfacial tension. Ethanol, the solvent used acts as an enhancer in the formulation, and it provides synergistic penetration.

Further, the addition of ethanol also provides soft flexibility and easy penetration into stratum corneum. The transfersome undergoes stress adjustment of carrier symphony to reduce the struggle of motility in the skin. This allows the transfersome to deliver the core drug associate across the skin effectively and reproducibility (B. Ita *et al.*, 2007).

The transfersome are drug mover system and work by two main factors (Figure 2): (a) High elasticity (deformability) of the vesicle bilayer and (b) Osmotic gradient across the skin. Because of high deformity properties of transfersomes, they will pass through stratum corneum as intact vesicles. With the support of edge activator, they generate trans-epidermal osmotic gradient and squeezes into the stratum corneum, carries the drug across the skin (Cevc and Blume, 2005). The trans-pore hydrostatic force is responsible for the penetration through the stratum corneum. In detail, the interaction between the lipid residue and the proximal water makes the lipid to attract the water molecule towards it's with hydration. With this attraction,

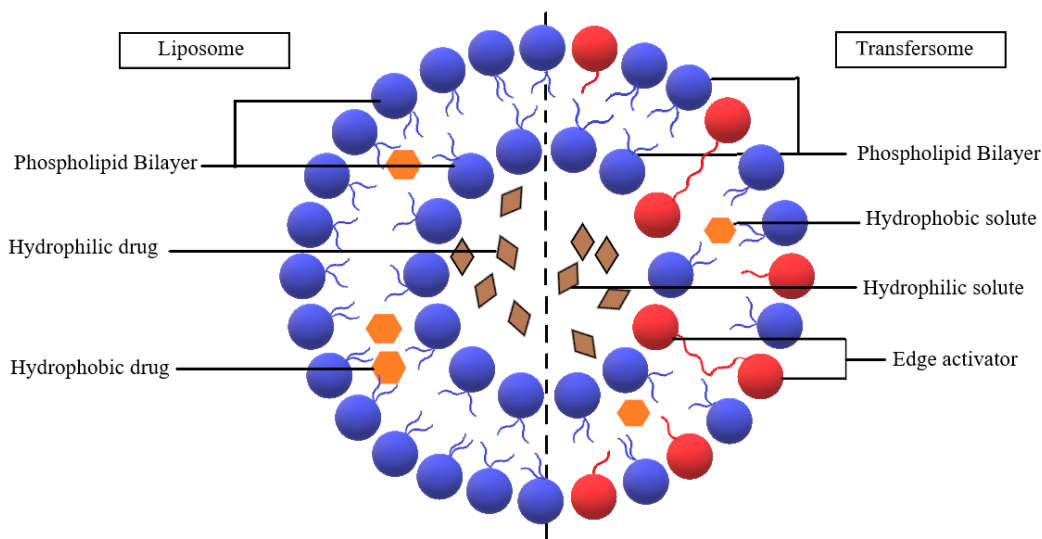


Figure 1: The detailed description of the composition of liposomes

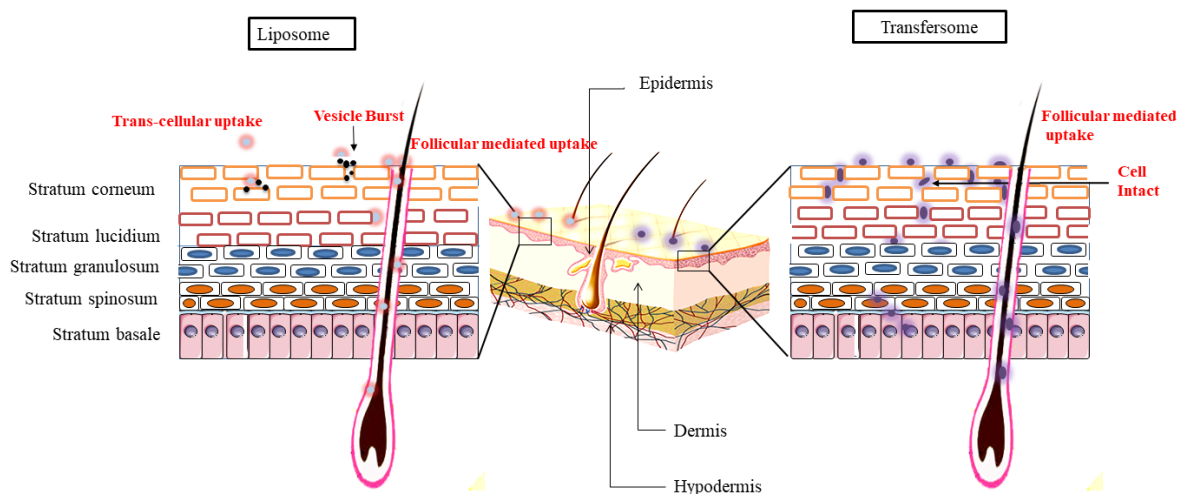


Figure 2: The skin permeation of liposomes works on two basic mechanisms

the lipid vesicle moves towards the site of higher water concentration. This difference in water content across the epidermis and stratum develops the transdermal (osmotic gradient- explain) (Cevca and Gebauera, 1998). This leads to the penetration of transfersomes across the skin. The epithelial obstacles are greatly biased by the flexibility of their membrane, which can be tallied by the relative ratio of surfactant and vehicle (Dubey and Mishra, 2006). The transfersome is adjusted to the change in the composition of membrane locally, and it can reversibly get back when they are forcedly attracted to narrow pores on the skin.

Transfersomes was first introduced by Cevc and Blume in 1992. Transfersomes is the first generation which has deformable vesicle and has the major edge activator. The edge activator is the single-chain surfactant, which the capacity to destabilize the lipid bilayer and enhances the deformability by decreasing the interfacial tension (Harper and Adami, 1993). There are five methods of preparation of transfersomes (a) Rotatory film evaporation method, (b) Reverse-phase evaporation method, (c) Vortexing sonication method, (d) Ethanol injection method, (e) Freeze-thaw method. The major ingredients used in the preparation (Garg et al., 2007).

Preparation of Transfersomes

Rotatory film evaporation method

This method is invented by Bangham, and it also said to be a hand-shaking method. Towards the preparation, the quality of the phospholipids plays a major role in forming the vehicle and surfactants the HLB scale is needed for the optimisation of the preparation. According to the HLB scale, the surfactants should have the hydrophile-lipophile balance character, which will be easy for penetration into the skin. The Tween-80(15-HLB), Span-80(4.3-HLB) in this the crude solvent which is combined of the solution of phospholipids and edge activator and completely dissolved in the methanol or in 2:1 v/v chloroform. It is then transferred into round-bottomed flask continuously rotated it should be kept under the constant temperature and reduced pressure at 40°C (Chen *et al.*, 2013). The film is formed on the sides of the wall on the flask. By using aqueous media, which has the drug film is hydrated with an appropriate buffer solution by rotating at 60 rpm. The vesicle size is allowed to swollen for 2 h at room temperature. The multi-lamellar lipid vesicle (MLV) is sonicated for superior vesicle. The size obtained by extruded through a sandwich of 200 and 450nm polycarbonate membrane.

Reverse-phase evaporation method

This method alters the viscous gel by arranging the vesicles. The two ingredients non-encapsulated and residual solvent may be used for in differentiable by centrifugation. In this method, the lipid is dissolved completely in the round bottom flask. The heating completely removed the solvent at 40°C under reduced pressure. The edge activator is added to the aqueous medium it is done under the nitrogen purging in order to take off the residual traces of the organic solvent (Naeff, 1996) based on the solubility the aqueous or lipid medium is added. After adding it is sonicated till a standardized dispersion, it should not be disturbed for 30min after sonication.

Vortexing sonication method

In this method, it is usually a combined process where mixed lipids, i.e. Phosphatidyl-choline, edge activator and the drug. These all three blended completely in phosphate buffer and allowed to attain milky suspension by Vortexing. After obtaining the suspension, the solution is allowed to sonicate followed by the extrusion process via the polycarbonate membrane. The mixing of the cationic lipids (DOTMA) combined with phosphate buffer solution to get the concentration of about 10 mg/mL continued to action by a count of sodium deoxycholate for the cationic transfersomes. The completed blend is vortexed and again sonicated. The extrusion is done by poly-carbonate (100 nm) filter

membrane (Zhong *et al.*, 2013).

Ethanol injection method

In 1990 the ethanol injection method was implemented in Industrial scale for encapsulating the liposomes of econazole (imidazole derivative) for the dermatomycosis. The principle behind this method was combining with high shear homogenization (Batzri and Korn, 1973). Ethanol injection method consists of three-stage (a) cross-flow injection using y connector, (b) the ultra-filtration is used to remove the organic solvent and concentration, (c) to control the liposomal size the high-pressure extrusion is used with polycarbonate (Mayer *et al.*, 1986). The preparation ethanol solution should have 20-40µmoles of egg lecithin/mL, which is rapidly injected via Hamilton syringe into 0.16M KCL for a maximum of 7.5% ethanol. Then the solution is purged with nitrogen. The 60mL solution completely converted into 1-2mL within the time rate 30-60 min by using the ultrafiltration device with a 43mm diameter XM-100A membrane with continuous rapid stirring under the N₂ pressure (10 lb/inch²). The radioactive phospholipid is passed through the filter. While passing none remains in the membrane. While rapid continuous stirring maintains low-pressure is needed to avoid the formation of larger, were the heterogeneous liposomes and the concentration if the phospholipids couldn't exceed 40mm (Hoenger and McIntosh, 2009).

Freeze-thaw method

The freeze-thaw extrusion method shows the positive result in the preparation of the liposome. This includes the exposure of the very low temperature for freezing continued by exposure towards very high temperature. The multi-lamellar vesicle was obtained by dispersing the dry lipid into 150mM NaCl and 20mM Hepes of pH-7.5 with vortex mixing. By freezing the MLV in Liquid Nitrogen and thawing in 40°C (water bath) the cycle of freeze-thawing is repeated continuously for five times. The frozen-thawed MLV is obtained

Characterisation of Transfersomes

Vesicle size

The computerized inspecting can analyse the size through photon correlation spectroscopy or dynamic light scattering (DLS)

Morphology and structure

Vesicle morphology can be determined using Transmission Electron Microscopy (TEM). The vesicles are diluted with water. Vesicles are mostly evaluated under the negative stain. On the holey film grid, the dilute suspension is kept stained by 1%

aqueous solution of phosphor-tungstic acid, which is allowed to dry and then observed (Hoenger and McIntosh, 2009). The remaining solution is completely removed by the filter paper to avoid the over stain.

Then it is allowed to dry and then examined under 20-80K fold enlargement in Tem with accelerating volt of about 120 KV (Jain *et al.*, 2005).

Entrapment efficacy

The percentage of transfersome drug entrapment was determined once after the preparation is done. The alcohol(absolute) is added and sonicated for 10 min to the transfersome preparation. The concentration of the drug in absolute alcohol can be found spectrophotometrically (Law and .Shih, 2001). The encapsulation efficacy expressed in percentage entrapment was calculated by

$$\text{Entrapment efficacy \%} = \frac{\text{Total drug} - \text{Free drug}}{\text{Total drug}}$$

Turbidity measurement

The transfersomes are diluted with distilled water to give the full transfersomes concentration of 0.312nM. Then the suspension is sonicated thoroughly for 5 min. The measurement of turbidity is done with ultraviolet-visible diode array spectroscopy at 400 nm (Fang *et al.*, 2001).

Transfersomes Applications On Skin Cancer

The overall applications of transfersomes in treating skin cancers are mentioned in Table 3.

Actinic Keratosis

Actinic keratosis is the normal skin cancer caused by long exposure to the sun; it forms on neck, balding scalp, chest, shoulder and arms. However, 75% of reports show that it exists on the neck and head. Actinic keratosis is generally characterised by the shape of the keratotic macules, plaques with superficial scales on a red base. Lesions are mostly itchy, which is asymptomatic (I.Schaefer *et al.*, 2014). Treatment of the actinic keratosis relays on the medical appearance of the lesions. In addition to the therapy, treatment options include excision surgery, photodynamic therapy and topical treatment (Imiquimod cream, 5-FU cream and ingenol mebutate gel).

(Khan *et al.*, 2014) have studied the 5-FU loaded transfersomal gel for skin cancer treatment. The 5-FU, which is an anti-neoplastic drug, is used for the treatment of actinic keratosis and non-melanoma administered topically. The 5-FU drug has poor percutaneous permeation through available creams, which is not suitable for the treatment of deep skin cancer. In this study, 5-FU, transfersomal gel was analysed for skin cancer treatment. By using

different formulations with varying concentration of tween-80 and span-80 (Edge activators), the transfersomes are prepared. The prepared transfersomal vesicles were characterised for particle size, shape, entrapment efficacy, deformability, *in vitro* and *in vivo* study. The prepared formulation was incorporated in 1 % Carbopol gel and evaluated for the efficiency on the skin cancer. 5-FU loaded transfersomes (TT-2) had a size range of 266.9 ± 2.04 nm, entrapment efficacy $69.2 \pm 0.98\%$ and highest deformability index 27.8 ± 1.08 . The transfersomes showed the maximum skin deposition (81.3%) and transdermal flux ($21.46 \mu\text{g}/\text{cm}^2\text{h}$). The prepared transfersomal gel showed a positive result compared with the marketed product (Khan *et al.*, 2014).

Basal cell carcinoma

The basal cell carcinoma shows clear changes in the environment. In North America white population, the frequency has raised 10% a year, which pretends to a lifetime risk of 30% of developing basal cell carcinoma. Basal cell carcinoma is mostly uncommon in the dark-skinned races and develops on type I skins (burns, never tans). Advanced basal cell carcinoma is reported more frequent after freckling in childhood (Zhao and He, 2010). On the other side of Non-ultraviolet ecological exposure, which has the interconnection with the amplified risk of the basal cell carcinoma includes high dietary energy, low intake of vitamins, various chemicals and dust. Patient with immuno-suppressive also has a high risk of basal cell carcinoma in transplant recipients, which is 10 times more than the general population (Hartevelt *et al.*, 1990).

(Fadel *et al.*, 2016) have studied the ICG (Indocyanine green), which is an IR-Fluorescent dye having potential as a photosensitizer in topical photodynamic for skin carcinoma. However, due to its high degradation rate, the merits have been hampered. In this study, the ICG was encapsulated into transfersomes (vesicle colloidal nano-carrier), to enhance its therapeutic effect. The prepared transfersomes were evaluated for entrapment efficacy, zeta potential, particle size, morphology, *in vitro* study and histopathology study on mice skin. The prepared transfersomal gel shows a particle size of 125nm with a negative zeta potential of -31mV. Authors found that transfersomes have shown the sustained release of ICG for a period of more than 2 hrs. After incorporating the prepared transfersomal ICG into a gel, they found the gel maintained normal histology on the mice skin post-irritation along with diode laser 820nm. They achieved 80% clearance by giving the transfersomal ICG for BCC patient with min-

Table 3: Studies on transfersomes for the applications in treating skin cancers

Drug	Year	Ingredients	Results
Indocyanine green (ICG)	2016	HEPES buffer, Sephadex G-100, soy-bean Phosphocholine, Sodium deoxycholate, carboxymethylcellulose sodium salt, chloroform, and ethanol.	The unstable photosensitizer molecule ICG is incorporated into the Transfersomal gel which has given positive result in the BCC. The entrapment efficacy is achieved in F1 (53.29±1.04) Transfersomes has the sustained capacity of releasing the ICG more than 2hrs. They achieved 80% of clearance in patient with minimal pain.by giving Transfersomal ICG gel. (Fadel, 2017)
5-FU	2015	Phospho-tidylcholine, Span-80, Tween-80, polycarbonate membrane filter.	The carbopol based 5-FU Transfersomal gel prepared by the tween-80 shows the better entrapment and edge activator than span-80. Through in vitro skin permeation the showed the better skin retention. 5-FU present in the deeper skin layer for long time. 5-FU loaded Transfersomal gel shows the good carrier for the skin layer for treatment of skin cancer. (Friedman-Kien, 1990)
Paclitaxel	2015	Soya lecithin, span-80, chloroform, Isopropyl alcohol and carbopol 940-gel.	Paclitaxel were actively optimised through the Box-Behnken design. This method is capable of achieving the more efficacy rate of skin AIDS-related KS. The Transfersomal gel showed more diffusion than control group. The fluorescence test shows the deeper observation in the depth of 1487.65 μm . This shows the promising route for the dermal chemotherapy. (Pathak <i>et al.</i> , 2016)
Cisplatin and Imiquimod	2013	Imiquimod pure drug 5%, soya lecithin, cisplatin, sodium cholate, PF-68, DDTC, carbopol-940 gel, organic solvent isopropanol, sodium chloride.	This combination drug shows the enhanced effect of cutaneous epithelial malignancies, therapy shows the potential carrier for topical drug delivery of therapeutic molecule. The combination drug remains same even after disperse in carbopol-940 gel. The retention of the drug in deeper stratum remains longer time. After administration of the topical gel erythema and necrosis were not found. This multi-drug topical delivery shows the better way for skin malignancies. (Gupta <i>et al.</i> , 2012)

imal pain (Fadel *et al.*, 2016).

Kaposi's sarcoma

Hungarian dermatologist Moritz Kaposi first discovered Kaposi's sarcoma in 1872. By that time the Human immunodeficiency virus and acquired immune deficiency syndrome are most prevailing, Kaposi's sarcoma remains the rare tumour. The propagated fulminate from Kaposi's sarcoma, which is associated with human immunodeficiency virus is referred to epidemic Kaposi's sarcoma to differentiate it from the transplant-related variant of neoplasm. In general Kaposi's sarcoma has been recognizing in homosexual men apart from human immunodeficiency virus disease epidemic (Friedman-Kien and Saltzman, 1990). In 1969, first Kaposi's sarcoma study was done in connection with immunosuppression in renal transplant.

(Pathak and Sharma, 2015) have taken the anti-cancer drug Paclitaxel, which has a very wide range of spectrum to AIDS-related Kaposi sarcoma. They made the formulation using soya lecithin, chloroform, isopropyl alcohol and span80 as an edge activator. The drug-loaded transfersomes loaded were loaded into the Carbopol 940 gel. The optimised formulation has an entrapment efficacy of 94.40 ± 2.29 %, vesicle size of 185.76 ± 2.15 nm and deformability index of 138.02. The prepared liposomes showed 94.69% drug permeation into the rat skin. Through confocal laser scanning microscopy technique, the authors observed the de-formability and squeezing of these nano-vesicles in different layers of the skin (Pathak and Sharma, 2015).

Melanoma

Melanoma is the tumour which arises from melanocyte and dendritic cell. It occurs due to the over exposure of the UV radiation, which leads to skin burns and tanning. This develops the DNA damage to a cell in the skin. The untreated DNA damaged cells form clusters of moles and pigment, which leads to the formation of the melanocyte. When melanocyte starts to spread, it projects dark spots and moles on the skin. The melanoma can grow deep under the blood vessels. The melanoma has the major pathways MAPK and PI3P (AKT). The two drugs approved by the USFDA are (a) BRAFI-vemurafenib and dabrafenib, (b) MEK-itrametib (Pollock *et al.*, 2003). Malignant melanoma is the most life-threatening cancer. It mainly develops on the melanocyte and has a high capacity to spread through the body parts. The major cause of malignant melanoma is sun exposure. The genetic factor plays a major role in developing the melanoma; approximately one-third

of the population has been affected by the malignant melanoma. It has two extreme conditions (a) patient with small skin lesions are mostly curable with surgical, (b) patient affected with metastatic disease to them therapeutic options are limited. In 2005 Australia report that 50-60/100000, are affected by the malignancy melanoma (Garbe and Schadendorf, 2008). In Slovenian, the human population both male and female in the year 2012 (male-23, 1/100000 and in female-23, 8/100000) the new strategy which holds for Slovenian has 700 new patients with malignancy melanoma (Orthaber *et al.*, 2017). In 2010 research 13200 new cases of malignant melanoma are found yearly. In 2006 study reports nearly 2-4% Asians and 1-2% black Asian Indians. Melanoma represents 1-8% in black people, 10-15% in Asian Indians and 19% Japanese (Narayanan *et al.*, 2010). However, to date, no transfersome products have been developed and tested in treating melanomas.

Squamous cell carcinoma

Squamous cell carcinoma is an epithelial malignancy which occurs in organs that are usually attached with squamous epithelial cells which involves more diverse anatomic sites, counting the skin, mouth, oesophagus, lung. Squamous cell carcinoma shows most of the common cancer in a metastatic spread worldwide. Human papilloma virus is carcinogenic, which causes all four sub-types. In fair-skinned people who burn and never get tanned are high risk on the increased rate of risk squamous cell carcinoma than those with dark races. It is reported that both past and strong exposure to the sun appears high predispose the population of skin cancer. Further Human papilloma virus involves in the multistep progress of carcinoma as co-factor with UV radiation, typically in a patient with low resistance (i.e.) limb transplant recipient and smoking, which enhances more risk of skin cancer.

(Gupta *et al.*, 2012) has developed pro-transfersomes for local delivery of cisplatin in cutaneous epithelial malignancy. Cisplatin loaded formulation of Pro-transfersomes is applied to the skin of the mice. After 45 days, the tumour volume was reduced compared to that of the control group. They evaluated in the fluorescence microscopy where it shows the deeper penetration on the stratum corneum. The cisplatin entrapment also shows the positive sign (97.97 ± 1.95 %). The result of the in vivo performance showed the positive outcome of increased therapeutic efficacy of a drug with low toxicity. Moreover, this method shows the enhancement accumulation on the skin is more, and they show an excellent opportunity for the

non-invasive drug with various size (Gupta *et al.*, 2012).

(Gupta *et al.*, 2011) has studied the cisplatin loaded Pro-Transfersomes gel for the squamous cell cancer. Cisplatin has a wide range used in various carcinomas, where they formulated Pro-Transfersomes and evaluated (in vitro permeation, drug deposition, anti-tumour effect, histopathology study and Geno-toxicity study). They followed the preparation method with slight modification. They observed *in vitro* result showed high ($p < 0.001$) compared to the drug solution using 0.9% NaCl. Anti-tumour activity checked in both control and treated animal group after 45 days, where Pro-Transfersomes group showed less size than the control group ($p < 0.001$). The cisplatin loaded Pro-Transfersomes gel with simple epithelial hyperplasia shows no growth of cancer. They found notably the induction of micronucleus in this formulation, which is treated with the injection (intra-peritoneal) cisplatin prepared saline solution is compared with the treated topical Pro -Transfersomes gel group. They found the positive and most suitable route of formulation helps in site-targeting and identified drug action. Finally, they identified the proper and better enhancement of the skin delivery for squamous cell carcinoma with the Pro-Transfersomes formulation (Gupta *et al.*, 2011).

Marketed Products And Regulatory Aspects

Many novel transdermal products have been developed and tested for treating various kinds of diseases and disorders. However, very few have seen the market face after regulatory approvals. The same is the position for transfersomes. According to a press release by IDEA AG, ketoprofen associated deformable vesicles (Diracetin®) received marketed by Swiss regulatory agency for treating osteoarthritis of the knee. Another drug-free product Flexiseq® (Pro BonoBio, UK) is commercially available for similar indication. Apart from these two products, none of the researched transfersomes are entered into the market. This fact now withstanding any trasfersomal product should pass safety and efficacy studies in clinical trials to show their potential in the commercial market. Various regulatory bodies such as USFDA, Japanese Ministry of Health, WHO, International Pharmaceutical Excipients council they categorised the nontoxic drug for the preparation. The USFDA mains the stringent excipient book which has names the 'Inactive Ingredient Guide' for the preparation (Owens, 2002).

CONCLUSION

Transfersomes is a novel, elastic vesicular drug carrier composed of phospholipids, surfactants and ethanol for enhanced transdermal delivery. They act as the reservoir which releases the drug slowly, and equally into the skin. It is clear from the literature that many studies (*in vitro* and preclinical) of the skin cancer use the application of the transfersomes for delivering the chemotherapeutic agents into the targeted site by overcoming skin barriers. However, the exact mechanism by which this transportation occurs is not well elucidated. Even though many studies have reported the application of transfersomes in treating skin cancer, no study was reported till date in clinical trials.

Further only two transfersome based products are currently available in the market (Ketoprofen and insulin). This is because of due to several issues such as safety, structural chemical stability, scale-up and large-scale manufacturing challenges. Among the various lipid vesicle, drug delivery system transfersome offers more permeability into the skin because of their extreme flexibility to squeeze into them self through the pore, which are much smaller than their diameter. Hence, the transfersomes will be a better option compared to another vesicular system for delivering the chemotherapeutic agents into various layers of skin cancer. We can anticipate that the number of transfersomes products for treating skin cancer will be developed in future and available in the market.

Conflict of Interest Statement

The authors declare that there are no conflicts of interest involved in this study. The authors alone are responsible for the content and writing of the paper.

Author's Contribution

Raahulan S was the lead author and synthesized the literature. Bharat Kumar Reddy. Sanapalli and Saikiran SS Pindiprolu were involved in drafting the paper. Karri V V S R provided conceptual input, design and critical revision of the manuscript. All authors read and approved the final document.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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