REVIEW PAPER

Transfersomes-based nanocarriers for anticancer drug delivery: a promising approach

Farnoush Mohamadpour[1], Zahra Chakeri[2], Mojgan Sheikhi[3], Saghi Naderpour[4,5], Milad Rahimzadegan[5], Alireza Sharafshah[6], Farshid Sefat[7,8*], Rahim Nosrati[6,9*]

[1] *Department of Pharmaceutics, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran*
[2] *Cardiothoracic Imaging Section, Department of Radiology, University of Washington, Seattle, WA, USA*
[3] *Department of Drug and Food Control, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran*
[4] *Faculty of Pharmacy, Eastern Mediterranean University Famagusta, North Cyprus via Mersin 10, Turkey*
[5] *Functional Neurosurgery Research Center, Shohada Tajrish Comprehensive Neurosurgical Center of Excellence, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
[6] *Cellular and Molecular Research Center, School of Medicine, Guilan University of Medical Sciences, Rasht, Iran*
[7] *Department of Bio-medical Engineering, School of Engineering, University of Bradford, Bradford, UK*
[8] *Interdisciplinary Research Center in Polymer Science & Technology (Polymer IRC), University of Bradford, Bradford, UK*
[9] *Student Research Committee, School of Medicine, Guilan University of Medical Sciences, Rasht, Iran*

ABSTRACT

Chemotherapy is typically used to treat cancer, but it can have a number of negative side effects. Nanocarrier-based drug delivery systems have gained much interest cancer treatment in recent years due to their advantages compared to conventional delivery systems. Recently, transfersomes (TFs) have been known to be the most outstanding innovative drug delivery systems that make them an attractive carriers for drug administration and cancer therapy. TFs have a bilayered structure that facilitates the encapsulation of lipophilic and hydrophilic drugs/agents with higher permeation efficiencies, offering a promising alternative to conventional liposomes as an anti-cancer drug delivery method. They are highly interesting for applications that involve controlled release. TFs are being explored as a complex system for drug delivery, with a focus on enhancing local drug penetration. This paper overview the current advancements in transfersomes-encapsulated with anti-cancer drugs for intelligent medication delivery to various cancers. In conclusion, this paper briefly discusses the prospects and problems of transfersomes-based anti-cancer drug delivery.

Keywords: *Nanocarriers; Transfersomes; Drug delivery; Cancer; Liposomes*

How to cite this article
Mohamadpour F, Chakeri Z, Sheikhi M, Naderpour S, Rahimzadegan M, Sharafshah A, Sefat F, Nosrati R. Transfersomes-based nanocarriers for anticancer drug delivery: a promising approach. Nanomed J. 2025; 12: 1-. DOI: 10.22038/NMJ.2025.86948.2203

INTRODUCTION

Cancer treatment

The term "cancer" describes a wide range of malignant conditions in which abnormal cells reproduce uncontrolled and have the potential to invade other organs. Masses of cancerous cells that grow out of control and eliminate neighboring healthy tissues are known as tumors [1, 2]. Tumor cells that have spread throughout the blood or lymphatic system are responsible for metastasis, or the growth of secondary cancers in other organs or tissues [3, 4].

*Corresponding author(s) Email: F.Sefat1@bradford.ac.uk; Rahim_nosrati@gums.ac.ir.
Note. This manuscript was submitted on March 31, 2025; approved on July 13, 2025.

The World Health Organization's (WHO) most recent statistics indicate that cancer has a remarkable frequency and mortality frequency that is on the rise [5]. Chemotherapy is typically used to treat cancer, but because the medications are not very precise, it can have a number of negative side effects [6, 7]. The majority of anticancer medications on the market today do not distinguish between malignant and healthy cells very well, which might restrict the maximal dosage of the medication and cause systemic toxicities and side effects [8]. Furthermore, a medicine must be administered in large doses in order to have quick clearance and extensive distribution into the targeted tissues and organs. This increases the cost of therapy and elevates the risk of adverse outcomes [9, 10].

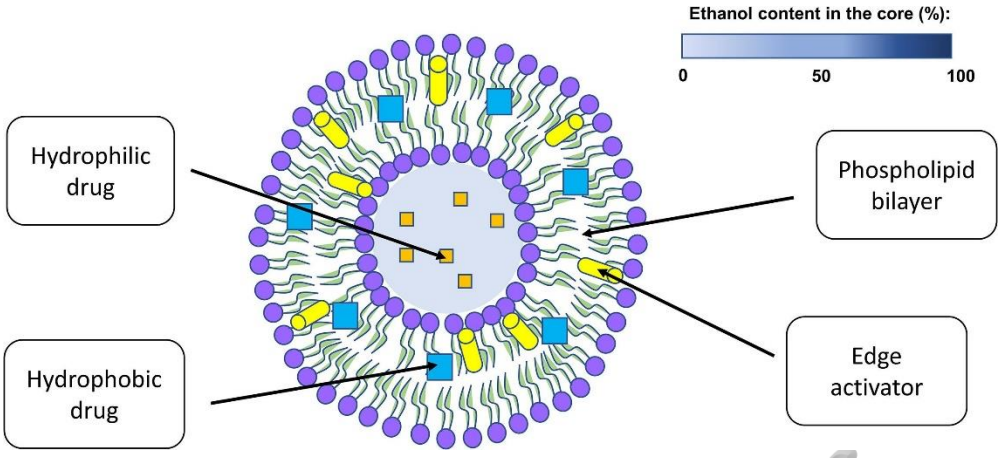

Fig. 1. Schematic showing the essential ingredients of a TFs: phospholipids, hydrophilic and hydrophobic medications, edge activator, and an interior core made of a combination of ethanol and water. Reprinted with permission from [18].

In fact, one of the most significant avenues for technological advances of the most advanced countries in the twenty-first century is nanotechnology, a vital field of study in technology and science that has been thoroughly investigated in the past 10 years [11]. Over the past ten years, pharmaceutical studies as well as clinical trials have shown the effectiveness of several drugs when administered *in vivo* through the application of pharmaceutical nanocarriers [12]. These carriers consist of vesicular and particulate systems, including micelles, liposomes, ethosomes, transfersomes (TFs), niosomes, dendrimers, and nanoparticles made of polymers, proteins, and lipids [13, 14]. Additionally, researched is on polymer–drug conjugates and antibody–drug conjugates [15].

Transfersomes: adaptable and flexible nano-vesicular carriers

Transfersomes/TFs (also known as transferosomes) are known as lipid-based vesicular carriers [16, 17] derived from the Greek term "soma," which means "body," and the Latin term "transferre," which means "to carry." [18, 19]. While the name transfersomes has been employed for over thirty years, it continues to be regarded as a new drug delivery mechanism due to the limited number of clinically available teransferosomal formulations [18, 20]. As a result, additional TFs would likely be marketed in the near future. TFs are made of four main components: phospholipids, an edge activator (EA), ethanol, and water (Fig. 1) [21, 22].

In this regard, phospholipids are typically including dipalmitylphosphatidylcholine, phosphatidylcholine, and distearylphosphatidylcholine. Surfactants or bile salts with a concentration of 10–25%, such as Tween 80 (T80), sodium deoxycholate, sodium cholate, Span® 80, and dipotassium glycyrrhizinate, are examples of EA. This component plays an essential role because it provides a wide radius of curvature that has the ability to weaken the lipid bilayer and enhance the flexibility of the lipid bilayer membrane of TFs. As a result, as the TFs move through the different layers of skin, they can compress naturally, which prevents the vesicles from rupturing. Water acts as a transport medium for ethanol, which is required in the TFs in smaller amount-typically below 10 percent [18, 22]. In the aqueous central cavity of TFs, hydrophilic drugs are encapsulated, while more hydrophobic drugs/active ingredients are incorporated into the phospholipid bilayer.

TFs have elasticity, flexibility, highly-deformability, and stress-responsivity [23] in contrast to liposomes with low lipid bilayer fluidity [24] or niosomes composed of non-ionic surfactants [25]. TFs can pass *via* the stratum corneum (SC) and enter the skin as intact vesicles smaller than 300 nm and in non-occlusive conditions. This preserves the gradient of trans-epidermal osmosis, allowing for elastic transport. Liposomes are among the most popular drug delivery devices. Their size and the lipid composition play a noteworthy role in permeation and treatment effects. In the case of niosomes, their ability to penetrate has been linked to decreased fluxes through the SC when compared to traditional liposomes, despite their higher stability and resistance to changes in osmolarity [26]. In comparison with niosomes and liposomes in liquid media, TFs appear to have demonstrated the highest colloidal stability in terms of zeta-potential at 4 °C and 25 °C [27], TFs have demonstrated

noticeable colloidal constancy (with no aggregation sign) for approximately some months, whereas liposomes and niosomes have demonstrated increased propensity for aggregation at the same temperatures and lower physical stability [28]. To ensure colloidal stability, TFs should have a zeta-potential that is either more than +30 mV or less than -30 mV; if not, there is a significant increase in the risk of aggregation [29].

The current review attempts to consider the researched TFs-based nanocarriers in the realm of anticancer drug delivery, their benefits and drawbacks, and future prospects.

This review article presents the current state of research concerning TFs-based nanocarriers for therapeutic agent's delivery to treat various cancers. The first section describes the properties of the general aspects of TFs, including their structure, synthesis methods, and properties, as well as the drug release mechanism. Then, a detailed discussion on the potential of TFs in the field of drug delivery for cancer treatment are presented. Finally, we summarize the challenges and future directions of drug delivery based on TFs as an emerging area of research.

Transfersomes for cancer drug delivery

Skin cancer

Today, one of the most widespread diseases in the world is skin cancer. Prolonged exposure to sunlight/ UV radiation and environmental pollution are the contributing factors. Today's world has seen a sharp rise in the incidence of skin cancer as a result of people's changing lifestyles. Skin cancer, specifically cutaneous malignant melanoma, is becoming more common than almost all other cancers. Melanoma continues to be a significant global issue, with current treatment options facing significant restrictions that emphasize the pressing need for innovative approaches [30, 31]. One promising strategy involves the targeted TFs of drugs through the skin to specifically attack cancer cells. Drugs with a higher molecular weight (above 500 Da) and ionized compounds should not be passed through the skin *via* the transdermal route, which is a non-invasive method suitable for systemic delivery. Numerous methods, including iontophoresis, microneedles, vesicular systems, penetration enhancers, and more, have been applied to increase the efficacy of the transdermal route [17]. However, they typically only penetrate the outer layers of the skin when applied topically. In transdermal drug delivery, vesicular delivery is becoming more and more important among the various methods [32]. Among them, TFs is most useful for transdermal delivery of substances with larger molecular weights due to its elastic and deformable nature, they can penetrate deeper skin layers through narrow pores that are significantly smaller than its size, come into contact with the systemic circulation, and then return to their original structure [33]. Some research were used TFs nanocarriers for transdermal delivery of anti-cancer natural active compounds for skin cancer therapy. This section, along with Table 1, provides a summary of various TFs that have been reported for use in skin cancer drug delivery.

Table 1. Transfersomes-based nanocarriers for skin cancer drug delivery

Transfersome components	Delivery agents	Target	Effect	Ref.
Apigenin /TFs	Apigenin	Skin cancer/ Mice	Improved skin permeation	[30]
Apigenin/ TFs gel /Con A		Melanoma (A375) cells	Enhanced skin targeting efficacy Cytotoxic effects on melanoma	[34]
Embelin/TFs Carbopol 934/H3/ TFs-based gel	Embelin	B16F10 melanoma cell/ Rat	Enhanced skin cancer therapy	[35]
RSV/TFs/ PC/ non-ionic EA	Resveratrol	B16F10 melanoma cell	Increase in accumulation Enhanced the stability, permeability, and uptake of drug	[36]
EGCG/TFs/HA	EGCG	HaCaT cells	Reduced lipid peroxidation, MMP expression, and intracellular ROS	[37]
Tea catechins (GTC)/TFs gel	Catechins		Improved skin penetration A remarkable decrease in IL-1β, IL-6, and TNF-α levels in mice	[38]
HK-loaded TFs	Honokiol (HK)	B16F10 melanoma cell	A prolonged release pattern Inhibiting TGF-β signaling, and decreasing CD47 and CD133 expression	[39]
RB/TFs microneedle array (TDMNs)	Rose Bengal (RB)		Superior intradermal transfer efficiency	[40]
Rtn-loaded TFs transdermal patches	Rutin (Rtn)		Significant drug release Increased inhibiting rate of cancer A high skin deposition	[41]

Transfersome components	Delivery agents	Target	Effect	Ref.
5-FU-loaded TFs	5-fluorouracil (5-FU)	HaCaT cell line	The greatest cytotoxicity on the HaCaT cell	[42]
Celecoxib/T20/TFs	Celecoxib	Human skin fragments	An optimal vesicle characteristics and uniformity Facilitated the drug permeation through the skin	[43]
CAR-loaded TFs	Carvedilol (CAR)	JB6 P+ and HaCaT cells	Induce cytotoxic effects Slowly drug penetration rates	[44]
CAR/TFs gel		Porcine ear skin model	Reduced tumor incidence and intensity Suppressed Ki-67 and COX-2 expression levels Not affected heart rate	[45]
CAR/TFs			Exhibited the maximum retention of the drug in the skin Reduced both acute and chronic skin inflammation of UV induction	[46]
PTX-CPP/TFs hydrogel		Xenograft B10F16 melanoma mouse model	Effectively pass through the SC Promoted effective delivery to tumor site Suppressed tumor development	[47]
SFN/TFs SFN/ethosomes	Sulforaphane (SFN)	SK-MEL 28 cells	Anticancer effects Enhanced percutaneous delivery	[48]
Cis-encapsulated TFs	Cisplatin (Cis)		Increased drug efficacy with reduced systemic toxicity	[49]
LR@TFs-CPP Gel	lycorine (LR)	Cutaneous squamous cell carcinoma (cSCC)	Anti-cSCC properties The skin and tumor permeability	[50]
Tamoxifen citrate/TFs gel	Tamoxifen citrate		Promising outcomes with a high drug release	[51]

Apigenin, a prevalent bioactive flavone detected in an extensive array of plants, fruits, and vegetables, has been recognized for its various pharmacological characteristics and anti-carcinogenic impacts. Apigenin serves as a chemotherapeutic agent for skin cancer therapy, both *in-vitro* and *in-vivo*. Jangdey et al's study aimed to utilize the Box-Behnken design in optimizing TFs that were developed using an altered rotary evaporation sonication system with the surfactant T80. The results showed an initial burst release followed by a controlled release, the optimized TFs (TW80-16) demonstrated a vesicle size 35.41 nm, the drug loading 8.042%, and an encapsulation efficiency (EE%) rate 84.24%. The permeation ability of apigenin was shown to be enhanced by this approach for prolonged periods of time, which may help cure skin cancer [30]. In another study, Jangdey *et al.* produced epigenin-loaded nano-TFs gel conjugated with Concanavalin-A (Con A) to specifically target melanocytes in the gel layer and treat UVB-induced skin carcinoma. The prepared formulation exhibited nano-sized vesicles (~179.0) with an EE% about 90%. The Con-A/TFs-based gel demonstrated cytotoxic effects on melanoma (A375) within a concentration ranging from of 0.4 to 2.0 mg/mL, while showing reduced toxicity towards HaCaT cells. The conjugated formulation exhibited an enhanced skin targeting efficacy both *in vitro* and *in vivo*, indicating a well-organized and cost-effective method for addressing skin cancer [34].

TFs-based vesicles carrying embelin, a naturally occurring benzoquinone derivative, was produced for transdermal medication delivery. Embelin, which comes from the dried fruit of *Embelia ribes*, is widely used because of its powerful anti-bacterial, anti-viral, anti-cancer, and anti-fungal properties. Employing the thin film hydration approach, embelin incorporated into several transferosomal formulations with varying ratios of Span-80 and Tween 80. A TFs-based gel with 2% embelin, carbopol 934 (at concentrations of 1%, 2%, 3%, and 4%), and propylene glycol (H3) was obtained as optimal formulation. The results showed that successful encapsulation of embelin in all formulations, with a maximum EE% 89.86%, suggesting the useful transdermal drugs delivery system for the skin cancer therapy [35]. Resveratrol (trans-3,5,4′-trihydroxystilbene, RSV), which is classified as a type of polyphenol, exhibits properties including antioxidant, anti-inflammatory, and anti-cancer effects, in addition to serving as a free-radical scavenger and a protector against cardiovascular ailments. Consequently, a RSV-encapsulated TFs was produced by phosphatidyl choline (PC) derived from a liposomal platform and non-ionic EA. The most favorable TFs production conditions involved using 5% PC/EA (3:1) and 5% ethanol in purified water, along with ultrasonic bath and rotating at 500 rpm, followed by high-pressure homogenization. The prepared TFs showed an EE% about 60%. Analysis of antioxidant functionality indicated that TFs exhibited a similar performance to free RSV. *In vitro* transdermal delivery analysis revealed a 27.59% increase in accumulation after 6 h with D1-20(W) formulation. Furthermore, cell viability tests demonstrated a 34.45% reduction in cytotoxicity for D3-80(W) formulation compared to

an equivalent concentration of RSV. As a result, the developed RSV-loaded TFs enhanced the stability, permeability, and entrance of drugs *via* the skin, even across the SC, owing to TFs deformability [36].

Epigallocatechin-3-gallate (EGCG) is known an effective antioxidant compound, most abundantly available in green tea. Avadhani *et al.* created TFs loaded with EGCG and hyaluronic acid (HA) to enhance the UV radiation-protective properties of both substances, while also providing anti-aging and antioxidant benefits. The nanosized TFs were produced using a thin-film-hydration method, utilizing sodium cholate and soy phosphatidylcholine, in conjunction with high-pressure homogenization. In HaCaT cells, it was shown that the improved TFs increased cell survival, reduced lipid peroxidation, MMP expression, and intracellular ROS. When compared to pure EGCG, the optimized EGCG/HA TFs showed considerably improved skin penetration and deposition of EGCG, demonstrating the potential use of the created TFs in sunscreen lotions and creams for improving UV protection [37]. Deka *et al.* developed a TFs herbal gel containing green tea catechins (GTC/TFs) to serve as an alternative to traditional invasive treatments of skin cancer and their associated side effects. The GTC/TFs was produced by the thin-film hydration technique, resulting in an EE% of 68.25%, and a drug loading of 10.41%. *In vitro* testing on B16F10 melanoma cell lines demonstrated favorable anticancer properties of the GTC/TFs. The treatment of skin cancer in mice as an animal model also showed a remarkable decrease in IL-1β, IL-6, and TNF-α levels, indicating both inhibiting and treating mice skin cancer of the formulation [38].

A naturally occurring bioactive substance called honokiol (HK) has been shown to have antineoplastic actions against melanoma. However, when taken orally, its bioavailability is very poor. The topical administration of HK presents a viable therapeutic option as an alternative. A modified scalable heating method was used to manufacture the HK-loaded TFs (HK-TFs). The cytotoxicity and cell uptake of HK-TFs were examined in B16F10 melanoma cells to scrutinize the influence of the complicated tumor microenvironment on the efficacy of HK. The improved formulation revealed an average size of 190 nm, a high EE% (89.9%), and a prolonged release pattern. HK-TFs demonstrated the immunosuppressive characteristics of B16F10 melanoma *in vitro* by inhibiting TGF-β signaling, and decreasing CD47 and CD133 expression (a hallmark of stem-like cells) [39].

Rose Bengal (RB) is a dye that is known for its selective toxicity towards melanoma cells. However, its therapeutic perspective is limited by its high water solubility and low permeability. In a research, RB-encapsulated TFs (RBTFs) were loaded into a trilayer dissolving microneedle array (RBTFs-TDMNs) to enhance intradermal delivery of RB for the treatment of melanoma. The RBTFs-TDMNs demonstrated sufficient strength to penetrate excised porcine skin, dissolve rapidly, and deliver RBTFs intradermally while preserving their physical and chemical properties. A dermatokinetic investigation revealed that RBTFs-TDMNs offer superior transfer efficiency in comparison with RBTFs dispersion and microneedles loaded with free drug, proposing its valuable potential for the topical treatment of melanoma [40]. Waldher *et al.* performed a study to improve the skin permeation of rutin (Rtn) by using TFs that integrated into transdermal patches (TPs) (Fig. 2).

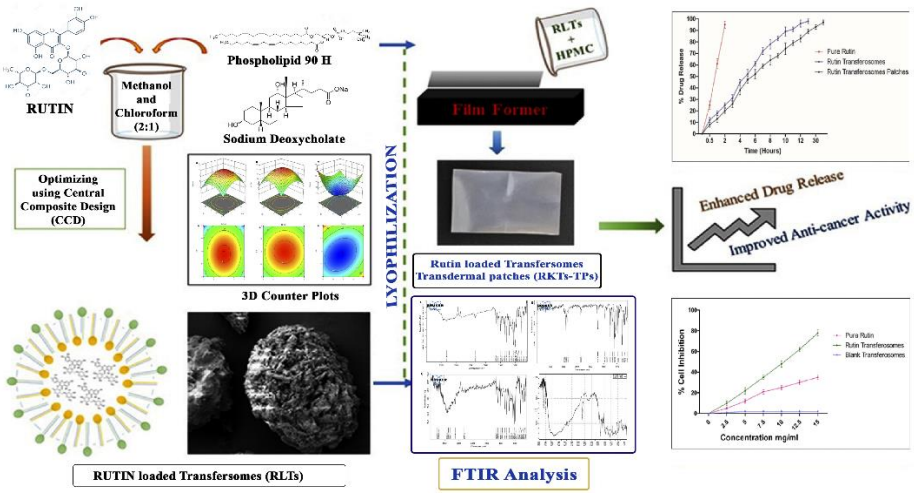

Fig.2. Development of rutin-loaded TFs to improve ex vivo membrane permeability and in vitro efficacy. Reprinted with permission from [41].

Rutin-loaded TFs (RtnTFs) were created using a central composite design (CCD) method and included different ratios of sodium deoxycholate and phospholipid 90H as variables. A greater Rtn encapsulation, enhanced formulation stability, and significant drug release were observed. Moreover, the RtnTFs exhibited an increased inhibiting rate B16–F10 melanoma cell line, and RtnTFs-loaded TPs cause a high skin deposition of approximately 0.921 ± 0.23 mg/cm^3 of Rtn. *Ex-vivo* skin diffusion tests showed a continues drug release of about 98% at 36 h. Skin irritancy assessments confirmed the compatibility of RtnTFs-TPs for dermal delivery, indicating high stability [41].

Based on the abovementioned research, the delay of drug release by topical delivery approach can enhance solubility and can be a hopeful approach for effective delivery of anti-tumor agents.

Topical 5-fluorouracil (5-FU) is used to treat non-melanoma skin cancer and actinic keratosis. Unfortunately, 5-FU does not penetrate the skin well, which reduces its effectiveness as an anticancer agent when applied topically. To that end, Alvi *et al.* synthesized 5-FU-loaded TFs, niosomes, and liposomes for topical application with EE% as high as 82.4%, 45.4%, and 43.4%, respectively. TFs were created using the solvent evaporation approach, whereas a reverse-phase evaporation was applied for liposomes and niosomes producing. , The skin permeability and retention revealed better permeability and retention when compared to the nonvesiculized dose form. After 72 hours, the IC_{50} values of free 5-FU (15.89 µmol/l) were significantly higher than those of niosomes (9.91 µmol/l), TFs (1.02 µmol/l), and liposomes (6.83 µmol/l). Comparing to liposomes and niosomes, 5-FU-loaded TFs showed the greatest cytotoxicity on the HaCaT cell line, concluding that vesiculization of 5-FU increases both its cytotoxic effect and topical distribution [42].

The topical application of celecoxib has proven to be a successful method of preventing the development of skin cancer and improving the efficacy of anticancer drugs in the management of skin cancer. An effective topical construction of celecoxib that could simplify skin delivery of the drug was designed by three types of vesicular carriers, such as liposomes with a surfactant, ethosomes, and TFs, with proper EA. It was observed that TFs significantly enhanced (2.0 fold) the drug dosage that entered the skin compared to a celecoxib suspension. Among ethosomes incorporating ethanol and Tween 20 (T20) as an EA those exhibiting optimal vesicle characteristics and uniformity with a 54.4% of EE, which could facilitate the drug permeation through the skin, confirming the role of ethanol and T20 as permeation enhancers [43].

Carvedilol (CAR) is a β-blocker has been found to inhibit UV-induced skin cancer. However, the systemic administration of CAR can lead to undesired cardiovascular effects. To address this issue, topical TFs-based CAR delivery system was evaluated in several researches. A CAR-loaded TFs was created utilizing different ratios of surfactants and phospholipids. The optimized TFs (F18), consisted of CAR, T80, and soy phosphatidylcholine, at a ratio of 1:0.5:3, exhibited an EE% of 93.7%. F18 effectively restricted the EGF-mediated JB6 P+ cells neoplastic transformation at non-toxic amounts of EGF, although higher amounts did induce cytotoxic effects in JB6 P+ and HaCaT (human keratinocytes). Rather than a free drug, CAR was released more slowly from F18 and penetrated into the skin of porcine ear. Moreover, once selected to reconstruct the human skin in its full-thickness, F18 exhibited reduced drug penetration rates whereas effectively mitigating UV-induced DNA damage, gene expression in inflammatory events, and apoptosis [44]. In another study, a topical CAR-encapsulated TFs (CARTFs) that were loaded into carbopol gel or into suspension, exhibited comparable drug penetration and deposition in porcine ear skin model. CARTFs gel (10 µM) notably decreased skin edema and also formation of cyclobutane pyrimidine dimerization in singular dose UV-exposed mice, while this gel delayed tumor onset, reduced tumor incidence and intensity, and suppressed Ki-67 and COX-2 expression levels, in prolonged UV exposure. The treatment of mice with repeated doses of CARTFs gel (100 µM) could not affected heart rate, while significantly enhanced the skin deposition of CAR, suggesting its potentials for skin cancer prevention with minimal systemic impact [45]. In a same study, TFs containing CAR with a ratio of 1:3:0.5 for drug:lipid:surfactant exhibited the maximum retention of the drug in the skin. When applied topically at a concentration of 100 µM, CARTFs did not cause skin sensitivity in laboratory and animal tests and 10 µM CARTFs effectively reduced both acute and chronic skin inflammation of UV induction [46].

Topical application of anti-cancer drugs is a viable therapy strategy for cutaneous melanoma. However, natural barriers between the skin and the tumor impair the effectiveness of drug administration. In a study to increase the

transdermal administration of paclitaxel (PTX) for the treatment of melanoma, Jiang *et al.* used a paintable oligopeptide hydrogel incorporating PTX-encapsulated cell-penetrating-peptide (CPPs)-modified TFs (PTX-CTs/Gel). The PTX-CTs/Gel functioned as a patch to successfully extend the duration of PTX-CTs on the skin when applied to the skin above the melanoma lesion. The PTX-CTs were able to effectively pass through the SC due to the EA-induced fluidity and penetration of TFs in the SC. Furthermore, the CPP alteration promoted effective transportation among the tumor cells and increased penetration of PTX-CTs in the skin and tumor stroma. In a xenograft B10F16 melanoma mouse model, PTX-CTs successfully suppressed tumor development when paired with systemically administered chemotherapy (Fig.3) [47].

Sulforaphane (SFN) is a versatile medication with multiple effects, and its ability to combat cancer is driving increased interest in its potential. SFN has demonstrated antiproliferative properties against melanoma and other forms of skin cancer in laboratory settings. Unfortunately, due to its specific physical and chemical properties, this natural compound cannot be effectively applied directly to the skin. Cristiano and coworkers applied ethosomes- and TFs-based nanocarriers for delivering SFN *via* the skin for the treatment of skin cancer. Ethosomes revealed better anticancer effects on SK-MEL 28 cells and an enhanced percutaneous delivery of SFN, indicating that ethosomes were the most suitable vesicles for delivering SFN topically in comparison with TFs [48].

Despite promising treatment of topical anticancer medications for managing cutaneous squamous cell carcinoma (cSCC), the limited skin absorption capacity is a main challenge. Gupta *et al.* assayed topical delivery of Cisplatin (Cis)-encapsulated TFs for cSCC therapy. The EE% of Cis was determined to be 98%, with skin penetration of 560.20 ± 7.89 μg/cm^2 confirmed by a fluorescent marker. *In vivo* evaluation of the system revealed increased drug efficacy with reduced systemic toxicity, suggesting an enhanced, targeted, and localized drug delivery for cSCC treatment [49]. Li *et al.* confirmed the effect of lycorine (LR) in the topical treatment of cSCC by incorporating it into a cell-penetrating peptide (CPP)-modified cationic TFs gel (LR@TFs-CPP Gel). They revealed the anti-cSCC properties of LR and the skin and tumor permeability of LR-loaded TFs [50]. Gayathri and Sangeetha assessed the transdermal delivery of Tamoxifen citrate using TFs gel which was composed of different ratios of drug concentrations and Carbopol. The optimized TFs formulation MG2, containing 0.1 g Tamoxifen citrate, demonstrated promising outcomes with a high drug release of 94.32% [51].

Breast cancer

Breast cancer (BC) is diagnosed as the most frequent cancer in women and ranks the second origin of cancer-related deaths in women [52, 53]. Therefore, prompt and effective treatment is important. This section and Table 2 provide an overview of various TFs that have been reported for drug delivery in BC.

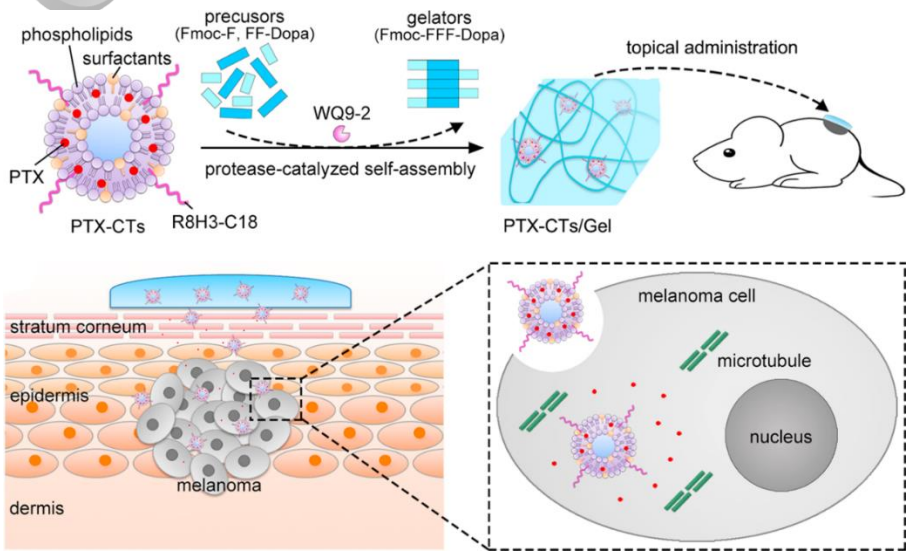

Fig. 3. The process of preparing and using PTX-CTs/Gel as a patch for the non-invasive treatment of melanoma. Reprinted with permission from [47].

Table 2. Transfersomes-based nanocarriers for breast cancer drug delivery

Transfersome components	Delivery agents	Target	Effect	Ref.
RLX-loaded TFs	Raloxifene hydrochloride (RLX)		Significant alterations in Rat skin	[54]
DOX/HA- GMS-TFs	doxorubicin (DOX)	MCF-7 cells	Increased in cellular uptake Enhanced transdermal penetration compatibility with mouse embryo fibroblast cells a low hemolysis rate	[56]
DOX/HA/TFs/microneedles complex (TFs/MNs)		Rat model of skin cancer	Develop lymphatic drug transport Successfully penetrate rat skin Increased the accumulation of DOX in lymph nodes	[57]
CUR-loaded oleic acid TFs	Curcumin (CUR)	MCF-7 cells	The *ex-vivo* permeation Effective cytotoxicity	[58]
4-OHT-loaded TFs/ emu oil	4-hydroxytamoxifen (4-OHT)	Mice model of BC	Similar effectiveness to orally administered TAM without skin irritation	[59]
4-OHT-loaded TFs/ratite oil		*Ex vivo* porcine skin/breast cancer	Anti-inflammatory qualities of ratite oils Enhanced penetration	[60]
DTX-CTs/Gel	Docetaxel (DTX)	Mice model of BC	Strong tumor and skin permeation Enhancing DTX accumulation in cancer cells	[61]
Mannosylated naringenin-loaded TFs (MA-NgTFs)	Naringenin		Enhanced of cellular uptake via mannose receptor-mediated TFs	[62]
HA/PVA/PVP- microneedle (MN) containing HA–GMS conjugated RB-TFs	Ribociclib (RB)	MDA-MB-231	Maintained the drug concentrations within the effective range Targeted delivery to tumor cells *via* CD44 as specific receptor of HA	[63]
iontophoresis-enhanced RSV-enclosed in TFs	RSV	BC rat model	A notable decrease in tumor volume and serum biomarker CA 15-3 High efficacy of transpapillary route delivery of RSV	[64]
iontophoresis-enhanced LPT and 5-FU encapsulated in TFs	Lapatinib (LPT)	MCF-7, MDA-MB-231 cells/ Rat skin	High efficacy of transpapillary route delivery of LPT	[65]
ERL-loaded TFs gel	Erlotinib (ERL)	Ductal carcinoma in situ	Superior effectiveness against MCF-7 Significantly lower IC_{50} values	[66]

Raloxifene hydrochloride (RLX) is approved to treat BC and decrease the invasive BC in high-risk women. RLX has extremely limited oral bioavailability. To overcome this problem, transdermal administration of RLX has been proposed. In this line, Mahmood *et al.* created RLX-loaded TFs for transdermal administration. The optimized TFs displayed spherical, unilamellar forms with an EE% of 91%, and transdermal flux of 6.5±1.1 μg/cm^2/hour. RLX-loaded TFs showed 6.25±1.50 drug permeability and 9.25±2.40 skin deposition that was better than traditional liposomes. Significant alterations in Rat skin structure were detected during the *ex vivo* drug diffusion research, indicating the higher potency of RLX-loaded TFs for oral medication administration [54].

Tumor-draining lymph nodes (TDLN) play a crucial role as primary metastatic foci in the progression and spread of various solid tumors. The use of the lymphatic system for drug delivery is considered beneficial for stimulating immune responses through vaccination or for the effective administration of chemotherapy to combat tumor metastasis. A novel TFs modified with HA was developed for the targeted delivery of doxorubicin (DOX) to the lymphatic system *via* the transdermal pathway for the management of tumor metastasis. DOX is a potent chemotherapeutic drug that is widely used in the treatment of various cancers [55]. The inclusion of DOX in HA-glycerol-a-monostearate(GMS)-TFs enabled efficient penetration into the deep layers of the skin, resulting in increased absorption by the lymphatics (Fig.4a). The optimized estimated values for EE% and loading capacity (LC) of HA-GMS-TFs were 60.86% and 4.63%, respectively. *In vitro* experiments showed that the transdermal penetration of DOX-loaded HA-GMS-TFs was 3 folds greater than the solution form. The modification with HA did not include the transdermal penetration efficiency of the TFs. Additionally, DOX-encapsulated HA-GMS-TFs resulted in a remarkable accumulation in lymph nodes *in vivo*, demonstrating good biocompatibility without cytotoxic effects. Moreover, HA-GMS-TFs significantly enhanced the endocytosis of breast tumor cells (MCF-7), leading to a 9-fold increase in

cellular uptake compared to unmodified TFs. HA-GMS-TFs also exhibited compatibility with mouse embryo fibroblast (MEF) cells and a low hemolysis rate of 4.65% compared to rabbit blood [56]. Another same research developed a new dissolving microneedle composed of HA, combined with TFs transdermal DOX delivery (Fig.4b). By leveraging the addition capabilities of microneedles and the lymphatic delivery potential of TFs, the TFs/microneedles complex (TFs/MNs) was anticipated to develop lymphatic drug transport. The findings demonstrated that the MNs could successfully penetrate rat skin and release DOX-loaded TFs into the dermis through self-dissolution. The DOX-TFs maintained their multilayer construction upon release from the dissolved MNs. The DOX-TFs/MNs formulation remarkably increased the accumulation of DOX in lymph nodes in comparison with diffusion through the epidermis, consequently elevating its bioavailability in the bloodstream. These results hold promise for enhancing chemotherapy of tumors *via* lymphatic drug delivery that can eradicate metastasized tumor cells present in draining lymph nodes [57].

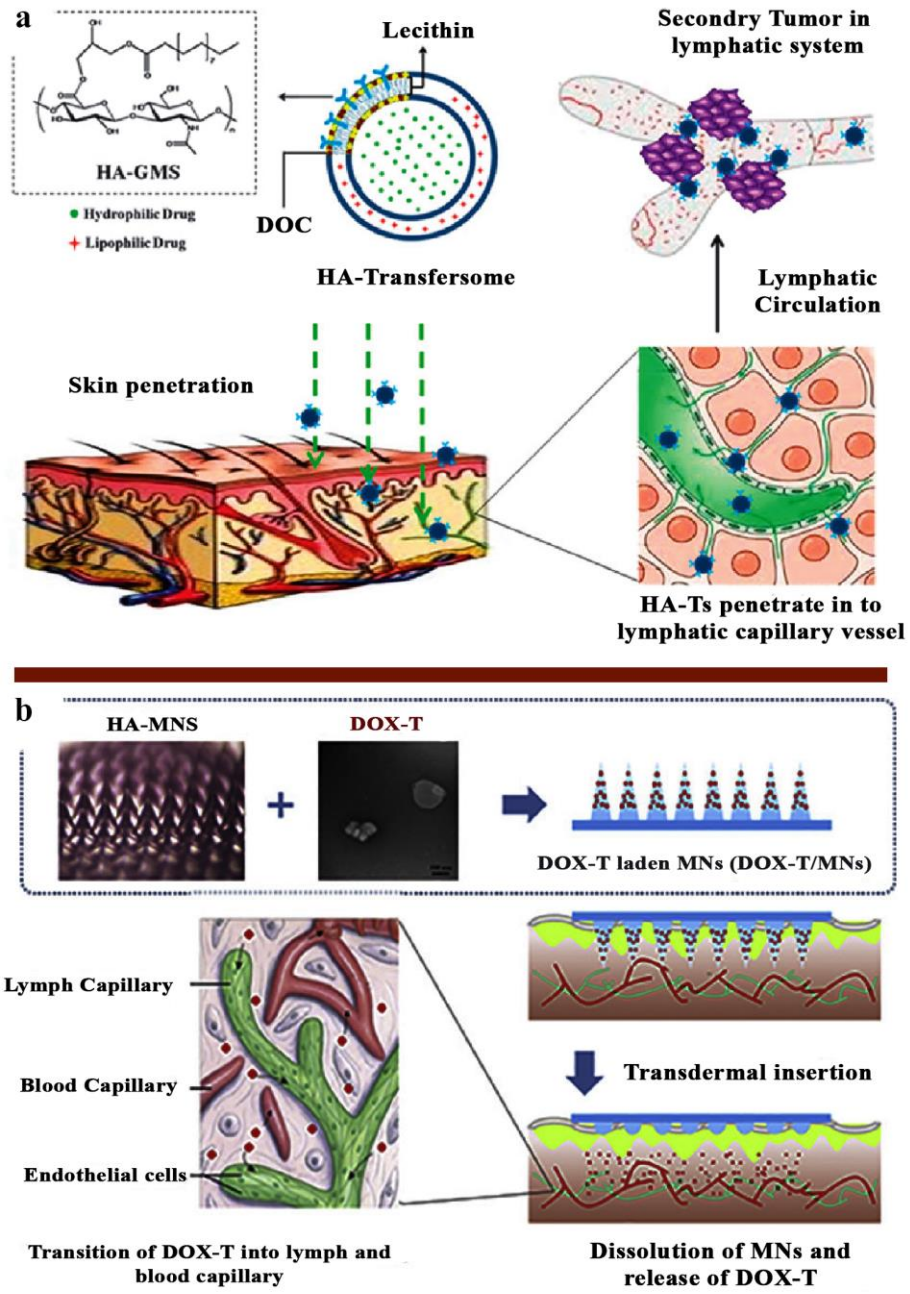

Fig.4. a) Schema of transdermal delivery of HA-modified TFs for tumor metastasis therapy; b) the mechanism of delivery of DOX *via* HA-based TFs/microneedle complex for the treatment of tumor metastasis. Reprinted with permission from [56] and [57].

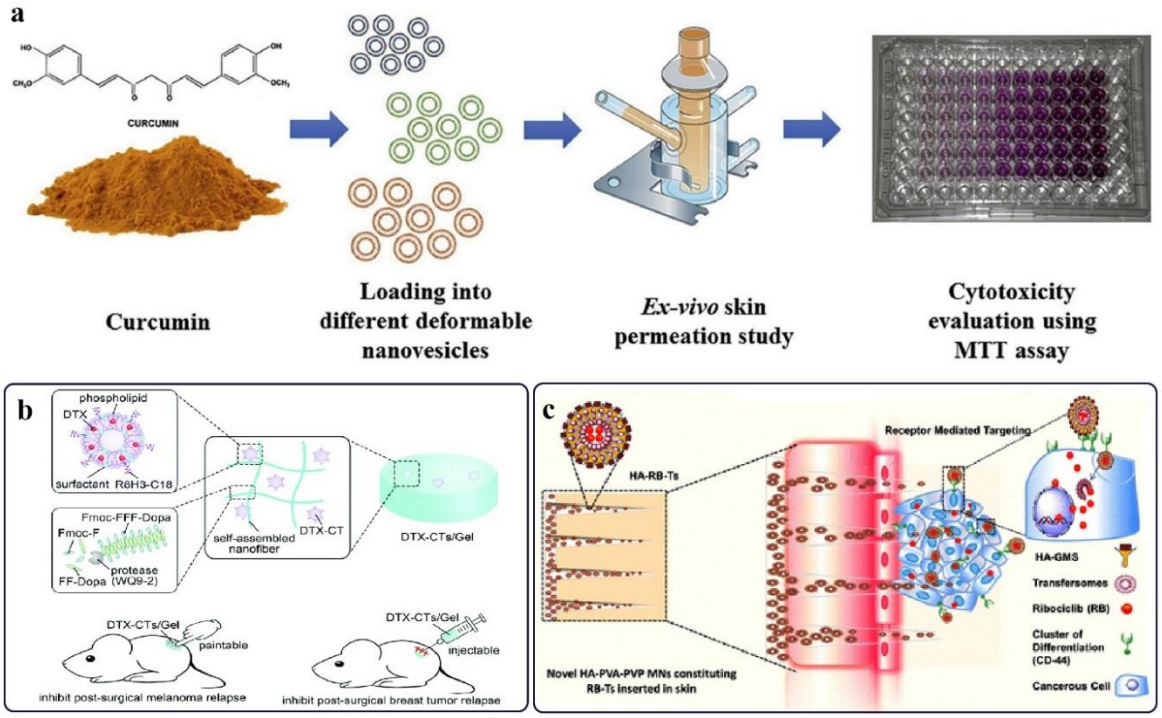

Fig. 5. a) Curcumin-loaded TFs as an impending delivery system for breast cancer treatment. b) A topical delivery manner of DTX utilizing DTX-CTs/Gel for the prevention the post-surgical melanoma and BC reappearance. c) The targeted delivery of Ribociclib-loaded TFs by a microneedle array and HA/CD44 interaction for breast cancer therapy. Reprinted with permission from [58] ,[61] and [63].

In order to target both surface and subcutaneous malignancies, such as BC, Abdel-Hafez *et al.* applied the transdermal method to give the poorly absorbable curcumin (CUR) into the circulation (Fig.5a). Thin film hydration and extrusion were used to manufacture CUR-loaded TFs by using EA included T80 and sodium cholate. The penetration-enhancing substances such oleic acid, Labrasol, Transcutol, and limonene were investigated for skin penetration efficacy. CUR, being hydrophobic, was efficiently encapsulated inside the lipid bilayers of the vesicles with high EE% of 93.91% and DL% of 7.04%. The *ex-vivo* permeation of CUR-TFs was confirmed on male albino mice's skin model. Cytotoxicity tests using MTT assay on MCF-7 cells showed that oleic acid TFs had an IC_{50} of 20 µg/ml, indicating the potential of these nanovesicles as an effect delivery system for BC treatment [58].

Oral tamoxifen (TAM), a selective antiestrogen, is used for the prevention and treatment of non-invasive BC. TAM, is not widely accepted owing to some severe side effects. The main metabolite of TAM, 4-hydroxytamoxifen (4-OHT), is responsible for the anticancer effect of TAM. The local application of 4-OHT in the breast through transdermal therapy can help to avoid the harmful effects of oral tamoxifen and still be effective. Sundralingam *et al.* assessed skin irritation and the effectiveness of 4-OHT-loaded TFs formulations developed with or without emu oil. A lower plasma concentration of 4-OHT and similar effectiveness to orally administered TAM without skin irritation was observed in mice model of BC that was administered with prepared TF, with or without emu oil, through local transdermal manner [59]. In another study, an efficient TFs system was prepared for delivering 4-OHT utilizing ratite oil as a carrier for treating BC. The anti-inflammatory qualities of ratite oils, along with their ability to function as penetration enhancers, introduce them as excellent candidates for incorporation into transdermal formulations. The optimized TFs containing 4-OHT were formulated, with and without ratite oils, by various molar ratios of soy phosphatidylcholine and sodium taurocholate as EA through the rotary evaporation/ultrasonication approach. The ratite oil TFs formulation exhibited the highest EE% (95.1 ± 2.70%) with a constancy for 4 weeks at 4 °C, while TFs lacking ratite oils were stable for 8 weeks. *Ex vivo* penetrability investigations using porcine skin confirmed the transdermal delivery of 4-OHT TFs formulations without emu oil, offering promise in BC treatment [60].

Residual microtumors remaining after surgery can lead to tumor recurrence, posing a significant challenge in cancer treatment. Liu *et al.* prepared a

nano-hybrid oligopeptide hydrogel to topically deliver the chemotherapeutic agent docetaxel (DTX), with the aim of preventing tumor recurrence after surgery (Fig.5b). The hydrogel, named DTX-CTs/Gel, was prepared by entrapping DTX in CCPs-modified TFs and embedding them in an oligopeptide gel. This formulation had the properties of being paintable and injectable, with the ability to stay longer at the application sites post-administration. The DTX-CTs released from the hydrogel displayed strong tumor and skin permeation ability, enhancing DTX accumulation in cancer cells and promoting cell death. The researchers demonstrated that the use of DTX-CTs/Gel for DTX delivery was effective in delaying tumor recurrence in mouse models of postoperative melanoma and BC [61].

Naringenin, a potent antioxidant abundant in citrus fruits, demonstrates significant potential in targeting skin carcinoma by scavenging reactive oxygen species (ROS). The created mannosylated naringenin-loaded TFs (MA-NgTFs) displayed vesicle sizes ranging from 102to 263, with an EE% from 72 to 82%. *In vitro* drug release analysis indicated percentages of 69.31%, 62.03%, 58.71%, and 65.02% for MA-NgTFs and marketed formulation dispersion, respectively. *Ex vivo* skin penetration and deposition investigations revealed that flux through the skin of mice was 6.5±3.07, with a drug retention percentage of 0.76±1.26, providing compelling evidence of cellular uptake *via* mannose receptor-mediated TFs [62].

HR+/HER2-metastatic BC (MBC) is a prevalent and serious ailment seen in women. The utilization of ribociclib (RB), an orally active CDK4/6 inhibitor, in endocrine therapy is a promising method for addressing HR+/HER2-MBC. Nevertheless, the current approach involving repetitive dosing over 3 to 6 cycles and non-targeted distribution of RB has resulted in severe side effects such as hepatobiliary, neutropenia, renal toxicities, and gastrointestinal, as well as QT interval prolongation. To tackle this issue, a HA/PVA/PVP-based microneedle (MN) array containing HA–GMS conjugated RB-TFs has been developed to deliver RB (Fig. 5c). The in the skin efficiently, enabling In this platform, HA-RB-TFs can penetrate to skin through MN-induced microchannels and targeted deliver to tumor cells *via* CD44 as specific receptor of HA, extending the drug release time by up to 6 times. Studies on pharmacokinetics and tissue distribution demonstrated that drug concentrations are maintained within the effective range for up to 48 h, reducing the dose administered thrice weekly, and reducing the risk of severe toxicities [63].

The local administration of drugs into the breast tissue is an interesting topic for the targeted administration of drugs. The administration of drugs through the mammary papilla offers advantages, as breast tumors often occur in the mammary ducts. A research study investigated the feasibility of using iontophoresis-enhanced transpapillary delivery of the RSV for treating BC. RSV was enclosed in TFs (RSV-TFs) to facilitate a gradual release of the drug by the biomaterial soya phosphatidylcholine (SPC). *In vitro* transpapillary iontophoresis experiments on porcine mammary papilla demonstrated increased permeation of RSV-TFs in comparison with passive diffusion. The optimized RSV-TFs administered *via* transpapillary delivery exhibited higher bioavailability values than pure RSV administered orally. In a chemically induced BC rat model, a notable decrease in tumor volume and serum biomarker CA 15-3 was observed, indicating the high efficacy of transpapillary route delivery of RSV in comparison to the oral route [64].

Combination chemotherapy, in which two or more anti-cancer drugs are used, has been a fundamental aspect of BC therapy due to its advantages over single-drug treatment. Fernandes *et al.* demonstrated the synergistic effects of Lapatinib (LPT) and 5-FU encapsulated in TFs on treating BC through the iontophoresis-induced transpapillary route. The IC_{50} values for 5-FU and LPT were determined to be 5.7 µg/ml and 19.38 µg/ml, respectively. The *ex vivo* rat skin permeation studies showed that drug-loaded TFs had high stability and superior penetrability compared to the solution of LPT and 5-FU, suggesting an efficient alternative therapy of BC [65]. Mangla *et al.* developed erlotinib (ERL)-loaded TFs gel (ERL@TFs gel) for the management of ductal carcinoma in situ. The process involved using a thin-film hydration method to create ERL-loaded TFs, which were then combined with a carbopol gel matrix to produce ERL@TFs gel. When compared to plain ERL, the optimized ERL@TFs gel demonstrated superior effectiveness against MCF-7 cell lines, showing significantly lower IC_{50} values, an improved safety profile with the ability to address ductal carcinoma in situ BC [66].

Lung cancer

Lung cancer is the second most common cancer and the leading cause of cancer-related mortality worldwide. A comprehensive overview of TFs nanocarriers for lung cancer is provided in this section, as well as in Table 3.

Table 3. Transfersomes-based nanocarriers for anti-cancer drug delivery

Cancer type	Transfersome components	Delivery agents	Target	Effect	Ref.
Lung cancer	Vinblastine-encapsulated TFs	Vinblastine	Human leukemia cell lines (HL-60, K562)	effective cytotoxic effects	[67]
	Uf-SUV/TFs	Vaccine	ErbB2-expressing cancer cells	effective antitumor effects against lung tumors	[68]
	PTX-loaded TFs Soya phosphatidylcholine	Paclitaxel (PTX)	MRC-5 SV2 cell line	a localized impact within the lungs toxicity towards lung cancer	[69]
	PDT + MPa-loaded nano-TFs	Methyl pheophorbide (MPa)	A549 and HeLa cells	a promising strategy for combating cancer through PDT	[70]
	ICG/TFs/gel + PDT	Indocyanine green (ICG)	Basal cell carcinoma (BCC)	the normal skin histology in mice following irradiation minimum pain during the management	[71]
	Purpurin-18 sodium salt (P18Na) and DOX-loaded nano-TFs + PDT	P18Na and DOX	HeLa and A549 cell lines	effectively transported DOX and P18Na pH-sensitive release	[72]
Sarcomas	MTM-loaded TFs	Mithramycin (MTM)	Chondrosarcoma and myxoid liposarcoma models	Strong anti-tumor effects Inhibited the signaling pathway facilitated by the pro-oncogenic factor SP1	[73]
Head and neck squamous cell carcinoma	Emodin-loaded TFs (NETFs)-ultrasound	Emodin	FaDu and CAL-27 cells	A remarkable dose-dependent ROS production Enhanced apoptosis Increased expression levels of caspase-3/9 genes	[60]
Oral cancer	5-FU and Etodolac co-loaded TFs Hydrogel	5-FU and Etodolac	FaDu cells	representing a synergistic impact of Et and 5-FU Exhibited lag time, similar flux, and penetration coefficient	[74]
Brain tumor	FA-modified TPGS TFs containing DTX	Docetaxel (DTX)	U-87 MG cells	A superior internalization FA-targeted GBM treatment	[75]

In first study, vinblastine was encapsulated in cholesterol-based liposomes, sodium cholate-prepared TFs using the thin film hydration procedure, and the lipids dimiristoylphosphatidylcholine (DMPC) and dipalmitoylphosphatidylcholine (DPPC). The findings demonstrated that the percentage of vinblastine encapsulated into TFs varied between 50% and 80% at a ratio between 0.05 and 0.09. It was found that the drug retention in TFs and liposomes depended on time. The preservation of medication in TFs was shown to be lower than in liposomes due to the presence of sodium cholate. The cytotoxic and cytostatic capabilities of vinblastine-encapsulated TFs and liposomes was confirmed on nine human cell lines [67].

Mucosal surfaces offer pioneering opportunities for vaccination, particularly the mucosa of the respiratory tract, which enables the development of non-invasive methods for vaccine delivery. This research project focused on using the nasal route to evaluate the efficacy of various di-epitopic liposomal constructs in inducing antitumor responses by prophylactic vaccination in mice. Intranasal delivery of liposomal vesicles containing specific epitopes and adjuvants with different sizes, structures, and compositions including multilamellar vesicles (MLV), small unilamellar vesicles (SUV), ultraflexible small unilamellar vesicles (Uf-SUV/TFs), and reverse-phase evaporation (REV) were assessed. The vaccines were delivered nasally to BALB/c mice, prior to the introduction of ErbB2-expressing cancer cells either intravenously or subcutaneously. Nasal management of the SUV vaccine demonstrated effective antitumor effects against lung tumors, with limited protection against subcutaneous tumors. Interestingly, unlike the total vaccine dose or adjuvant concentration, the structure, size, and flexibility of the liposomes and TFs did not significantly influence vaccine-induced immunity or antitumor responses against lung tumors [68]. A straightforward method was utilized in the development of innovative proTFs tablets containing paclitaxel (PTX) for pulmonary drug delivery. The extensive surface region of the pulmonary system serves as an advantageous location for the deposition of anti-cancer

medication, resulting in a localized impact within the lungs. A PTX-loaded TFs Soya phosphatidylcholine was designed for lung cancer treatment. The hydrated TFs formulations (F3, F6, and F9) showed PTX entrapment levels of 93–96% with a toxicity towards lung cancer MRC-5 SV2 cell line (about 60, 68, and 67% cell viability) while being non-toxic to normal lung fibroblast cells (MRC-5) [69].

Photodynamic therapy (PDT) is a targeted therapy that does not require invasion. Photosensitizers (PSs), crucial components in PDT, tend to aggregate directly due to their lipophilic nature. Methyl pheophorbide (MPa)-loaded nano-TFs were produced using sonication, which displayed a gradual release of the drug over 48 h in a natural environment, making it suitable for controlled drug release in PDT, and leading to enhanced photodynamic effects and decreased adverse effects. The formulations exhibited minimal toxicity in the absence of light but demonstrated anti-cancer effects upon light exposure. Notably, nano-TFs with the smallest size revealed a higher level of photodynamic activity, indicating that the MPa-loaded nano-TFs system presents a promising strategy for combating cancer through PDT [70]. Indocyanine green (ICG) is a fluorescent dye that emits near-infrared light and shows promise for use as a photosensitizer in PDT for skin conditions. Of note, its effectiveness has been limited due to its rapid degradation. To address this issue, a study encapsulated ICG in colloidal TFs. A preliminary clinical trial was carried out to evaluate the efficacy of ICG/TFs in PDT for basal cell carcinoma (BCC). The ICG/TFs exhibited a particle size of approximately 125 nm and a sustained release of ICG for over 2 h. When ICG/TFs was incorporated into a gel formulation, it preserved the normal skin histology in mice following irradiation with an 820 nm diode laser. Additionally, PDT using ICG/TFs achieved an 80% clearance rate for BCC patients, with minimum pain described during the management [71]. In another study, purpurin-18 sodium salt (P18Na) and DOX-loaded nano-TFs were produced for a dual approach combining PDT and chemotherapy against cancer. The release of P18Na and DOX from the nano-TFs showed a consistent pH-responsive pattern, releasing gradually in normal conditions and rapidly in acidic pH. Consequently, the nano-TFs effectively transported DOX and P18Na to cancer cells with minimal leakage, demonstrating pH-sensitive release within the cancer cells. The finding confirmed a photo-cytotoxicity in HeLa and A549 cell lines [72].

Other cancers

Sarcomas

Sarcomas represent a diverse collection of cancerous growths that develop from mesenchymal stem/progenitor cells (MSCs), impacting mesodermal tissues like bones, cartilage, fat, or muscles. While these malignancies account for a mere 1% of total cancer diagnoses worldwide, the percentage increases to 12%–15% when considering pediatric cases. This underscores the necessity for more effective treatment strategies as a promising therapeutic option, our focus was on mithramycin (MTM), a natural antibiotic known for its potential anti-tumor properties but also for causing significant systemic toxicity. Hence, the utilization of nano-delivery systems to encapsulate MTM could potentially widen its therapeutic gateways. The comprehensive data on the use of TFs for the delivery of anti-cancer drug is presented in the following report and can also be found in Table 3. Estupiñán *et al.* developed innovative PLGA polymeric TFs (MTM-loaded TFs) through ethanol injection and thin film hydration techniques. The optimized MTM-loaded TFs displayed an EE 87% with a strong anti-tumor effects against adherent and cancer stem cell-enriched tumorsphere cultures of chondrosarcoma and myxoid liposarcoma models. Moreover, MTM delivered *via* nanocarriers effectively hinders the signaling pathway facilitated by the pro-oncogenic factor SP1 [73].

Head and neck squamous cell carcinoma

Non-invasive sonodynamic therapy (SDT) employs low-intensity ultrasound to induce chemical agent sensitizers for cancer treatment in a targeted manner. Pourhajibagher *et al*. explored the impact of ultrasound in combination with emodin-loaded TFs (NETFs) on head and neck squamous cell carcinoma (HNSCC) cell lines (Table 3). The final formulation exhibited a high drug-loading capacity and EE% with a significant hemolytic activity. The cytotoxicity test showed 97.3% and 98.2% cytotoxicity with 10×10^{-4} g/L of NETFs combined with ultrasound irradiation for about 5 minutes (frequency: 1 MHz and intensity: 2 W/cm^2) on CAL-27 and FaDu cell lines, respectively. Moreover, a remarkable dose-dependent ROS production, an enhanced apoptosis, and increased expression levels of caspase-3/9 genes were observed in NETFs group [60].

Oral cancer

Oral cancer (or mouth cancer) is among the most prevalent malignancies with a rising incidence rate. An amalgam of 5-FU and Etodolac (Et) is an efficient chemotherapy approach for treating oral cancer. Et is a cyclooxygenase-2 inhibitor and enhances the sensitivity of cancer cells to chemotherapeutic agents. In a study, TFs co-loaded 5-FU and Et formulations were created through the thin-film hydration system. The maximum EE% was ~37% for 5-FU and ~80% for Et (1:1). In a cell viability assay on FaDu cells with varying concentrations of Et and 5-FU, a combination index of 0.36 was observed, representing a synergistic impact. The uptake of Et/5-FU-loaded TFs by FaDu cells was meaningfully higher than that of free form. The TFs hydrogel containing HPMC (2% w/w) exhibited lag time, similar flux, and penetration coefficient to that of drug-loaded TFs when tested on excised porcine buccal tissue, suggesting a targeted delivery in the management of oral cancer (Table 3) [74].

Brain tumor

Glioblastoma multiforme (GBM) is acknowledged as an initial central nervous system (CNS) tumor that is highly prevalent and deadly. The treatment of this tumor is hindered by the challenges associated with breaching the blood-brain barrier (BBB) and by the nonspecificity of chemotherapeutic agents towards tumor cells. Luiz *et al.* developed folate (FA)-modified TPGS TFs containing docetaxel (DTX/TFs/FA) to enhance the treatment of GBM (Table 3). The optimized formulation examined a small particle size (below 200 nm) and high EE% (about 79% for DTX/TFs and 75.6% for FA-modified DTX/TFs). Additionally, DTX/TFs/FA revealed the significant capability to decrease the viability of U-87 MG cells compared to the DTX commercial formulation and TF-DTX. The *in vitro* cellular uptake demonstrated a superior internalization of DTX/TFs/FA into U-87 MG cells compared to DTXTFs (72% and 63%, respectively, after 24 h), proposing the potential of folate modification for targeted GBM treatment [75].

Challenges of TFs-based anticancer drug delivery

Various factors could affect the anticancer drug delivery by TFs. The ratio of phospholipid/EA can affect the entrapment efficiency, vesicle size, and permeation ability. A higher surfactant concentration can decrease TFs size and reduce the EE% via the generation of pores within the TFs membrane and the leakage of the encapsulated drug. In addition, increasing EA content may lead to pore formation in the bilayer and a reduced permeation ability of the TFs. The selection of a suitable solvent is another critical factor. A proper solvent can enhance the penetration of the drug into the SC by different mechanisms, such as increasing the drug solubility in TFs, altering the solubility properties of the target tissue, and improving the drug partitioning into the membrane. Despite this, a high concentration of ethanol in the TFs may lead to a decrease in the %EE. The suitable pH of the hydration medium can also influence the entrapment of the drug into TFs and the permeation of the drug into the cell membrane. Another main drawback of TFs is related to the loading of hydrophobic drugs into the TFs, which can interfere with their elastic and deformability properties.

Transdermal delivery systems are a promising approach for anticancer drug delivery as they are minimally invasive methods without first-pass effects. However, the main challenge with transdermal delivery systems is the barrier function of the skin, which prevents the transdermal delivery of therapeutic agents and needs to be overcome. Molecules with a molecular weight of more than 500 Da and ionized compounds generally cannot pass through the skin. Therefore, only a limited number of drugs can be administered this way. Encapsulation of drugs in TFs is one of the possible approaches to overcome the above-mentioned challenge. Compared to liposomes, TFs are known to be the most outstanding innovative transdermal drug carrier to date.

When TFs reach the skin pores, they can change their membrane flexibility and spontaneously pass through the skin pores. In addition, TFs are elastic and extremely deformable. Therefore, they can deform and compress as an intact vesicle to easily pass through even very narrow pores that are significantly smaller than their size. The high deformability of the vesicles facilitates the transport of drugs through the skin without measurable losses and can be used for both topical and systemic treatments. They are also able to enhance transdermal flow and improve the site specificity of therapeutic agents.

Phospholipids as a component of TFs can facilitate their formability. In this context, the use of natural phospholipids is preferable due to their biocompatibility and biodegradability. However, the difficulty in achieving the purity of natural phospholipids may be a further obstacle in the use of TFs as a transdermal drug delivery system.

According to current evidence, most studies utilizing TFs-based anticancer drug delivery systems

have been conducted in vitro, with only a limited number progressing to preclinical in vivo models. These early-stage studies, while promising, indicate that TFs are still far from entering clinical evaluation for oncology. Several key factors contribute to the current absence of clinical trials for TFs-based cancer therapies. First, TFs are primarily optimized for transdermal or localized delivery, which suits conditions such as osteoarthritis but poses challenges in treating cancers, particularly systemic or metastatic types, that require precise tumor targeting, deep tissue penetration, and prolonged systemic circulation. Second, regulatory and safety considerations are more demanding in oncology. Clinical translation of TFs is limited by the need for comprehensive data on biodistribution, immunogenicity, and long-term toxicity. Compared to more established nanocarriers like PEGylated liposomes, TFs lack the extensive clinical data required to meet these regulatory standards. Third, pharmacokinetic limitations restrict the applicability of TFs in cancer therapy. Their short systemic circulation time and limited passive or active tumor-targeting capabilities reduce their effectiveness in treating internal or deep-seated tumors. Lastly, formulation-related challenges such as scale-up, reproducibility, and variability across patients in skin permeability and tumor physiology further hinder their clinical development in oncology. Nonetheless, future advances including ligand-functionalized TFs, hybrid delivery systems (e.g., microneedle- or iontophoresis-assisted delivery), and application in localized cancers (such as melanoma and oral squamous cell carcinoma) may overcome current limitations and facilitate the translation of TFs into clinical oncology trials.

CONCLUSIONS AND FUTURE PERSPECTIVES

In the arena of drug delivery, TFs have attracted significant attention in recent years. They are being explored as a complex system for drug delivery, with a focus on enhancing local drug penetration.

As previously mentioned, TFs are highly interesting for applications that involve controlled release. They act as carriers for various substances such as medications, chemicals, peptides, and proteins, protecting them from degradation. Researchers have developed numerous formulations of TFs for the treatment of infections, cancer, bone-related issues, and tissues.

The penetration through skin can be a challenge of TFs-based anti-cancer drug delivery. Two key factors influence the passage of TFs through the skin and delivery to tumor site, including the level of flexibility and the partition coefficient of the active substance. The primary mechanism involved is the passive movement of TFs through the SC, driven by the gradient of water content between SC (15%) and the epidermis (75%) [76].

This report discussed the use of TFs for delivering drugs and bioactive materials to various cancers. These techniques are currently in the initial phases of development. Altering the surface properties of TFs can be a key factor in improving their performance, enhancing biocompatibility, and bio-functionality.

Future advancements in TFs could involve optimizing the concentration of therapeutic agents and achieving specific and reversible binding to target sites using targeting molecules like aptamers or peptides. Moreover, enhancing uptake can be achieved by modifying TFs with positively charged nanoparticles or peptides that can interact with negatively charged receptors on cells or tissues. The increasing number of documents related to TFs indicates their potential to serve as innovative and efficient smart drug delivery systems in the future.

ACKNOWLEDGMENTS

The authors acknowledge the financial support from Guilan University of Medical Sciences (GUMS), Rasht, Iran (grant no. IR.GUMS.REC.1402.198).

CONFLICTS OF INTERESTS

All authors declare no financial/commercial conflicts of interest.

DECLARATION OF GENERATIVE AI TOOLS

During the preparation of this work, the authors were not used any AI-assisted technologies.

AUTHORSHIP CONTRIBUTIONS

The specific contributions made by each author:
F.M, Z.Ch: Original draft, Writing & editing
M.Sh, S.N: Graphical abstract, figures and tables
M.R, A.Sh: Writing & Critical revision
F.S, R.N: Conception and design of study, Writing & Critical revision
All authors read and approved the final manuscript.

REFERENCES

1. Wang J-J, Lei K-F, Han F. Tumor microenvironment: recent advances in various cancer treatments. Eur Rev Med Pharmacol Sci. 2018; 22(12).
2. Arneth B. Tumor microenvironment. Medicina. 2019; 56(1): 15.
3. Rahman M, Mohammed S. Breast cancer metastasis and the lymphatic system. Oncol Lett. 2015; 10(3): 1233-1239.
4. Majidpoor J, Mortezaee K. Steps in metastasis: an updated review. Med Oncol. 2021; 38(1): 3.

5. Mattiuzzi C, Lippi G. Current cancer epidemiology. J Epidemiol Glob Health. 2019; 9(4): 217-222.
6. A Baudino T. Targeted cancer therapy: the next generation of cancer treatment. Curr Drug Disc Technol. 2015; 12(1): 3-20.
7. Anand U, Dey A, Chandel AKS, Sanyal R, Mishra A, Pandey DK, et al. Cancer chemotherapy and beyond: Current status, drug candidates, associated risks and progress in targeted therapeutics. Genes & Diseases. 2022; 10(4): 1367-1401.
8. Liu Z, Delavan B, Roberts R, Tong W. Lessons learned from two decades of anticancer drugs. Trends Pharmacol Sci. 2017; 38(10): 852-872.
9. Wen H, Jung H, Li X. Drug delivery approaches in addressing clinical pharmacology-related issues: opportunities and challenges. The AAPS journal. 2015; 17: 1327-1340.
10. Dawidczyk CM, Kim C, Park JH, Russell LM, Lee KH, Pomper MG, et al. State-of-the-art in design rules for drug delivery platforms: lessons learned from FDA-approved nanomedicines. J Controlled Release. 2014; 187: 133-144.
11. Haleem A, Javaid M, Singh RP, Rab S, Suman R. Applications of nanotechnology in medical field: a brief review. Glob Health J. 2023; 7(2): 70-77.
12. Dutta B, Barick K, Hassan P. Recent advances in active targeting of nanomaterials for anticancer drug delivery. Adv Colloid Interface Sci. 2021; 296: 102509.
13. Bandopadhyay S, Manchanda S, Chandra A, Ali J, Deb PK. Overview of different carrier systems for advanced drug delivery. Drug Delivery Systems: Elsevier; 2020: 179-233.
14. Sudhakar K, Fuloria S, Subramaniyan V, Sathasivam KV, Azad AK, Swain SS, et al. Ultraflexible liposome nanocargo as a dermal and transdermal drug delivery system. Nanomaterials. 2021; 11(10): 2557.
15. Estanqueiro M, Amaral MH, Conceição J, Sousa Lobo JM. Nanotechnological carriers for cancer chemotherapy: The state of the art. Colloids Surf B Biointerfaces. 2015; 126: 631-648.
16. Simrah, Hafeez A, Usmani SA, Izhar MP. Transfersome, an ultra-deformable lipid-based drug nanocarrier: an updated review with therapeutic applications. Naunyn-Schmiedeberg's Arch Pharmacol. 2024; 397(2): 639-673.
17. Stefanov SR, Andonova VY. Lipid nanoparticulate drug delivery systems: Recent advances in the treatment of skin disorders. Pharmaceuticals. 2021; 14(11): 1083.
18. Fernández-García R, Lalatsa A, Statts L, Bolás-Fernández F, Ballesteros MP, Serrano DR. Transferosomes as nanocarriers for drugs across the skin: Quality by design from lab to industrial scale. Int J Pharm. 2020; 573: 118817.
19. Shahidulla S, Yameen SH. Transferosomes-a highly permeable nanocarriers of drugs for transdermal drug delivery. World J Pharm Pharm Sci. 2022; 11(9): 509-527.
20. Matharoo N, Mohd H, Michniak-Kohn B. Transferosomes as a transdermal drug delivery system: Dermal kinetics and recent developments. Wiley Interdiscip Rev Nanomed Nanobiotechnol. 2024; 16(1): e1918.
21. Modi C, Kanada K, Prajapati B, Vaghela S, Chadha H. Transfersomes as Transporters via Lipid-Based Drug Delivery System. Lipid-Based Drug Delivery Systems: Jenny Stanford Publishing; 2024: 185-212.
22. Das B, Nayak AK, Mallick S. Transferosomes: a novel nanovesicular approach for drug delivery. Systems of Nanovesicular Drug Delivery: Elsevier; 2022:103-114.
23. Dey S, Hasnain MS, Jha SK, Sahoo N, Nayak AK. Transferosomes: a novel nanotechnological approach for transdermal drug delivery. Advanced and Modern Approaches for Drug Delivery: Elsevier. 2023:199-221.
24. Zhao J, Mao S. Tuning the membrane fluidity of liposomes for desirable in vivo fate with enhanced drug delivery. Adv Biomembr Lipid Self-Assem: Elsevier. 2021 (34):67-106..
25. Li D, Martini N, Liu M, Falconer JR, Locke M, Wu Z, et al. Non-ionic surfactant vesicles as a carrier system for dermal delivery of (+)-Catechin and their antioxidant effects. J Drug Targeting. 2021; 29(3): 310-322.
26. Moammeri A, Chegeni MM, Sahrayi H, Ghafelehbashi R, Memarzadeh F, Mansouri A, et al. Current advances in niosomes applications for drug delivery and cancer treatment. Materials Today Bio. 2023; 23: 100837.
27. Chamel S, Mishra A, Gull A. Transferosomes as innovative drug delivery systems for enhanced antifungal therapy: A comprehensive review. J Drug Deliv Sci Technol. 2024: 105545.
28. Moqejwa T, Marimuthu T, Kondiah PP, Choonara YE. Development of stable nano-sized transfersomes as a rectal colloid for enhanced delivery of cannabidiol. Pharmaceutics. 2022; 14(4): 703.
29. Leonyza A, Surini S. Optimization of sodium deoxycholate-based transfersomes for percutaneous delivery of peptides and proteins. Int J Appl Pharm. 2019; 11(5): 329-332.
30. Jangdey MS, Gupta A, Saraf S, Saraf S. Development and optimization of apigenin-loaded transfersomal system for skin cancer delivery: in vitro evaluation. Artif Cells Nanomed Biotechnol. 2017; 45(7): 1452-1462.
31. Iqubal MK, Khan MA, Agarwal NB, Ali J, Baboota S. Nanoformulations-based advancement in the delivery of phytopharmaceuticals for skin cancer management. J Drug Deliv Sci Technol. 2021; 66: 102912.
32. El-Zaafarany GM, Nasr M. Insightful exploring of advanced nanocarriers for the topical/transdermal treatment of skin diseases. Pharm Dev Technol. 2021; 26(10): 1136-1157.
33. Priyanka K, Singh S. A review on skin targeted delivery of bioactives as ultradeformable vesicles: Overcoming the penetration problem. Curr Drug Targets. 2014; 15(2): 184-198.
34. Jangdey MS, Kaur CD, Saraf S. Efficacy of Concanavalin-A conjugated nanotransfersomal gel of apigenin for enhanced targeted delivery of UV

induced skin malignant melanoma. Artif Cells Nanomed Biotechnol. 2019; 47(1): 904-916.
35. Jain S, Jain N. PUB040 formulation and evaluation of embelin loaded transfersome for effective treatment of skin cancer. J Thorac Oncol. 2017; 12(11): S2378.
36. Wu PS, Li YS, Kuo YC, Tsai SJJ, Lin CC. Preparation and evaluation of novel transfersomes combined with the natural antioxidant resveratrol. Molecules. 2019; 24(3): 600.
37. Avadhani KS, Manikkath J, Tiwari M, Chandrasekhar M, Godavarthi A, Vidya SM, et al. Skin delivery of epigallocatechin-3-gallate (EGCG) and hyaluronic acid loaded nano-transfersomes for antioxidant and anti-aging effects in UV radiation induced skin damage. Drug Deliv. 2017; 24(1): 61-74.
38. Deka T, Das MK, Das S, Das P, Singha LR. Box-behnken design approach to develop nano-vesicular herbal gel for the management of skin cancer in experimental animal model. Int J Appl Pharm. 2022; 14(6): 148-166.
39. Ezzeldeen Y, Swidan S, ElMeshad A, Sebak A. Green synthesized honokiol transfersomes relieve the immunosuppressive and stem-like cell characteristics of the aggressive B16F10 melanoma. Int J Nanomedicine. 2021: 5693-5712.
40. Demartis S, Anjani QK, Volpe-Zanutto F, Paredes AJ, Jahan SA, Vora LK, et al. Trilayer dissolving polymeric microneedle array loading Rose Bengal transfersomes as a novel adjuvant in early-stage cutaneous melanoma management. Int J Pharm. 2022; 627: 122217.
41. Wadher K, Trivedi S, Rarokar N, Umekar M. Development and assessment of rutin loaded transfersomes to improve ex vivo membrane permeability and in vitro efficacy. Hybrid Adv. 2024; 5: 100144.
42. Alvi IA, Madan J, Kaushik D, Sardana S, Pandey RS, Ali A. Comparative study of transfersomes, liposomes, and niosomes for topical delivery of 5-fluorouracil to skin cancer cells: Preparation, characterization, in-vitro release, and cytotoxicity analysis. Anti-Cancer Drugs. 2011; 22(8): 774-782.
43. Bragagni M, Mennini N, Maestrelli F, Cirri M, Mura P. Comparative study of liposomes, transfersomes and ethosomes as carriers for improving topical delivery of celecoxib. Drug Deliv. 2012; 19(7): 354-361.
44. Chen M, Shamim MA, Shahid A, Yeung S, Andresen BT, Wang J, et al. Topical delivery of carvedilol loaded nano-transfersomes for skin cancer chemoprevention. Pharmaceutics. 2020; 12(12): 1-17.
45. Shamim MA, Yeung S, Shahid A, Chen M, Wang J, Desai P, et al. Topical carvedilol delivery prevents UV-induced skin cancer with negligible systemic absorption. Int J Pharm. 2022; 611: 121302.
46. Shamim MA, Shahid A, Sardar PK, Yeung S, Reyes J, Kim J, et al. Transfersome Encapsulated with the R-carvedilol Enantiomer for Skin Cancer Chemoprevention. Nanomaterials. 2023; 13(5): 929.
47. Jiang T, Wang T, Li T, Ma Y, Shen S, He B, et al. Enhanced transdermal drug delivery by transfersome-embedded oligopeptide hydrogel for topical chemotherapy of melanoma. ACS Nano. 2018; 12(10): 9693-9701.
48. Cristiano MC, Froiio F, Spaccapelo R, Mancuso A, Nisticò SP, Udongo BP, et al. Sulforaphane-loaded ultradeformable vesicles as a potential natural nanomedicine for the treatment of skin cancer diseases. Pharmaceutics. 2019; 12(1): 6.
49. Gupta V, Karthikeyan C, Trivedi P. Localized delivery of cisplatin for the effective management of squamous cell carcinoma from protransfersome formulation. Arch Pharmacal Res. 2012; 35(5): 851-859.
50. Li Y, Tai Z, Ma J, Miao F, Xin R, Shen C, et al. Lycorine transfersomes modified with cell-penetrating peptides for topical treatment of cutaneous squamous cell carcinoma. J Nanobiotechnology. 2023; 21(1): 139.
51. Gayathri H, Sangeetha S. Pharmaceutical development of tamoxifen citrate loaded transferosomal gel for skin cancer by doe approach. J Posit Sch Psychol. 2022: 1879–1890.
52. Yousefi M, Nosrati R, Salmaninejad A, Dehghani S, Shahryari A, Saberi A. Organ-specific metastasis of breast cancer: molecular and cellular mechanisms underlying lung metastasis. Cell Oncol. 2018; 41(2): 123-140.
53. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018; 68(6): 394-424.
54. Mahmood S, Taher M, Mandal UK. Experimental design and optimization of raloxifene hydrochloride loaded nanotransfersomes for transdermal application. Int J Nanomedicine. 2014; 9: 4331-4346.
55. Catuogno S, Esposito CL, De Franciscis V. Aptamer-mediated targeted delivery of therapeutics: An update. Pharmaceuticals. 2016; 9(4): 69.
56. Kong M, Hou L, Wang J, Feng C, Liu Y, Cheng X, et al. Enhanced transdermal lymphatic drug delivery of hyaluronic acid modified transfersomes for tumor metastasis therapy. ChCom. 2015; 51(8): 1453-1456.
57. Yang H, Wu X, Zhou Z, Chen X, Kong M. Enhanced transdermal lymphatic delivery of doxorubicin via hyaluronic acid based transfersomes/microneedle complex for tumor metastasis therapy. Int J Biol Macromol. 2019; 125: 9-16.
58. Abdel-Hafez SM, Hathout RM, Sammour OA. Curcumin-loaded ultradeformable nanovesicles as a potential delivery system for breast cancer therapy. Colloids Surf B Biointerfaces. 2018; 167: 63-72.
59. Sundralingam U, Chakravarthi S, Radhakrishnan AK, Muniyandy S, Palanisamy UD. Efficacy of emu oil transfersomes for local transdermal delivery of 4-oh tamoxifen in the treatment of breast cancer. Pharmaceutics. 2020; 12(9): 1-19.
60. Pourhajibagher M, Etemad-Moghadam S, Alaeddini M, Bahador A. Modulation of the triggered apoptosis by nano emodin transfersome-mediated sonodynamic therapy on head and neck squamous

cell carcinoma cell lines. Photodiagnosis Photodyn Ther. 2021; 34: 102253.
61. Liu C, Ma Y, Guo S, He B, Jiang T. Topical delivery of chemotherapeutic drugs using nano-hybrid hydrogels to inhibit post-surgical tumour recurrence. Biomater Sci. 2021; 9(12): 4356-4363.
62. Verma N, Saraf S. Development and optimization of mannosylated naringenin loaded transfersomes using response surface methodology for skin carcinoma. Int J Appl Pharm. 2021; 13(2): 235-241.
63. Sharma M, Mittapelly N, Banala VT, Urandur S, Gautam S, Marwaha D, et al. Amalgamated microneedle array bearing ribociclib-loaded transfersomes eradicates breast cancer via CD44 targeting. Biomacromolecules. 2022; 23(3): 661-675.
64. Gadag S, Narayan R, Sabhahit JN, Hari G, Nayak Y, Pai KSR, et al. Transpapillary iontophoretic delivery of resveratrol loaded transfersomes for localized delivery to breast cancer. Biomater Adv. 2022; 140: 213085.
65. Fernandes NB, Velagacherla V, Spandana KJ, N B, Mehta CH, Gadag S, et al. Co-delivery of lapatinib and 5-fluorouracil transfersomes using transpapillary iontophoresis for breast cancer therapy. Int J Pharm. 2024; 650.
66. Mangla B, Mittal P, Kumar P, Javed S, Ahsan W, Aggarwal G. Development of erlotinib-loaded nanotransferosomal gel for the topical treatment of ductal carcinoma in situ. Nanomedicine. 2024; 19(10): 855-874.
67. Maswadeh H, Demetzos C, Dimas K, Hatziantoniou S, Georgopoulos A, Rallis M, et al. Accumulation of vinblastine into transfersomes and liposomes in response to a transmembrane ammonium sulfate gradient and their cytotoxic / cytostatic activity in vitro. Anticancer Res. 2001; 21(4 A): 2577-2583.
68. Kakhi Z, Frisch B, Heurtault B, Pons F. Liposomal constructs for antitumoral vaccination by the nasal route. Biochimie. 2016; 130: 14-22.
69. Khan I, Apostolou M, Bnyan R, Houacine C, Elhissi A, Yousaf SS. Paclitaxel-loaded micro or nano transfersome formulation into novel tablets for pulmonary drug delivery via nebulization. Int J Pharm. 2020; 575: 118919.
70. Yeo S, Yoon I, Lee WK. Design and Characterisation of pH-Responsive Photosensitiser-Loaded Nano-Transfersomes for Enhanced Photodynamic Therapy. Pharmaceutics. 2022; 14(1): 210.
71. Fadel M, Samy N, Nasr M, Alyoussef AA. Topical colloidal indocyanine green-mediated photodynamic therapy for treatment of basal cell carcinoma. Pharm Dev Technol. 2017; 22(4): 545-550.
72. Yeo S, Kim MJ, Yoon I, Lee WK. pH-Responsive Nano-transferosomes of Purpurin-18 Sodium Salt and Doxorubicin for Enhanced Anticancer Efficiency by Photodynamic and Chemo Combination Therapy. ACS Omega. 2023; 8(18): 16479-16490.
73. Estupiñán Ó, Rendueles C, Suárez P, Rey V, Murillo D, Morís F, et al. Nano-encapsulation of mithramycin in transfersomes and polymeric micelles for the treatment of sarcomas. J Clin Med. 2021; 10(7): 1358.
74. Bollareddy SR, Krishna V, Roy G, Dasari D, Dhar A, Venuganti VVK. Transfersome Hydrogel Containing 5-Fluorouracil and Etodolac Combination for Synergistic Oral Cancer Treatment. AAPS PharmSciTech. 2022; 23(2): 70.
75. Luiz MT, Viegas JSR, Abriata JP, Tofani LB, Vaidergorn MdM, Emery FdS, et al. Docetaxel-loaded folate-modified TPGS-transfersomes for glioblastoma multiforme treatment. Mater Sci Eng C. 2021; 124: 112033.
76. Sala M, Diab R, Elaissari A, Fessi H. Lipid nanocarriers as skin drug delivery systems: Properties, mechanisms of skin interactions and medical applications. Int J Pharm. 2018; 535(1-2): 1-17.