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Research Article

5-Fluorouracil-Loaded Transfersome as Theranostics in Dermal Tumor of Hypertrophic Scar Tissue

Z. Zhang,[1] X. Wang,[1] X. Chen,[1] Y. Wo,[2] Y. Zhang,[3] and Ewelina Biskup[4]

[1] *Department of Dermatology, Shanghai Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai 200011, China*
[2] *Department of Human Anatomy, Histology and Embryology, School of Medicine, Shanghai Jiao Tong University, Shanghai 200025, China*
[3] *Department of Plastic and Reconstructive Surgery, Shanghai Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai 200011, China*
[4] *University Hospital of Basel, Petersgraben 4, 4051 Basel, Switzerland*

Correspondence should be addressed to X. Chen; xdchen@medmail.com.cn

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To investigate the ability of transfersomal gel carrying the antiscarring agent (5-FU) to permeate hypertrophic scars *in vivo* and *in vitro*, scar permeation studies were performed after the agent was labeled with the fluorescent agent, rhodamine 6GO. Laser confocal microscope was employed to dynamically observe the effects of transfersomal gel carrying 5-FU at different time points. High-performance liquid chromatography (HPLC) was used to analyze the contents of the agent in the scar tissues at different hours after administration. Scar elevation index (SEI) was used to evaluate the changes of the ear scar models in rabbits. Compared with the PBS gel of 5-FU, the transfersomal gel displayed greater permeation rate and depth, as well as a higher content retention of the agent in scar tissues. Local administrations of the agent for some certain periods effectively inhibited the hyperplasia of ear scars in rabbits. Transfersomes can be chosen as a potential transdermal drug delivery system.

1. Introduction

Hypertrophic scars (HS) are common pathological scars, which result from the excessive healing of wounds and lead to malformed appearance, paresthesia, and even organic dysfunctions. The current nonsurgical treatment for hypertrophic scar focuses on medication, predominantly local injection, and cutaneous application. However, local injections cause obvious pain and require long-term repetitive course, which is associated with a poor compliance. Topical application is easy to operate and has low drug side effects, but the penetration efficacy depends on the permeability of the agent, as well as being limited due to the thickening and dense structure of HS dermis layer and the decreasing of skin's appendages [1]. The major barrier in transdermal delivery is the stratum corneum, skin's intrinsic barrier against diffusion of hydrophilic agents. Therefore, in order to improve the HS management, the efficiency of antiscarring agents has to be enhanced. This can be achieved by augmenting the drug's transdermal delivery, for example, in a high-performance vesicular carrier, so that it can accumulate at an effective local concentration in the target tissues. A number of vesicular systems have been described, for example, liposomes, niosomes, and elastic liposomes such as ethosomes and transfersomes. Transfersomes are novel, self-optimizing lipid carriers for enhanced transdermal drug delivery, composed of active surface agents embedded in a common liposome [2]. Their main feature is their deformability, resulting in a good permeability through spaces 5 to 10 times smaller than their own diameter. Transfersomes have been studied extensively in the recent years, mostly in *in vitro* conditions. They have been reported to be extremely effective for low and high molecular weight drugs, since they permeate the keratinocyte barrier of skin to enter the dermis along the osmotic gradient due to their low surface tension [3, 4].

A number of agents have been studied and reported to have the ability to treat hyperplastic scar, for example, corticosteroids, interferon, and 5-fluorouracil (5-FU) [5–8]. 5-FU is a pyrimidine analogue with antimetabolic activity. It inhibits the *in vivo* production of thymine nucleotide, blocks the transformation of deoxyuridine nucleotide to deoxythymine nucleotide, inhibits the synthesis of DNA, and interferes with proteins' metabolism. 5-FU has found an increasing application in the clinic as an inhibitor of excessive scar tissue hyperplasia. The agent is being injected into multiple points of scar tissues [9], resulting in reported improvement of scar thickness, color, and hardness in hypertrophic scars [8, 10]. However, local 5-FU injections induced several side effects, such as pain at administration, pigmentation, ulceration, purpura on injection sites, tissue collapse, and so forth. Therefore, it is crucial to develop a safer and more effective 5-FU administration system that would allow the drug to permeate HS tissues circumventing the adverse symptoms.

In our previous studies we investigated the efficacy of transfersome suspensions in HS. We observed a high permeability in hypertrophic scar tissues compared to hydroethanolic solutions. We also reconfirmed what has been reported before: transfersomes are vesicles with an excellent permeability and high retention effect due to their dynamics and high lipid affinity [11]. However, suspensions were not convenient for application, since their adhesion is poor. Gel form would be more practicable, since it adheres to the site of action, allowing for a sufficient time for distribution and absorption of the drug in local tissues, as well as for a controlled release rate of the drug with the aid of hydrated gel layer [12]. In this study, we transformed the 5-FU transfersome suspension into gel. We aimed to investigate its deformation ability, skin irritation, HS permeation, deposition, and interference *in vivo* and *in vitro*. Finally, we evaluated 5-FU transfersome gel for its efficacy in HS treatment.

2. Materials and Methods

2.1. Materials

2.1.1. Human Hypertrophic Scar Samples. This study was performed in accordance with the 1975 Declaration of Helsinki ethical guidelines and approved by Shanghai Ninth People's Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China. Samples were collected from 4 patients who underwent resection and dermoplasty for hypertrophic scald scars (chest and shoulder) in our reconstructive surgery department of Shanghai Ninth People's Hospital. All patients were female of 22–31 years of age, with a disease course of 6 months–1 year, who never received pharmacologic or radiation HS therapy. Those scars were intact with areas of >5 cm × 5 cm but no ulceration. General anesthesia was used to eliminate the interference of local anesthetics.

2.2. Methods

2.2.1. Preparation of Transfersomal Gel of 5-FU. A modified preparation protocol by Touitou et al., called improved film dispersion method [13], was used to prepare 5-FU transfersomal gels. 0.03% (w/v) rhodamine 6GO, 0.4% (w/v) 5-FU, and 4% (w/v) phospholipid transfersomes' suspension were prepared with 160 mg carbopol. 400 mg glycerin was added to grind carbopol. Triethanolamine was added to adjust PH to 7.4. After swelling for 2 hours, 4.2 mL deionized water was added. A 3.2% (w/v) carbopol, 8% (w/v) glycerol 2.5 g carbopol gel was accurately weighed and homogenously mixed in 2.5 mL nanoscale 5-FU transfersomes to obtain rhodamine 6GO labeled 5-FU transfersomal gel with a final 5-FU concentration of 0.4% (w/v).

To bind rhodamine to rhodamine 6GO 5-FU suspension PBS pH 7.4 (0.8% (w/v)) was mixed with an equal volume of the carbopol gel under continuing stirring to form rhodamine 5-FU gel (Rho 5-FU CG) (rhodamine 0.03% (w/v)). It has been shown that rhodamine is not changing the affinity or pharmacokinetics of 5-FU [13].

2.2.2. Characterization of Transfersomal Gel. 5-FU TG was diluted 10 times with distilled water. 20 μL was dripped on the carbon film of copper net, air-dried overnight, stained with 3% phosphorus acid buffer (pH = 6.0) at room temperature, and then observed under Philips TEM JEM2010 electron microscope. The particle size and polydispersity index (PDI) of 5-FU TG were measured at room temperature using particle sizer system (PSS).

2.2.3. Determination of 5-FU Retention as per 5-FU-Rhodamine-TG Permeating Scars for 6 h. Modified Franz drug percutaneous permeability diffusion tester was applied to perform *in vitro* permeation experiment. Two groups were included: Rho 5-FU TG group and Rho 5-FU CG group. Hypertrophic scar tissues were prepped into round samples with 2 cm in diameter, of which hypertrophic scar samples were 3.5 mm deep. The samples were fixed in between administration room and reception tank with stratum corneum facing the administration room. PBS (PH = 7.4) was added into reception tank to cover the skin samples completely. Gels were placed into administration room (0.5 g gel: 5-FU was 1 mg), scars permeation initiated: effective scar diffusion area 2.8 cm^2, volume of reception tank 6.5 mL, temperature of water bath 37 ± 0.5°C, and 200 ± 1 rpm constant speed electromagnetic stirring. At 6 h, residual drugs on scar surface were removed and dried; samples were placed in 10 mL PBS to swell on a shaking table overnight (120 r/min). HPLC was used to determine total retention amount of 5-FU in tissues after the PBS wash. Retention amount per tissue unit was compared between groups as follows: retention amount in per unit tissue = total retention amount in tissues/thickness of tissues.

2.2.4. Determination of Depth and Intensity of 5-FU TG Labeled with Rho Permeating Hypertrophic Scars. In a modified Franz drug percutaneous permeability diffusion tester, 0.5 g 5FU-gel was coated on scars stratum corneum of and tested at 200 r/min, 37 ± 0.5°C. Gel preparations were taken as control at 1 h and 6 h. Drugs retained in the surface of scars were softly rinsed with 37°C double distilled

water and dried. Samples were soaked in 4% paraformaldehyde solution overnight, followed by 30% sucrose solution overnight. Finally, samples were cut into size of 0.5 cm × 0.3 cm × 0.2 cm, embedded at optimal cutting temperature (OCT), then placed into a freezing microtome, and cut into slices of 7 μm thickness. Histomorphology and fluorescent distribution were observed with confocal laser scanning microscopy (CLSM). Distribution range and intensity of fluorescence were analyzed and calculated (image analysis software, Release Version 4.0 SP2) in high power field (<100 times). Standard values were converted to pixel values of fluorescence depth (unit length of Rho penetrating the scars) to provide semiquantitative expression of transfersomes permeability.

2.2.5. Construction of Rabbit Ear Hypertrophic Scar Models. Rabbit ear hypertrophic scar models were constructed (Morris's method) [14]. The experiment was carried out with the approval of the Animal Experimentation Ethics Committee School of Medicine, Shanghai Jiao Tong University. Six New Zealand white rabbits were anesthetized with 0.15 mL SU-MIAN-XIN and 0.2 mL ketamine per 100 g. After conventional disinfection and draping, round skin defects (1 cm in diameter) were prepped (reaching perichondrium, 1.5 cm). Five wound surfaces were constructed in each ear, with a total of 60. After complete hemostasis, all the wound surfaces were dressed with sterile gauzes for one day; the wound healing was monitored closely. Vernier caliper was used to record the thickness of scar and surrounding tissues at 4 weeks. Scar wound surfaces with ratios greater than 150% were regarded as successfully constructed hypertrophic scar models.

2.2.6. In Vivo Permeation of Rhodamine in Hypertrophic Scar Tissues under CLSM. An ear of each big-ear white rabbit was randomly selected to receive Rho 5-FU TG on the successfully constructed scar wounds. The respective other ear was treated with Rho 5-FU CG and massaged for 30 min. The ear was given, once a day, 0.05 g each time (working concentration of 5-FU 0.2 g/day/scar) [15]. The experiment was repeated 6 times. Permeation depth of the two gel preparations in hypertrophic scars in rabbit ears was observed at 1 h and 6 h under a laser confocal microscopy (LCM): removing of residual drugs with PBS, scar samples preparation as 1.5 cm × 0.5 cm × 0.3 cm, embedding at OCT, freezing at −20°C, and microtomy into longitudinal slices with 10 μm thickness. The images were processed by image management software, Release Version 4.0 SP2.

2.2.7. Histological Examination of SEI after Hematoxyline & Eosin (HE) Staining. HE staining was used to observe morphologic changes of 5-FU TG on hypertrophic scars in rabbit ears. 14 days after drug administration, fresh scar tissues in rabbit ears in each group were internally fixed in 4% paraformaldehyde for 48 h. 20~30% sucrose solution was used for gradient dehydration for 24, placed into paraffin blocks, frozen for 30 min, and cut into 5~10 μm slices. After HE staining, SEI (scar elevation index) was detected under inverted phase contrast microscope [16]. SEI of facies ventralis in rabbit ears after 14 days of continuous administration of drugs in each group was calculated as SEI = dermal thickness on segmental venter in rabbit ears after wound healing/dermal thickness of adjacent normal tissues (Figure 1).

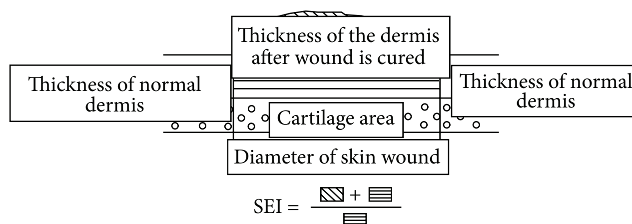

Figure 1: SEI reflects the scars' hyperplasia. SEI: dermal thickness after wound healing/dermal thickness of adjacent normal tissues. SEI > 1.5 indicates hypertrophic scars.

2.2.8. MTT. Fibroblasts derived from scar tissues were enzymatically digested and cultivated in DMEM with 10% fetal bovine serum at 37°C in a humidified incubator with 5% carbon dioxide [16]. Only low-passage cultures (passage 4 to passage 6) were analyzed in this study in experimental groups as follows: 5-FU TG and 5-FU CG, TG, CG, and control. Briefly, after incubation for 24 h, 48 h, and 72 h, fibroblasts were washed three times with culture medium. 100 μL 3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide MTT (5 mg/mL in PBS) was added for 4 h of incubation. After a PBS wash, 100 μL DMSO was added to each well; plates were agitated at 80 rpm for 15 min. The absorbance was measured at 490 nm with an ElX-800 Microelisa reader. Each sample was evaluated four times and expressed as mean ± standard deviation (SD).

2.3. Statistical Analysis. Continuous variables were reported as mean ± SD. Then intercomparison was performed with a one-way ANOVA. SPSS Version 18 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. A P value of <0.05 was chosen to indicate statistical significance.

3. Results

3.1. Preparation and Characterization of Transfersomes. Transmission electron microscope (TEM) images in Figure 2 present prepared transfersomes as small, unilamellar, liposomal, spherical, or elliptical vesicles, with homogeneous distribution and an average particle diameter of 91.6 ± 3.5 nm (n = 16). Particle size of 5-FU TG was 84.5 ± 7.1 nm (n = 16) measured by particle size analyzer, which was consistent with the TEM results. PDI (polydispersity index) of transfersomal gel measured by a laser particle size analyzer (λ = 632.8 nm) at 25°C was 0.143 ± 0.022.

3.2. In Vitro 5-FU Retention of in Hypertrophic Scar Tissues. At 6 h of transfersomal gel permeation, retention amount of 5-FU in hypertrophic scar tissues was detected by HPLC. The results were expressed by retention amount per tissue

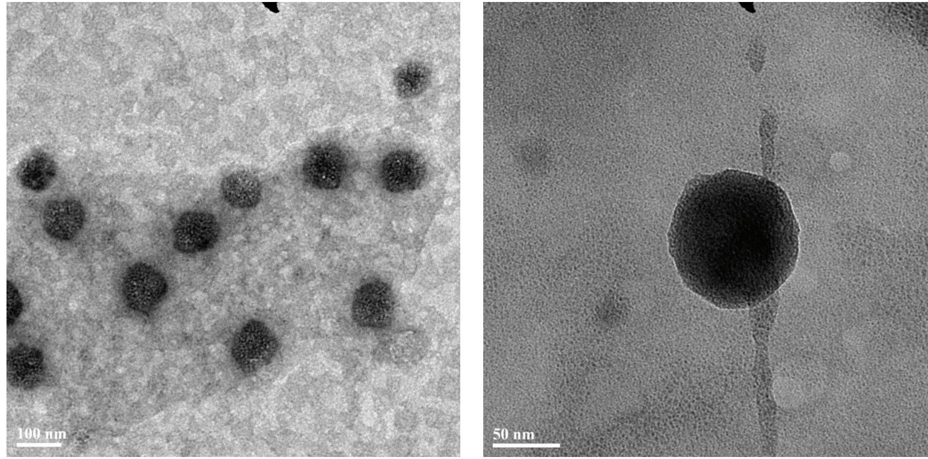

FIGURE 2: 5-FU TGs in transmission electron microscopy (TEM): spherical or elliptical vesicles, sizes < 100 nm ($n = 16$).

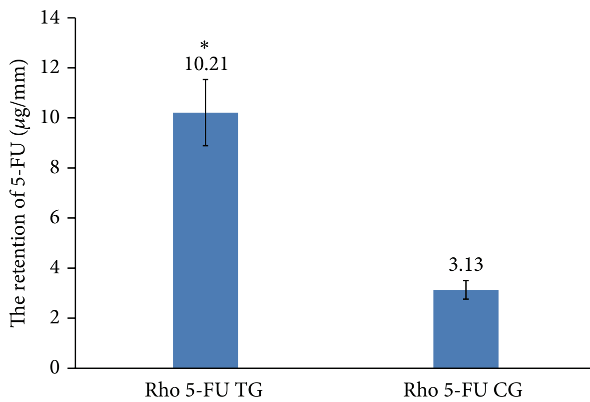

FIGURE 3: The retention of 5-FU (6 h administration) in each of the gel groups ($n = 3$). Values were expressed as mean ± SD ($n = 6$). $^*P < 0.05$ compared with 5-FU CG using one-way ANOVA. The retention of 5-FU in tissues of TG after 24 h was higher than that in 5-FU CG. 5-FU TG: 5-FU transfersomal gel. 5-FU CG: 5-FU PBS gel.

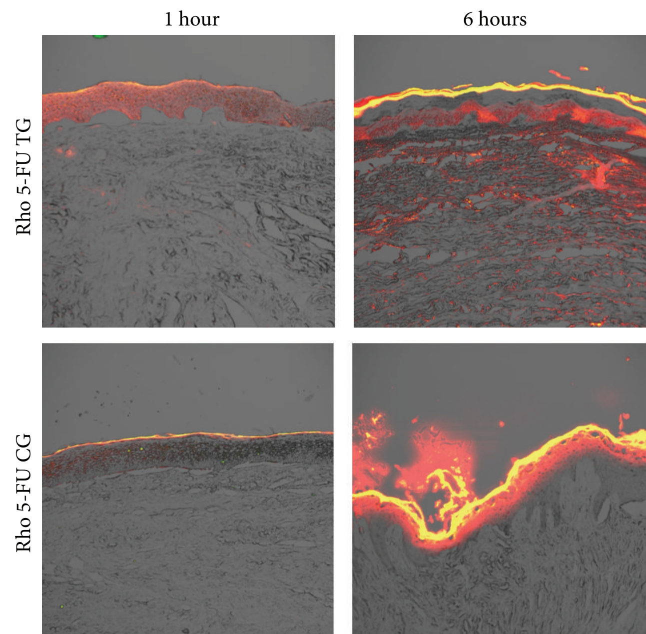

FIGURE 4: CLSM images of cross sections in each gel group containing rhodamine 6GO at 1 h and 6 h.

unit. Retention amount in Rho 5-FU TG group was higher (10.21 ± 1.32 μg/mm ($n = 3$)) than in Rho 5-FU CG (3.13 ± 0.37 μg/mm). The difference had statistical significance (Figure 3), indicating that transfersomal gel carries drugs and permeates and is retained in skin tissues.

3.3. Permeation Depth and Fluorescent Intensity of Transfersomal Gel in Hypertrophic Scar Tissues. Rho labeled 5-FU TG and CLSM were used to assess scar permeability of two kinds of liposome gels (Figure 4) at 1 h and 6 h of percutaneous scar permeation. Compared with control group, fluorescence intensity in Rho 5-FU TG group increased significantly over time. At 1 h, stratum corneum was permeated by Rho 5-FU TG, which was distributed in the entire epidermal layer, while the fluorescence in Rho 5-FU CG group is only distributed in shallow parts of the epidermal layer and did not permeate stratum corneum. At 6 h, gels in each group permeated epidermis; however the fluorescence in Rho 5-FU TG group was distributed in the entire tissue and the fluorescent intensity was decreasing from dermis to epidermis, with the strongest fluorescence areas in the epidermal layer. Permeation in each group was analyzed by a quantitative analysis of the images (Figure 5). Permeation of Rho 5-FU TG (215.07 ± 6.93, $n = 6$) was greater than Rho 5-FU CG (108.01 ± 4.08) with a statistical significance ($P < 0.05$).

3.4. Postoperative Observation of Hypertrophic Scar Tissues in Rabbit Ears. On day 14, scar wounds gradually healed with scabs at the surface and no significant eminence. On day 28, wounds showed a complete epithelization. Macroscopic findings included obvious eminence in skin and thick, hard hyperplasia (Figure 6).

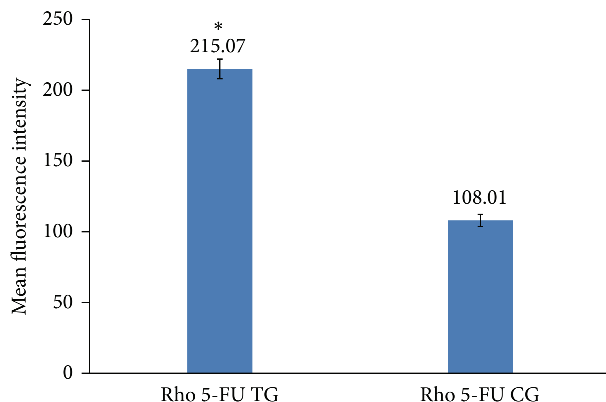

Figure 5: Fluorescence analysis in human skin and hypertrophic scars (ROI chest and shoulder) in both gel groups containing rhodamine 6GO at 6 h ($n = 6$).

3.5. In Vivo Permeability of Rho Transfersomal Gel in Rabbit Ear Hypertrophic Scar Tissues. Depth of Rho permeation in hypertrophic scars of rabbit ears at two time points (1 h and 6 h) was compared. At 1 h after administration, Rho in Rho 5-FU TG group and Rho 5-FU CG group gathered in superficial skin layer, with Rho 5-FU TG group showing the tendency of permeating to dermal layer. At 6 h, Rho 5-FU TG group gel permeated into the superficial layer of dermis and diffused to deep layers.

5-FU CG group gels were retained in superficial skin layers, with no trend of a continuous permeation at 6 h after administration. These results indicate that transfersomal gel percutaneously permeates into dermal layer of body scar tissues (Figure 7).

3.6. In Vivo Observation of Histomorphologic Changes of 5-FU TG. After HE staining, morphologic changes of rabbit ear hypertrophic scar tissues and their surrounding normal tissues were observed on day 14 after administration; SEI in each group was compared. Numerous fibroblasts were seen in dermal layers in both groups with disordered arrangement and circinate structure, while superficial collagen fibers showed nodular or circinate arrangement. Hyperplasia and several new chondrocytes (reconstruction of cartilage tissues) were found in central scar tissues (Figure 8). SEI was higher in Rho 5-FU CG than in Rho 5-FU TG with a statistical significance ($P < 0.05$) (Figure 9).

3.7. MTT. Compared to all control groups, without 5-FU, each experimental group with 5-FU (10 μg/mL) inhibited the fibroblasts' activity. This observation was most obvious at 24 h after administration, with a significant accentuation at 72 h (A values in each experimental group were significantly reduced, with a statistical significance ($P < 0.05$)).

4. Discussion

Over the past few decades, there was a tremendous development of new technologies that enhance the dermal permeation of drugs. The application of transdermal drug delivery is usually limited by the inability to permeate into deep skin and retention in superficial skin, with merely local effects [17–19]. Liposomes for external use have proven to be one of the main focuses in delivery vesicles research [4, 20, 21]. These are hollow spheres, encapsulated by a bimolecular lamellar lipid membrane, with diameters of 50~1000 nm. Both liposoluble and hydrosoluble drugs can be transported via liposomes, which thus are being exploited for the transdermal permeation of drugs. Drugs in liposomes can be transdermally absorbed by hydration, fusion, permeation, and other rationales. They can also directly reach lower skin through sebaceous glands, sweat glands, and even hair follicles. Further features of liposomes such as a good biodegradability, sustained release, or nontoxicity are attractive for transdermal drug transportation.

Protocols for liposomes with improved components, which greatly improve their ability of transdermal drug delivery, have been reported. The most representative ones are transfersomes, also known as deformed liposome, developed by Cevc and Blume [2], enriched with surfactants such as sodium cholate, Tween-80, and Span-80, which reduce the surface tension of phospholipid bilayer, improving flexibility, and increasing the deformability. Therefore, transfersomes can penetrate small junctions of a diameter 1/5~1/10 of its own size. Compared with common liposomes, transfersomes can permeate to stratum corneum or even deep dermis, thus shortening the physical distance of absorbing drugs into bloodstream. They also improve the retention of drugs in local skin, greatly enhancing the targeting of drugs. Transfersomes are thus currently considered one of the most ideal lipidic drug carriers for transdermal administration.

Our previous studies have found that permeability of ethosomes with a particle diameter of 65 nm is higher than that of 200 nm [1]. Particle size of transfersomes prepared in our study was uniformly 91.6 ± 3.5 nm under TEM and was 84.5 ± 7.1 nm ($n = 16$) by particle size analyzer; PDI was 0.143 ± 0.022. Production principle of transfersomes was to add surfactants to the basic liposomes, which enhances the deformability on the basis of lipid affinity at liposome level. This morphology allows permeating the interspace of immature keratinocytes in scar surface. In addition, gel preparation includes adding carbomer into liposomes, which reduces the aggregation of transfersome suspension vesicles, increases their stability, and effectively improves the permeability of drugs.

For percutaneous administration for therapy of skin diseases, the permeation of drugs into skin tissue through intercellular spaces, hair follicles, sweat glands, and other subsidiary organs and retention in local tissues are premises for the drugs' therapeutic effect. In our *in vitro* scar permeation experiment, we observed the process of wound repair in skin tissue accompanied by an excessive hyperplasia, collagen, and extracellular matrices thickening dermal layer, as well as reduction of hair follicles, sweat glands, and accessory structures. In our study, modified Franz diffusion cell model was used to conduct *in vitro* scar permeation experiments. We also assessed the permeability and retention performance in tissues of transfersomal gel preparations in *in vivo* rabbit ear hypertrophic scar permeation experiment. In the *in vitro*

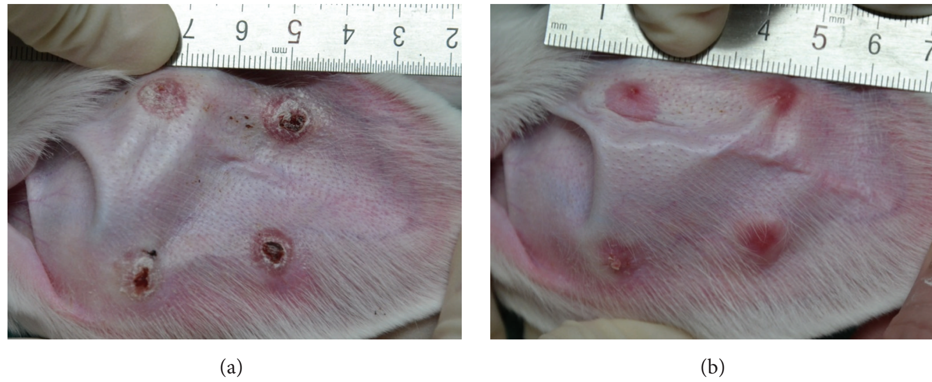

Figure 6: Rabbit ear hypertrophic scar model: (a) 2 weeks post-op; (b) 4 weeks post-op.

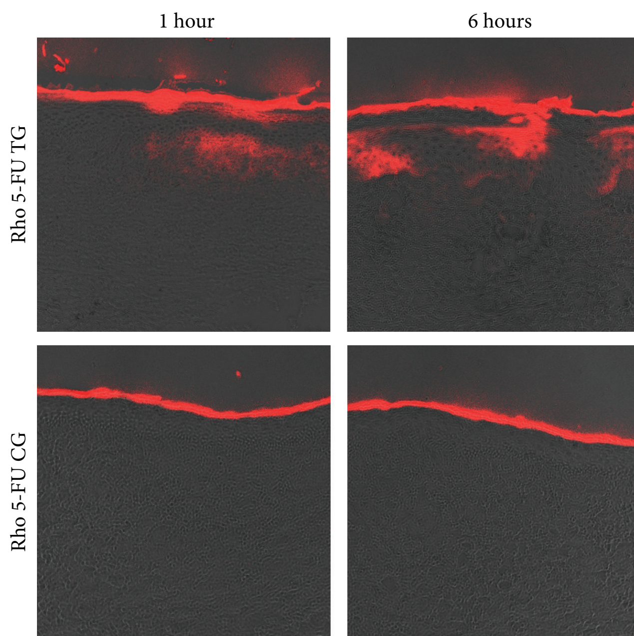

Figure 7: *In vivo* permeation of rhodamine gels. Red areas show rhodamine fluorescence in hypertrophic scar tissues in rabbit ears at 1 h and 6 h after administration. Compared with control group, at 6 h after administration, transfersomal gel percutaneously permeated into deep dermal layers.

human hypertrophic scar tissue permeation experiment, we found that penetration depth and intensity in 5-FUTG group labeled with rhodamine were better than those in 5-FU CG group ($P < 0.05$) (Figure 4). *In vivo* rabbit ear hypertrophic scars permeation experiment revealed the same results (Figure 7). Liposomes with small particle sizes were able to transport 5-FU by means of phospholipids fusing in keratinocyte membrane, giving a nanosmall size effect with a good permeability and retention. So far, liposomes were applied in form of a suspension, which cannot be maintained on the body surface for a long time. We transformed it into a gel and labeled it with rhodamine. We found that, at 1 h after administration, transfersomal gel had the tendency of permeating to dermal layers; at 6 h after administration, it was able to permeate to superficial dermis and diffuse to deeper areas. The permeative capability of transfersomes was still intact in the gel form. Transfersomes percutaneously permeated into the dermal layer of body scar tissue in rabbit ears and allowed 5FU to reach the target site. This indicates that transfersomes in gel form are very good penetration enhancers. Gel form seems to retain the features of phosphatides fusion and nanosmall size effect to change space conformation in nanoscale space [5–7].

In vitro drug retention in scar tissues after 6 h was detected by HPLC. The content of 5-FU in scar tissues in 5-FU TG group was significantly higher than that in 5-FU CG group ($P < 0.05$). Under LCM, at 6 h after either *in vitro* or *in vivo* drug administration, 5-FU transfersomal gels labeled with rhodamine exhibited greater fluorescence intensity in scar tissues compared to the control group. Our previous studies have found that, compared with other liposomes, transfersomes themselves have a larger deformability, resulting in a higher entrapment rate.

SEI in each group was measured by HE staining and tissue sections. In our *in vivo* experiments, SEI of 5-FU Gel and 5-FU TG were significantly reduced compared with that in control group. This indicates that transfersomal gel transported more 5-FU into scar tissues, while effectively inhibiting the hyperplasia of scar tissues. This allowed preventing the cytotoxic effects of 5FU via activation of intracellular superoxide radicals specifically on normal human epidermal keratinocytes. Additionally, transfersomes have no damaging effect on the drugs' structure or efficacy, enabling drugs to be released and act after entering the target tissue. Gel form allows carbomer to disperse in lipid vesicles, reducing the tendency of heavy flocculation caused by the collision between vesicles due to Brownian motion. Thus, the gel form is more stable and easier to store than a suspension, has good air permeability, and can form drug reservoirs on skin layer, which enables the slow release of drugs. Additional features are a good adhesiveness, which prolongs the time of retention on the body surface, making gel preparation convenient for external use. MTT experiment showed that 5-FU can directly inhibit the proliferation activity of fibroblasts, but gels and transfersomes have no obvious effect on proliferation activities and on 5-FU efficacy (see Figure 10). *In vivo*, after

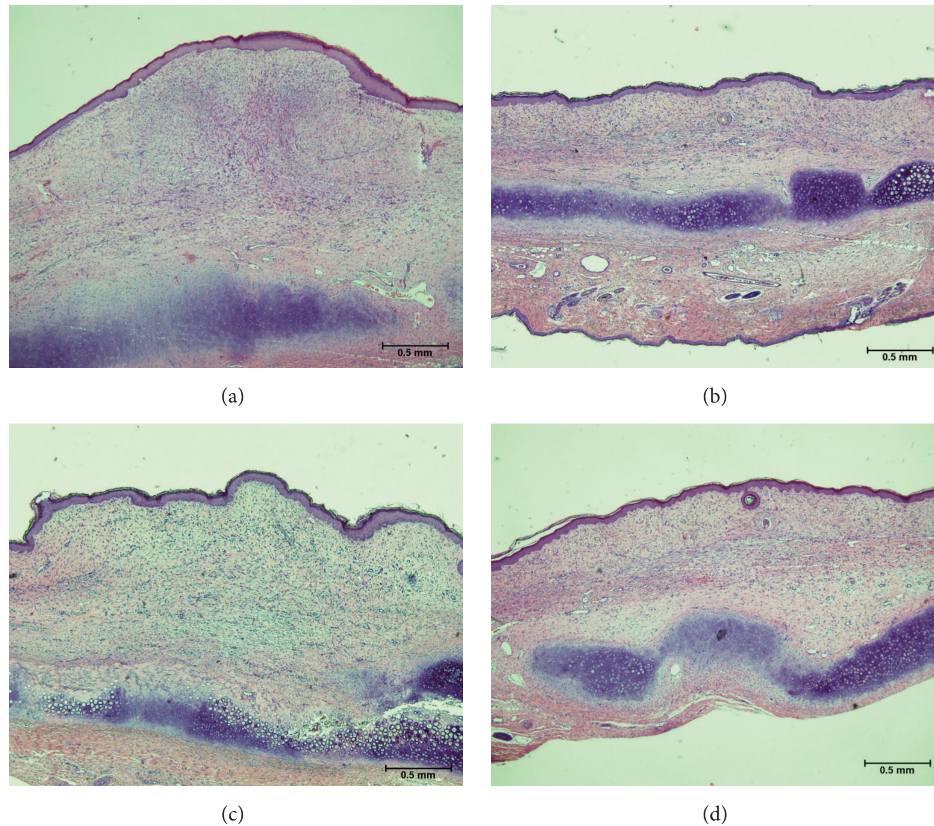

Figure 8: Morphological changes of hypertrophic scars in rabbit ears in both groups on day 14 after administration. (a) and (c) showed the comparison of the hypertrophic scars of before and after administration. Similarly, (b) and (d) showed the changes of 5-FU TG group. Numerous fibroblasts and collagen fibers with disordered arrangement were seen in dermal layer in (a) and (b). Hyperplasia of dermal tissue in 5-FU TG group was alleviated more than in Rho 5-FU CG group.

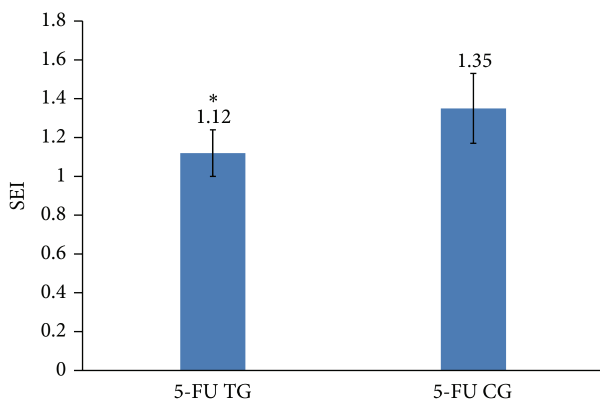

Figure 9: Comparison of hypertrophic scar index in rabbit ears of each group 14 days after administration, $P < 0.05$.

external use of gel preparations on scars in rabbit ears, no significant changes in appearance and no pruritus or allergic reactions were observed.

The strong advantage in our study is the use of not only *ex vivo*, but also *in vivo* animal model experiments. We studied

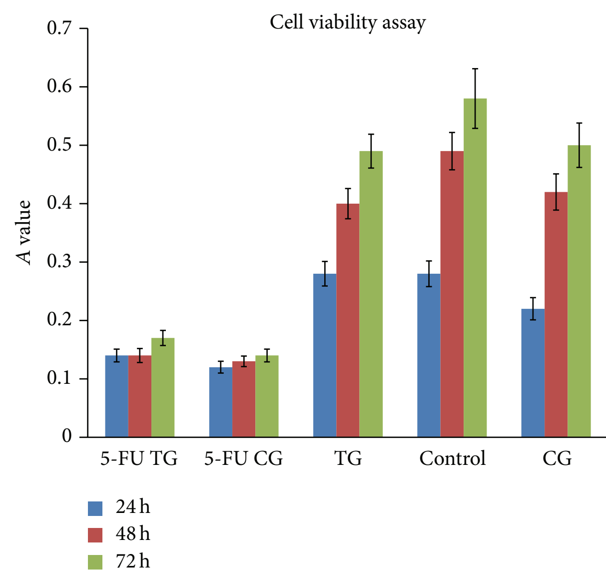

Figure 10: Effects of gels on cell proliferation. Data expressed as the mean ± SD are representative of 4 independent experiments with similar results. Cell survival was assessed at 24 h, 48 h, and 72 h ($P < 0.05$).

transfersome penetration in human skin (*ex vivo*) and rabbit ears (*in vivo*) hypertrophic scar tissue. We could conclude that transfersomes are superior in 5-fluorouracil delivery compared to standard solutions. Our results thus pave the way for further, phase III clinical studies. Furthermore, we have been using a gel form of 5-FU, which is a novel medium that we described before [11]. 5-FU EGs is durable and usable. This provides a feasible condition for hypertrophic scar treatment in the rabbit ear model.

Our results allow the conclusion that 5-FU transfersomal gel is a safe, convenient, and effective drug carrier for transdermal administration.

5. Conclusion

Transfersomes hold a variety of advantages, such as non-toxicity, good compatibility with organisms, and penetration enhancement. They can wrap hydrosoluble or liposoluble drugs. The gel preparation can increase drugs' accumulation on the local skin without affecting the structure of transfersomes and transported drugs, enhance the targeting of drugs towards skin, and reduce the stimulation of drugs themselves to skin. Thus, studies of transdermal scar permeation with the transfersomal gels as a drug carrier have important significance.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' Contribution

Z. Zhang and X. Wang contributed equally to this paper.

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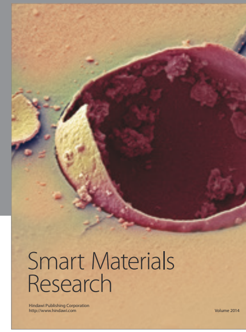
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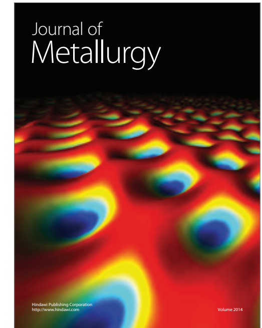
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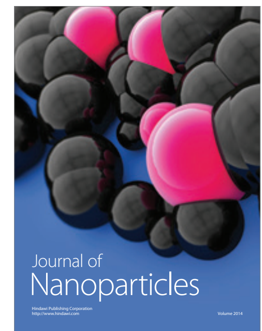
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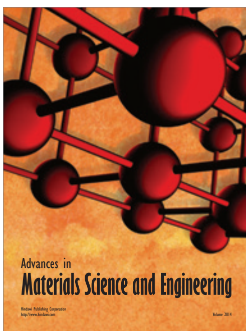
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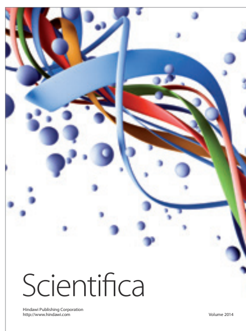
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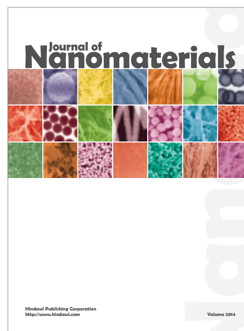
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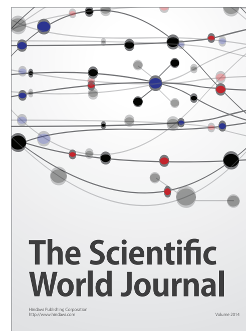
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