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RESEARCH ARTICLE

Targeting Potential of Zinc Oxide Nanoparticles and Finasteride-loaded Nano Lipidic Carriers-infused Topical Gel - *In vitro* and *In vivo* Skin Permeation Studies

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ABSTRACT

Background: There is an unmet clinical need to develop topical carriers for finasteride to reduce its systemic side effects in the treatment of androgenic alopecia (AGA). Zinc oxide (ZnO) nanoparticles have also emerged as an influential agent in hair biology.

Aim: The main focus of the work was to develop a novel formulation to explore the potential of ZnO nanoparticles in combination with NLCs of finasteride (FIN) for topical delivery.

Method: ZnO nanoparticles were synthesized by precipitation method and were subsequently incorporated within the Carbopol gel. The ZnO nanoparticles and the gel were evaluated for their physicochemical characteristics. In vitro release study was performed for the determination of release of the drugs from the gel and ex vivo study was conducted for the determination of penetration of the NLCs and ZnO nanoparticles into the skin.

Result: The particle size of the nanoparticles was found to be 200 nm. The pH, viscosity and spreadability of the gel was observed to be 6.13±2.11, 35,845.3±6.97 cps at 5 rpm and 17.14±2.32 respectively. Ex vivo drug permeation and skin distribution studies of the NLC gel formulations carried on rat dorsal skin indicated 25.763±0.2 µg/cm² and 19.375±1.2 µg/cm² of FIN and ZnO in 12 hr respectively.

Conclusion: The results indicated the potential of developed systems for topical drug delivery for treatment of androgenic alopecia.

Keywords:
Metal nanoparticles, drug delivery, nanostructured lipid carriers, alopecia, hair.

Introduction

Androgenic alopecia is a patterned loss of hair, and is primarily mediated through the dihydrotestosterone (DHT) hormone. The conversion of testosterone to dihydrotestosterone (DHT) is facilitated through the enzyme Type-II 5-α reductase, which is expressed in hair follicles and other androgen dependent tissues, and is reported to be critical in androgenic alopecia. The characteristic feature of alopecia is the absence of hair

Abbreviations: NLC - Nanostructured lipid carriers; ZnO - Zinc oxide; FIN – Finasteride; DHT – Dihydrotestosterone; HF - Hair follicle; AGA - Androgenetic alopecia; SEM - Scanning Electron Microscopy; FTIR - Fourier transmission electron microscopy.

from an otherwise normally hairy area, and it is reported that more than 25% of women in developed countries are affected by this condition[1]. Male androgenetic alopecia is typically hair loss in a noncicatricial pattern with the change of terminal hair to vellus hair. Studies indicate that the hair integrity hinges on nutritional intake, and an inadequate and unbalanced diet may lead to disproportionate hair loss [2,3]. With regard to hair loss, hair also needs adequate nutrition for its proper growth and development, and is also affected by various nutritional deficiencies. Various micronutrients have also been studied as etiological factors of hair loss [4].

Zinc, an essential trace element plays a significant influence on nearly all aspects of the metabolism within the body. In the hair follicles (HF), zinc acts as a potent inhibitor of endonucleases, the vital constituent of the apoptotic machine, given the critical role of keratinocytes apoptosis in HF regression during the involution phase of the hair cycle catagen. Thus, by inhibiting endonucleases, zinc has been proposed as a strong candidate for reducing or delaying HF regression. Zinc has also been reported to suppress the expression or activity of several enzymes like tyrosinase, the rate-limiting enzyme of HF melanogenesis. Zinc has a significant role in the body and is critical in the action of more than 200 enzymes. Zinc is a potent inhibitor of hair loss and inhibits hair follicle regression, and enhances the hair follicle recovery[5,6]. Zinc is also vital for DNA stability and hair repair parameters, since the epithelial hair matrix is one of the most rapidly proliferating and most damage-sensitive tissues in the mammalian organism [7,8].

ZnO nanoparticles have been investigated through in vitro and in vivo studies and it was reported that the penetration of topical ZnO nanoparticles was confined to the superficial layers of stratum corneum with absence of nanoparticles in the viable epidermis [9,10]. In the present work, ZnO nanoparticles have been synthesized by precipitation methods and characterized by various techniques. The 5-α reductase enzyme inhibitors such as finasteride and dutasteride are used in the treatment of AGA. Finasteride inhibits type II-5α reductase and reduces the conversion of testosterone to DHT [11,12]. The most severe adverse effects of finasteride are sexual dysfunction, mental issues and increased risk of high-grade prostate cancer in males and its administration is restricted in pregnancy or women with personal or family history of breast or ovarian cancer. On continuous oral administration for long term, there can be loss of hairs, which are gained within 12 months. The long-term oral use of finasteride is limited owing to the ensuing systemic side effects. There remains a need to explore the potential carriers to facilitate its topical application to reduce the systemic side effects.

Nanocarriers have the ability to improve the pharmacokinetics and viability at the targeted site, demonstrating their utility in the current circumstance. In recent years, the application of nanotechnology for drug delivery has advanced at an exponential rate [13]. The potential of nano lipid carriers (NLC) to cross the skin barrier, to penetrate the stratum corneum, and diffuse into the underlying regions are some of the major safety concerns with reference to their topical cosmetic applications. Although there is depression of the melting point of NLCs, they retain the solid state at the body temperature [14]. In comparison to other lipidic formulations, they exhibit better physical stability profile over time and have high entrapment of both lipophilic and hydrophilic drugs. During the preparation of NLCs, the lipid matrix comprises a mixture of solid lipid and liquid lipid and their ratio can range from 70:30 upto a ratio of 99.9:0.1. The primary advantage of NLCs is the prospect to use lower temperatures [15–18].

The objective of the present study was to explore the potential of a combination of finasteride containing NLCs and ZnO nanoparticles loaded within the gel formulation. As finasteride inhibits the conversion of testosterone to DHT and ZnO shows the synergistic effects on hair follicles, the novel formulation may prove beneficial in the treatment of AGA.

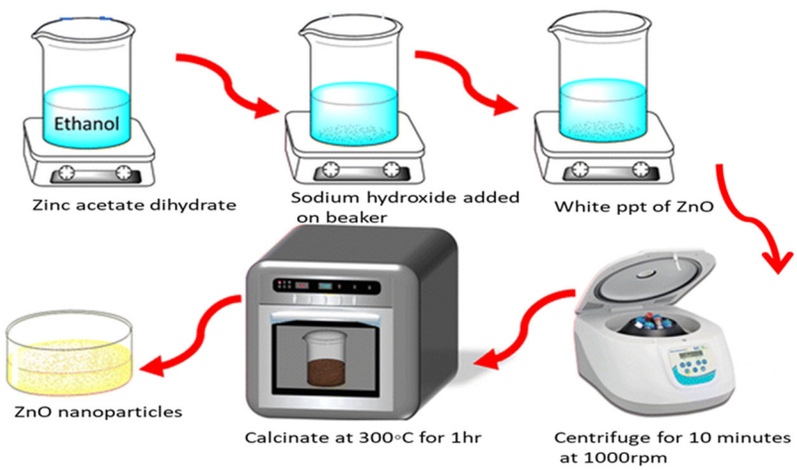


Fig. 1. Preparation of zinc oxide nanoparticles.

Materials

The drug finasteride (FIN) was received as a gift sample from Cipla Ltd. L.B.S. Marg, Vikhroli (W), Mumbai, India. Fenugreek oil was purchased from Parvati Gramodyog Herbal Product, Faridabad, Haryana India. Compritol ATO-888 (Molychem), Soya phosphatidylcholine (SPC) (Molychem), and Zinc acetate dihydrate (Rankem), NaOH (Molychem). All the other reagents, solvents, and chemicals used in the experiment were of analytical grade.

Method

Method of preparation zinc oxide (ZnO) nanoparticles

ZnO nanoparticles were prepared by precipitation method[19]. 20mM zinc acetate dihydrate and 50 mL ethanol, were mixed at room temperature with vigorous stirring for 15 min. Subsequently, 0.5 gm NaOH was added and stirred for 2 hours. The white precipitate of ZnO was formed. The mixture was centrifuged at 5000 rpm for 10 min and washed twice with distilled water. For drying, the white precipitate was calcinated at 300ºC for 1 hour to form powdered form of ZnO nanoparticles.

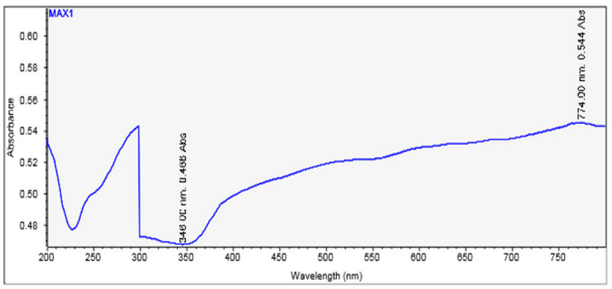


Fig. 2. Absorption maxima of ZnO nanoparticles.

Method of Preparation of FIN loaded NLCs

Finasteride loaded NLCs were prepared by microemulsion method [20] with some modifications. Solid lipid (Compritol ATO-888) was melted and liquid lipid (Fenugreek oil) was added to the liquefied solid lipid. The lipid mixture was heated to 80ºC [21] and FIN was added. The aqueous phase was heated with soya phosphatidylcholine to the same temperature. The lipid mixture was added slowly to the aqueous phase with continuous stirring for 30 minutes at 2000 rpm. The microemulsion formed was filtered and quickly added to cold water (0–4◦C), in the ratio of 1:50, forming an NLC dispersion system. Temperature difference between the cold water and the microemulsion is a significant factor for preparing small-particle-sized NLCs.

Preparation of gel formulations

A homogenous hydrogel matrix (Carbopol 934) loaded with FIN NLCs and ZnO nanoparticles was prepared. Briefly, 20% w/v aqueous dispersion of Carbopol 934 was prepared. The polymer was permitted to wet completely and dispersed on the magnetic stirrer. During the entire process, it was ensured that any non-dispersible lumps were not formed. Subsequently, FIN NLCs with ZnO nanoparticles were introduced discreetly into the polymer solution in different beakers at 800 rpm for 15 min for their proper dispersion into a homogenous NLC loaded hydrogel. Triethanolamine was added gently to the mix with stirring for 15-20 min to neutralize the Carbopol and form a gel (at pH 5.4-6.8).

Characterization of ZnO Nanoparticle

UV spectroscopy: UV–visible spectra was measured in the range of 200–600 nm using UV-spectrophotometer (UV-1800 Shimadzu, Japan) to confirm the absorbance of ZnO nanoparticles and to investigate the changes in absorbance induced by differences in reaction circumstances. Synthesized ZnO nanoparticles were dispersed in distilled water and sonicated for 15 minutes. The dispersion was used for UV-spectroscopic measurements. The spectrum has an absorption peak in the range of 360–380 nm, which is the characteristic band for pure ZnO.

SEM: Scanning electron microscopy (SEM) was used to examine the surface morphology of ZnO nanoparticles. The SEM images of the ZnO nanoparticles were obtained using a ZEISS Sigma Scanning Electron Microscope.

FT-IR: The IR analysis of ZnO nanoparticles was carried by Fourier Transform Infrared (FT-IR) spectrometer (FTIR-8400S, Shimadzu, Kyoto, Japan) utilising KBr pellets approach at room temperature in order to detect the characteristic functional groups present on the surface of the ZnO. Samples were combined with KBr and compacted into a thin pellet, which was used for recording measurements in the designated frequency range (4000–400 cm^{-1}).

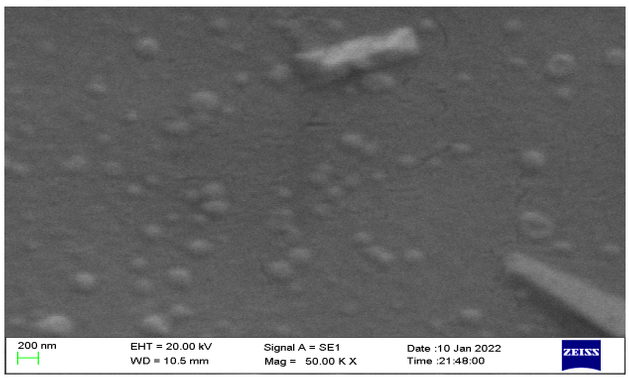


Fig. 3. SEM analysis of ZnO nanoparticles.

Characterization of Gel Formulation

Determination of pH value

The pH of the gel-formulations of FIN NLCs with ZnO nanoparticles at room temperature were assessed with a

digital pH meter (Remi, Model 1010). The measurements were conducted in triplicate.

Drug content

The gel (1g) was dissolved in methanol (100 mL). The resultant solution was shaken mechanically for 2 hours and subsequently filtered through a 0.45 μm membrane filter. The drug was quantified by UV-simultaneous measurement [22].

Viscosity study

The rheological attributes of a formulation are significant for the topical drug delivery applications. It is important for its efficacy in delivering molecules onto the skin and/or retention on the skin. The permeation across the skin is influenced by the viscosity of the gel matrix as this regulates the drug release into the receptor medium. The release of the drug from the formulation is governed by its components and the consistency of the formulation [23,24]. The viscosities of the gel-formulation of FIN NLCs with ZnO nanoparticles was measured with Brookfield LDV prime I viscometer.

Spreadability

The spreadability study of all the gel-formulations of FIN NLCs with ZnO nanoparticles was performed using a modified parallel plate method. Sample (0.5 g) was placed on the lower plate (glass slide) of the device. Subsequently, a weight of 20 g was placed over the pan that was attached to the upper slide [23,25] and the time required by the upper plate to transverse the entire length of the lower plate was measured. The spreadability was calculated as:

$$S = \frac{M \times L}{T}$$

Where,

S = Spreadability (g.cm/sec)

M = Weight attached to the upper slide

L = Length of the slide

T = Time taken for physical detachment of the slides

In-vitro drug release study

In-vitro release of FIN and ZnO from the gel-formulations was determined by the dialysis sac method at room temperature. The dialysis tube (D9652-100FT, Sigma-Aldrich) was filled with gel (1 gm) and suspended into a vessel containing dissolution media (a mixture of methanol and phosphate buffer saline [pH 5.8]). The assembly was placed on a shaker bath at 80 rpm. At designated time intervals, aliquots (0.1 ml) were withdrawn from the receptor compartment and replaced with an equivalent quantity of fresh media. The cumulative amount of drug released was quantified by the UV-spectrophotometric simultaneous method. The studies were conducted in triplicate. The release kinetics of the developed formulations were assessed by the curve fitting technique [26,27].

Ex-vivo skin permeation and retention study

Ex-vivo drug permeation and skin retention studies of the gel formulation was carried out on shaved dorsal skin excised from Wistar rat. The protocol for the *ex-vivo* experiments was approved by the Institutional Animal Ethical Care Committee (IAEC) of University Institute of Pharmacy, Pt. Ravishankar Shukla University, Raipur, India (IAEC/Pharmacy/2021-22/04). Modified static Franz diffusion cell having a contact surface area of 1.3 cm^2 was used for the experiments. Phosphate buffer saline (pH 5.8) was used to wash the skin several times. A skin patch (diameter - 15 mm) was placed between donor and receptor compartment and the stratum corneum was positioned to face the donor compartment. The formulation (with approx. 50 mg drug) was placed into the donor compartment. The experiments were conducted with sink conditions maintained with PBS (pH 6.5) at 250 rpm and body temperature of mice was mimicked through the media stabilized at 36±2°C. Samples (1 mL) were withdrawn from the receptor compartment at predetermined time intervals (0, 1, 2, 3, 4, 5, 6, 7 and 24 hours), and were replaced with equivalent volume of fresh media. The samples were quantified by a spectrophotometric method. The skin was removed from the diffusion cell after 24 hours. The residual gel at the external surface of the excised skin was completely washed with water (15 ml). Subsequently, the drug in the rinsed sample was extracted with methanol (10 ml). The skin used in the study was homogenized in media (5 ml) and the drug was extracted with methanol (5 ml). The extracts were centrifuged (10000 rpm, 15 min) and the supernatants were quantified spectroscopically. These experiments were conducted in triplicate to measure the drug retention in the skin[28,29].

Result

Characterization

UV spectroscopy

UV–visible spectra were measured in the range of 200–600 nm using UV spectrophotometer (UV-1800 Shimadzu, Japan) to confirm the absorbance of ZnO nanoparticles and to investigate the changes in absorbance induced by differences in reaction circumstances. The UV-VIS absorption spectra of ZnO nanoparticles are depicted in Fig. 2. The spectrum shows a typical absorption peak of ZnO at 300 nm.

SEM

Scanning electron microscopy was used to study the surface morphology of the ZnO nanoparticles. The current investigation indicated that the ZnO nanoparticles were spherical and the particle size was approximately 200 nm.

FTIR

FTIR is a useful technique to confirm the characteristic behaviour of oxides. Figure 4 displays the FTIR spectra of the prepared ZnO nanoparticles in the range of 4000–400 cm^{-1}. Several absorption bands were also observed at 874, 1670, and 2165 cm^{-1}.

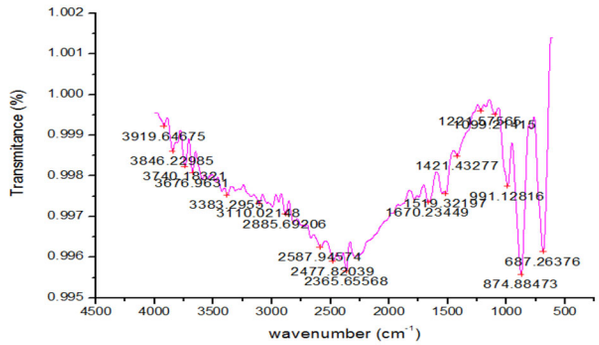


Fig. 4 FTIR of ZnO nanoparticle.

pH of the gel formulation

The pH of the gel formulation loaded with lipid carriers was determined at room temperature and was found to be 5.13±1.21.

Drug content

The content of FIN and ZnO in the gel was observed to be 98.14±1.23% and 98.12+1.34 % respectively.

Viscosity and spreadability

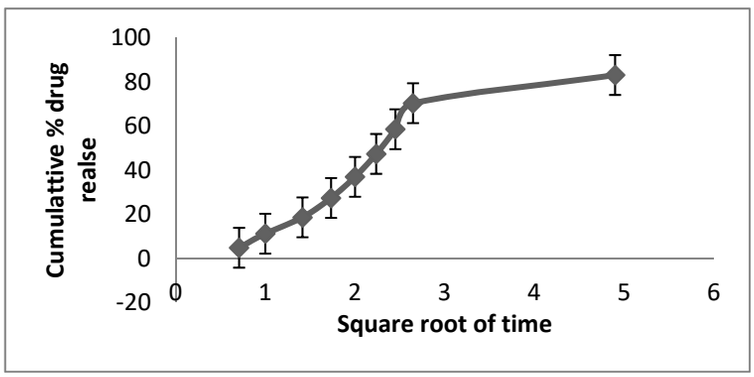
The FIN NLCs and ZnO nanoparticles loaded gel were evaluated for viscosity and spreadability characteristics. The spreadability of the gel formulation was found to be 17.14±2.32 g.cm/s and viscosity was 35,845.3±6.97 cps at 5 rpm.

In-vitro drug release

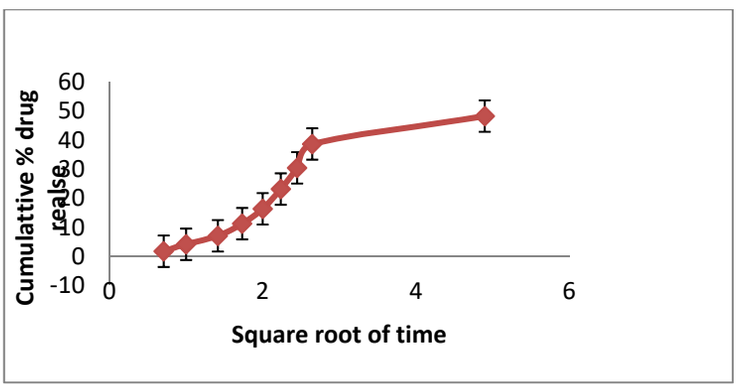
The *in vitro* release studies indicated the Higuchi kinetic model to be the best fit (R^2=0.85 for FIN and R^2=0.871 for ZnO nanoparticles). The nonlinear drug release pattern suggested the noncompliance to zero-order kinetics.

Ex-vivo skin permeation and retention

Ex-vivo drug permeation and skin distribution studies were conducted on rat dorsal skin and the amount of FIN and ZnO release was found to be 25.763 ±0.2 µg /cm^2 and 19.375 ±1.2 µg/cm^2 respectively. The drug retention within the epidermis was sufficient to provide the desired action at the drug activity site. However, a significantly reduced quantity of FIN and ZnO, were obtained in the receptor compartment from NLC gel formulation indicating the minimal amount of both activities in systemic circulation. This signified that the developed gel formulations may be anticipated to have limited systemic evasion of both active [30].



(a)



(b)

Fig. 5. *In-vitro* drug release profile of FIN (a) and ZnO nanoparticles (b) from gel formulation.

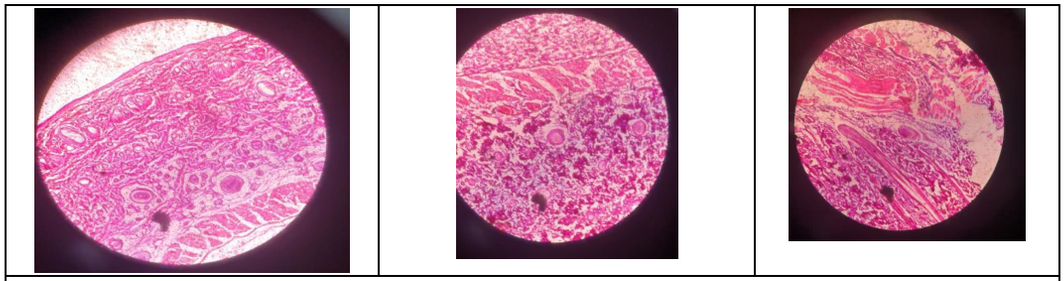


Fig. 6. Retention of NLCs on dorsal skin of rat.

Table 1. R^2 values of different releasing models.

Drug	Zero order	First order	Korsemeyer-Peppas	Higuchi kinetics
FIN	0.249	-5.62	-2.29	0.845
ZnO	0.525	-1.62	0.080	0.871

Discussion

The UV-VIS absorption spectral studies displayed an absorption peak which can be attributed to the inherent band-gap absorption of ZnO caused by electron transitions from the valence band to the conduction band. The spectrum has an absorption peak in the range of 360–380 nm, which is the characteristic band for pure ZnO. The FTIR spectra in the range of 4000–400 cm^{-1} indicate high purity of the obtained ZnO nanoparticles. The absorption bands observed at 874, 1670, and 2165 cm^{-1} were likely related to C-O and O-H bonds absorbed from the atmosphere as CO_2 and H_2O.

The pH of the gels was observed to be 5.13±1.21, which is acceptable for topical formulations. The content of both FIN and ZnO in the gel formulation was high at 98%. The viscosity study was conducted to examine the rheological attributes of the topical gel, and it allied with the spreadability of the formulation and contact time on the skin surface. A gel with good spreadability can be effectively applied to the affected part of the skin to accomplish patient compliance.

The *in vitro* release studies indicated that the release of FIN and ZnO followed the Higuchi kinetics. The nonlinear release pattern showed that the developed formulation did not comply with zero-order kinetics. The most critical rate limiting mechanisms used in the sustained controlled-release formulations are diffusion, swelling, and erosion or burst release. Results indicated that diffusion was the prominent mechanism of drug release from the developed formulations and was best characterized by Fickian diffusion. However, if the system undergoes swelling, other mechanisms may influence drug release.

Conclusion

In the present research work, a novel topical gel formulation which contains NLC loaded with Finasteride and ZnO nanoparticles was prepared. ZnO nanoparticles were prepared by precipitation method and characterized by SEM and FT-IR for determination of surface morphological features and presence of functional groups respectively. On other hand, NLCs were prepared by microemulsion method. The *ex-vivo* study indicated that the developed gels are likely to transport the actives with restricted systemic escape and are a carrier with targeting potential for the treatment of AGA. Following their release from the lipid carrier-loaded gel, both FIN and ZnO reside primarily in the epidermis by the virtue of higher hydrophilicity of the dermis, and this further confine extensive partitioning of hydrophobic activities. It penetrates moderate medications into the follicle in the viable epidermis and blocks the action of 5-α reductase enzyme present in hair bulb. The present study indicates that the developed formulations are promising for topical therapies of androgenic alopecia. Further, the methods utilized for the preparation of formulations are simple and easily scalable. Additional *in-vivo* studies are required in animal models to quantify the results, ascertain its reproducibility and establish the treatment protocols.

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Reference

1. Oh JW, Kloepper J, Langan EA, Kim Y, Yeo J, Kim MJ, et al. A guide to studying human hair follicle cycling in vivo. Journal of Investigative Dermatology. 2016;136(1):34–44.
2. El-Esawy FM, Hussein MS, Ibrahim Mansour A. Serum biotin and zinc in male androgenetic alopecia. Journal of Cosmetic Dermatology. 2019;18(5):1546–9.
3. Luana AFA, Ushirobira CY, Barbosa DPP, Souza PEN De, Leles MIG, Cunha-filho M, et al. Novel iron oxide nanocarriers loading finasteride or dutasteride: Enhanced skin penetration for topical treatment of alopecia. International Journal of Pharmaceutics. 2020;587(May):1–10.
4. Rushton DH. Nutritional factors and hair loss. Clinical and Experimental Dermatology. 2002;27(5):396–404.
5. Deshwali Sanjay, Kare Pawan Kumar, Agrawal B.K. AA. Study of serum zinc, copper and ferritin levels in alopecia patients. International Journal of Advanced Research in Biological Sciences. 2015;2(7):12–20.
6. Slominski A, Wortsman ÃJ, Plonka PM, Schallreuter KU, Paus R, Tobin DJ. Hair Follicle Pigmentation. Journal of Investigative Dermatology. 2004;(124):13–21.
7. Abdel Fattah NSA, Atef MM, Al-Qaradaghi SMQ. Evaluation of serum zinc level in patients with newly diagnosed and resistant alopecia areata. International Journal of Dermatology. 2016;55(1):24–9.
8. Jiang J, Pi J, Cai J. The Advancing of Zinc Oxide Nanoparticles for Biomedical Applications. Bioinorganic Chemistry and Applications. 2018;18.
9. Leite-Silva VR, Lamer M Le, Sanchez WY, Liu DC, Sanchez WH, Morrow I, et al. The effect of formulation on the penetration of coated and uncoated zinc oxide nanoparticles into the viable epidermis of human skin in vivo. European Journal of Pharmaceutics and Biopharmaceutics. 2013;84(2):297–308.
10. Srivastava V, Gusain D, Sharma YC. Synthesis, characterization and application of zinc oxide nanoparticles (n-ZnO). Ceramics International. 2013;39(8):9803–8.
11. McElwee KJ, Shapiro JS. Promising therapies for treating and/or preventing androgenic alopecia. Skin therapy letter. 2012;17(6):1–4.
12. Kaufman KD, Olsen EA, Whiting D, Savin R, Devillez R, Bergfeld W, et al. Finasteride in the treatment of men with androgenetic alopecia. Journal of the American Academy of Dermatology. 1998;39:578–89.
13. Vaseem M, Umar A, Hahn Y. ZnO nanoparticles: Growth, Properties, and Applications. Vol. 5, Metal Oxide Nanostructures and Their Applications. 2010. 1–36.
14. Pardeike J, Hommoss A, Müller RH. Lipid nanoparticles (SLN, NLC) in cosmetic and pharmaceutical dermal products. International Journal of Pharmaceutics. 2009;366(1–2):170–84.
15. Yazdani-Arazi SN, Ghanbarzadeh S, Adibkia K, Kouhsoltani M, Hamishehkar H. Histological evaluation of follicular delivery of arginine via nanostructured lipid carriers: a novel potential approach for the treatment of alopecia. Artificial Cells, Nanomedicine and Biotechnology. 2017;45(7):1379–87.
16. Cevc G, Blume G. New, highly efficient formulation of diclofenac for the topical, transdermal administration in ultradeformable drug carriers, Transfersomes. Biochimica et Biophysica Acta - Biomembranes. 2001;1514(2):191–205.
17. Wang W, Chen L, Huang X, Shao A. Preparation and Characterization of Minoxidil Loaded Nanostructured Lipid Carriers. American Association of Pharmaceutical Scientists. 2017;18(2):509–16.
18. Das S, Chaudhury A. Recent advances in lipid nanoparticle formulations with solid matrix for oral drug delivery. AAPS PharmSciTech. 2011;12(1):62–76.
19. Girigoswami K, Viswanathan M, Murugesan R, Girigoswami A. Studies on polymer-coated zinc oxide nanoparticles: UV-blocking efficacy and in vivo toxicity. Materials Science and Engineering C. 2015;56:501–10.
20. Id IC, Yasir M, Verma M, Singh AP. Nanostructured Lipid Carriers : A Groundbreaking Approach for Transdermal Drug Delivery. Tabriz University of Medical Sciences. 2020;10(2):150–65.
21. Shamma RN, Aburahma MH. Follicular delivery of spironolactone via nanostructured lipid carriers for management of alopecia. International Journal of Nanomedicine. 2014;9:5449–60.
22. Balakrishnan P, Shanmugam S, Lee WS, Lee WM, Kim JO, Oh DH, et al. Formulation and in vitro assessment of minoxidil niosomes for enhanced skin delivery. International Journal of Pharmaceutics. 2009;377(1–2):1–8.
23. Sethuraman N, Shanmuganathan S, Sandhya K, Anbarasan B. Design, development and characterization of nano structured lipid carrier for topical delivery of aceclofenac. Indian Journal of Pharmaceutical Education and Research. 2018;52(4):581–6.
24. Loo CH, Basri M, Ismail R, Lau HLN, Tejo BA, Kanthimathi MS, et al. Effect of compositions in nanostructured lipid carriers (NLC) on skin hydration and occlusion. International Journal of Nanomedicine. 2013;8:13–22.
25. Sandeep DS, Mahitha M, Meghna S. Development, Characterization, and In vitro

Evaluation of Aceclofenac Emulgel. Asian Journal of Pharmaceutics. 2020;14(3):330.

26. Madheswaran T, Baskaran R, Thapa RK, Rhyu JY, Choi HY, Kim JO, et al. Design and in vitro evaluation of finasteride-loaded liquid crystalline nanoparticles for topical delivery. AAPS PharmSciTech. 2013;14(1):45–52.
27. Dokka S, Cooper SR, Kelly S, Hardee GE, Karras JG. Dermal delivery of topically applied oligonucleotides via follicular transport in mouse skin. Journal of Investigative Dermatology. 2005;124(5):971–5.
28. Eroğlu İ, Azizoğlu E, Özyazıcı M, Nenni M, Gürer Orhan H, Özbal S, et al. Effective topical delivery systems for corticosteroids: dermatological and histological evaluations. Drug Delivery. 2016;23(5):1502–13.
29. Mahmoud NN, Alkilany AM, Dietrich D, Karst U, Al-Bakri AG, Khalil EA. Preferential accumulation of gold nanorods into human skin hair follicles: Effect of nanoparticle surface chemistry. Journal of Colloid and Interface Science. 2017;503:95–102.
30. Sturikova H, Krystofova O, Huska D, Adam V. Zinc, zinc nanoparticles and plants. Journal of Hazardous Materials. 2018;349:101–10.