Design of Finasteride-Loaded Nanoparticles for Potential Treatment of Alopecia

Luís V. Roque^a Inês S. Dias^b Nuno Cruz^c Ana Rebelo^a Amílcar Roberto^a
Patrícia Rijo^{a, d} Catarina P. Reis^{d, e}

^aCBIOS, Research Center for Biosciences and Health Technologies, Universidade Lusófona de Humanidades e Tecnologias, ^bUniversidade Lusófona de Humanidades e Tecnologias, ^cFaculty of Sciences, Universidade de Lisboa, ^diMed.Ulisboa, Instituto de Investigação do Medicamento, Faculty of Pharmacy, Universidade de Lisboa, and ^eIBEB, Biophysics and Biomedical Engineering, Faculty of Sciences, Universidade de Lisboa, Lisbon, Portugal

Keywords

Alopecia · Nanotechnology · Drug · Prolonged drug release · Toxicity · Efficacy and safety testing

Abstract

Background/Aims: Androgenetic alopecia is an extremely common dermatological disorder affecting both men and women. Oral finasteride (FNS), a synthetic 4-aza-3-oxo-steroid compound with poor aqueous solubility, blocks the peripheral conversion of testosterone to dihydrotestosterone (DHT) in a significant reduction in DHT concentration, achieving satisfactory results in alopecia treatment. However, its oral intake generally causes severe side effects. Considering that there is currently no scientifically proven treatment, new drug delivery systems able to improve alopecia therapy are urgently required. **Methods:** In this study, polymeric nanoparticles have been proposed as a new carrier for topical delivery of FNS in hair follicles. **Results and Conclusions:** Polymeric nanoparticles, prepared by using a modified method of the emulsification/solvent diffusion, showed a mean particle size around 300 nm, which may be sufficient for reaching the dermis and hair follicles and

negative zeta potential values. Scanning electron microscope measurements showed that all the polymeric nanoparticles exhibited a spherical shape and a smooth surface regardless of their composition. A high encapsulation efficiency was achieved for FNS ($79.49 \pm 0.47\%$). In vitro release assays in physiological conditions demonstrated that nanoparticles yielded a prolonged release of FNS for 3 h. Skin assays through an in vitro permeation study demonstrated that nanoparticles had low levels of penetration of FNS, improving its time residence onto the skin. All excipients used in nanoparticle composition and in 3 different vehicles were safe. These results suggest that the proposed novel formulation presents several good characteristics indicating its suitability for dermal delivery of FNS for alopecia treatment.

© 2017 S. Karger AG, Basel

Introduction

Androgenetic alopecia is an extremely common dermatological disorder affecting both men and women. This disease is characterized by a reduction of frontal hair

in temporal region, resulting in a gradual decrease in the hair diameter [1].

Some of these follicular disorders involve changes in the protein synthesis of follicular cells [2]. These changes occur because of dihydrotestosterone (DHT) or 5 α -DHT, which is a derivative of testosterone, and causes change in the synthesis of proteins in the follicular cells. Testosterone is an endogenous hormone produced by the adrenal gland and is converted into the skin, liver, and prostate DHT by the action of 5 α -reductase. In human tissue, there are 2 types of 5 α reductase, type I and type II. Type I is most evident on the skin and in the outer sheath of the hair follicle root. Type II is most apparent in the prostate and seminal vesicle. In the hair follicle, type II is more active in the dermal papillae and the outer sheath of the hair follicle root, which has been described as causes of androgenic alopecia [3].

Finasteride (FNS), a synthetic azosteroid with potent selective antagonist action on 5 α -reductase type II enzymes, acts by irreversibly binding to the enzyme, preventing the conversion of testosterone to DHT, its active metabolite. FNS was initially approved for the reduction of prostate size associated with urinary obstruction (benign prostatic hyperplasia), specifically 1 mg/day for the treatment of benign prostatic hyperplasia and 5 mg/day for prostate cancer. In recent years, it was found that oral administration of FNS was useful in the treatment of various dermatological and follicular disorders, particularly in androgenetic alopecia [2].

Taking into account the synthesis of DHT, the use of FNS may be very useful because it will lead to an inhibition of the conversion of testosterone into DHT, thereby reducing the effects on the protein synthesis in follicular cells. However, the oral intake of FNS causes some adverse side effects, such as impotence, erectile dysfunction, and impaired reproductive function. Therefore, it is necessary to find another route to administer FNS into the system without those adverse side effects. On the other hand, most topical products available in the market for alopecia are formulated with the actives dissolved in water-alcohol solutions. However, due to the low permeability through the keratin layer, only a fraction of the applied drug dose actually reaches the target site, penetrating the pores and hair follicles. As a result, those products do not exceed consumer expectations, leading in some cases to lack of adherence to treatment.

Thus, recent studies have confirmed the hypothesis that nanoparticles can penetrate effectively and remain in the pilous follicles for longer periods [4]. Besides, nanoparticles may have the advantage of achieving solubilization

of hydrophobic drugs, reduction of a drug dose used and, in some cases, prevention or elimination of side effects [5–8].

In this study, PLGA nanoparticles loaded with FNS were produced by the modified method of spontaneous emulsification with diffusion of the solvent and characterized. PLGA is one of the most used polymers, mainly because of its biodegradability, biocompatibility capacity, and sustained drug release [9–11]. A second objective of this study was to incorporate these nanoparticles in 3 different types of topical formulations: lotion, shampoo, and the solution. All topical formulations were fully characterized including the human safety testing of the used excipients and nanoparticles, but always without FNS.

Materials and Methods

FNS and the Pluronic® F68 were obtained from Sigma-Aldrich (St. Louis, MO, USA). PLGA (50/50) was obtained from Purac (Gorinchem, The Netherlands). Sodium polyacrylate or Carbopol®940 was obtained from Fagron (Barcelona, Spain). All other reagents were of analytical grade.

Preparation of PLGA Nanoparticles of FNS

PLGA nanoparticles with FNS were prepared using a modified method of the emulsification/solvent diffusion [12]. Briefly, PLGA and FNS were dissolved in a mixture of acetone:ethanol. This ratio between FNS and PLGA was determined by previous works [13, 14]. The previous solution then was added drop by drop to an aqueous solution of Pluronic® F68 at 0.1% (w/v), with a constant stirring speed of 800 rpm, using the RT15 power IKA® WERKE (IKA, Königswinter, Germany), at room temperature. Nanoparticle suspension was formed instantly and the aqueous layer became milky. After the last drop was added, the stirring continued for additional 15 min. Subsequently, the organic solution was removed using a rotary evaporator at 40°C for 20 min. After evaporation of the solvent, there was a colloidal suspension of PLGA nanoparticles with FNS in water. Empty nanoparticles were also prepared, i.e., without the FNS.

Preparation of the Topical Formulations

A typical formulation of shampoo can usually contain up to 30 compounds, which include cleaning agents (surfactants), conditioning agents, additives that modify the effect of surfactant, preservatives, scents, dyes, and additives [15–17]. Throughout the development of the formulation, different formulations were prepared and tested. All compositions were firstly optimized. This formulation resulted from the mixture of 2 phases where the first phase included 0.2 g of methylparaben in 15.68 mL of glycerol with 35 g of sodium lauryl ether sulfate and 0.5 mL of essence of menthol. The second phase contained 0.48 mL of citric acid buffer in 46.64 mL of water.

In case of the lotion, the first phase contained a homogeneous mixture of 0.05 g of Carbopol® 940 dissolved in 70 mL of water. The second phase included 0.5 mL of essence of menthol and 6.45 g of propylene glycol in 23 mL of isopropyl alcohol.

Finally, a solution was made of 16.3 mL of water, 20 g of propylene glycol and 63.2 mL of ethanol.

Characterization of the Formulations

Viscosities of the formulations were accessed using a Brookfield Rotating Viscometer (Middleboro, MA, USA) with a spindle 2 with a rotation speed of 20 rpm and at a temperature $22.0 \pm 2.0^\circ\text{C}$. Mean viscosity was recorded in triplicate. The pH value and the organoleptic and tactile aspects were also accessed [18].

Particle Size Analysis

Mean particle size and polydispersity index (PI) of the nanoparticles were measured in hydrated particulate systems (diluted aqueous solutions, 1:1) with a Coulter Nanosizer Delsa Nano™ C (Coulter Beckman, Fullerton, CA, USA). A low value of PI factor indicates a more stable and less dispersed nanoparticle distribution in size. All experiments were made at room temperature, with angle detection of 165°C and in triplicate.

Zeta Potential Analysis of the Nanoparticles

Zeta potential was measured in Delsa Nano™ C (Coulter Beckman, Fullerton, CA, USA) by electrophoretic mobility technique. Each analysis was carried out at 25°C with an angle of detection of 90°C and in triplicate.

Particle Morphology and Surface

Morphology of the particles was assessed by scanning electron microscopy before the centrifugation procedure. An aliquot of each sample was placed in a glass coverslip and left to dry in a desiccator for scanning electron microscopy analysis [19]. The material was then coated with a thin layer of gold (with 500 nm of thickness) and observed on a JEOL 5200 LV scanning electron microscope (JEOL Ltd., Tokyo, Japan) at an accelerating voltage of 20 kV. Images were digitally recorded.

Determination of FNS Encapsulation Efficiency

After the preparation of the nanoparticles, the supernatant was centrifuged 13,158 g for 45 min and the amount of free FNS present in the supernatant was analyzed by a spectrophotometric method at 210 nm (UV-visible spectrophotometer, Evolution 300, Hertfordshire, England). Measurements were made in triplicate and a standard calibration curve was made with a FNS standard solution. The established linearity range was 5–25 $\mu\text{g/mL}$ ($R^2 = 0.9995$). The precision, accuracy, and repeatability of the method were also determined [20, 21].

In vitro Drug Release

An amount of nanoparticles containing 5 mg of FNS were placed in a recipient containing 10 mL of PBS buffer with a pH 7.4 and without enzymes and following the sink conditions (USP XXX), respecting FNS solubility. The assay was maintained at $25.0 \pm 2.0^\circ\text{C}$ and in a constant stirring (130 rpm) in a multipoint laboratory magnetic stirrer (Thermo Scientific Variomag Telesystem Stirrers; Langensfeld, Germany). In appropriated time intervals, aliquots of the release medium were taken and replaced immediately with fresh PBS buffer. The aliquots were then centrifuged at 13,158 g for 10 min and the FNS concentration was determined in triplicate using the UV-visible spectrophotometer and using a standard calibration curve determined before ($R^2 = 0.9995$). Possible interferences were taken into account.

In vitro Permeation Test

After incorporation of the nanoparticles in the 3 different formulations (1:1), in vitro diffusion studies with Franz cells were conducted using a PDMS membrane as a simplified model of human epidermis [22]. The effective diffusion area was 0.95 cm^2 and the mean volume of the receptor chamber was, approximately, 4.0 mL. The receptor chamber was filled with PBS at pH 7.4 and stirred constantly at 130 rpm. The receptor compartments of the diffusion cells were maintained at 32°C using a recirculating water bath. Donor compartments were covered by glass slips. Samples were taken at regular intervals for UV spectrophotometry assay and replaced immediately with an equal volume of fresh PBS. A standard calibration curve was performed with the FNS solution in PBS at pH 7.4. All experiments were performed 5 times. The multiple steady-state fluxes of FNS and the percentage of drug permeation were determined.

In vitro Safety Assessment

Preliminary cytotoxicity studies were first performed using *Saccharomyces cerevisiae*. The study consisted of exposition of test substances to yeast cells growing in cuvettes containing complete YPD medium (1% yeast extract, 0.5% peptone, 2% glucose, and 1.5% agar). Cell cultures of *S. cerevisiae* were prepared by transferring a cell colony growing on a Petri dish into a 50-mL sterile Erlenmeyer with 20 mL of YPD medium and incubated at 30°C with agitation (80 stirring cycles per min) for 24 h, which leads to approximately 1.0×10^7 cells/mL. Afterwards, an equal volume of the yeast suspension (20–50 μL) was diluted with YPD medium in disposable cuvettes in order to obtain an initial absorbance for the tested samples and control, not higher than 0.15 at 525 nm.

The final volume in all cuvettes containing the substances to be tested together with YPD medium and yeast cell cultures was 2 mL. The control groups were prepared with medium without any exposing agent. Growth curve in the logarithmic phase for each tested group as well as for the control group was conducted by correlating the logarithm of the cell concentration (number of cells per mL) against the time of incubation. Each group was tested in 4 replicates. Cell concentrations were calculated by equation 1 at 525 nm [23–27].

$$C = \frac{A - 0.0327}{6.8219 \times 10^{-8}} \quad (1)$$

The growth inhibition in percent was calculated from the linear slope in the logarithmic phase for each tested substance and concentration for the cytotoxicity evaluation toward *S. cerevisiae*.

In vivo Safety Assessment

Safety of the formulation excipients was made by the Occluded Patch Test method [28–30]. A convenience sample of 10 healthy volunteers was selected, all female, between 21 and 27 years of age (23 ± 2). Volunteers were selected after informed consent, according to inclusion criteria previously established and in full compliance with all ethical standards from the Declaration of Helsinki and its amendments. Noninclusion criteria were being pregnant and/or in lactation, nonsmokers (more than 2 years of not smoking), presence of cutaneous signs in the experimental area, existence of any type of topical treatment in the 8 days preceding the study, intense exposure to UV radiation,

Fig. 1. Scanning electron microscopy image of PLGA nanoparticles before loading (**a**, magnification 15,000 $\times$) and after loading (**b**, magnification 20,000 $\times$).

and regular practice of physical exercise. Some restrictions were imposed, in particular, not to wash the anatomical area subject to measurement with soap or shower gel and no application of cosmetics in these areas in the 24 h preceding the measurements. Evaluations were carried out in the laboratory under controlled temperature and humidity ($22.0 \pm 1.0^\circ\text{C}$; RH $60 \pm 3\%$) in the absence of heat and forced convection according to previously published methods [29, 30]. In this study, we aimed to make the assessment of excipients present in all 3 formulations: shampoo, lotion, and solution with and without empty nanoparticles, but in all cases without FNS. Two adhesives were applied in the lumbar area of each of the volunteers during 24 h. These adhesives had semiocclusive devices which contained 100 μL of each formulation (lotion with empty PLGA nanoparticles, lotion without nanoparticles, solution with empty PLGA nanoparticles, solution without nanoparticles, shampoo with empty PLGA nanoparticles, and shampoo without nanoparticles) in the respective areas. FNS was not included in any situation. Distilled water was used as a negative control. The location of each sample was randomized among volunteers and visual observations were carried out for 2 h after removal of the adhesives. Noninvasive measurements were performed using skin biometrics equipment to assess erythema through a Chromameter CR400 (Minolta, Japan) and the transepidermal water loss (TEWL) using Tewameter TM300 (CK Electronics, Germany). For both analysis, especially TEWL analysis, an acclimation period of at least 15 min to 1 h was required. During this analysis, any thermally or emotionally induced sweating was completely avoided.

All the tests were based on previous studies [31–33] and received the approval of the local ethical committee.

Statistics

All results are expressed as means $\pm$ SD. One-way ANOVA analysis was applied to demonstrate statistical differences in all tested parameters. All analyses were performed using a software program (GraphPad Prism 5®, GraphPad Software, San Diego, CA, USA) with a statistical significance level of 0.05.

Results and Discussion

Spherical PLGA particles were obtained using standardized and reproducible conditions (Fig. 1). Size particle range was around 316.5 ± 14.44 nm (PI 0.114) and 185.3 ± 9.32 nm (PI 0.122) for the nanoparticles with and without FNS, respectively. According to previous studies, particle size should be between 200 and 400 nm in diameter to penetrate through the hair follicles [34]. On the other hand, the zeta potential of FNS-loaded nanoparticles was negative (-5.71 ± 0.431 mV), probably due to the presence of terminal carboxylic groups of PLGA. Concerning this subject, it is well-known that the investigation of nanoparticle interactions with skin is a complex process [35]. The behavior of the skin surface seems to be similar to the functioning or artificial ion exchange membranes, designed to attract positively or negatively materials to their pores [36]. Hence, there are both indiscriminate and specific charge adsorption processes on the skin surface [36, 37]. In a dry state and neutral pH, the skin surface is negatively charged because of the carbohydrate content, residues of proteins in the outer membranes of the cells, and active ions pumps. Consequently, it is expected to observe a great adsorptivity of cationic solutes to the skin surface, but curiously anionic nanoparticles also strongly interact with the skin [37–39]. Here, the composition of the skin may confer some positive charge because of some surface lipids covering the stratum corneum. Previous studies have shown that negatively charged carriers like NLC or SLN generally interact with lipids in the outermost skin layers, resulting in an occlusive effect [37, 40]. Those negatively charged

Fig. 2. Release profile of FNS from the nanoparticles.

NLC and SLN carriers may increase the drug penetration in the stratum corneum and retention in the skin, forming an intact film on the skin surface [40]. Other nanoparticles like polymeric nanoparticles may accumulate in skin appendages such as hair follicles, which can act as a long-term reservoir. We suspect that our negatively charged nanoparticles may retain FNS in the target area, i.e., within the hair follicles [36, 41]. The encapsulation efficiency of FNS into PLGA nanoparticles was high, around $79.49 \pm 0.47\%$, suggesting that the interaction established between PLGA and FNS was very probable. It has been documented by a number of investigators that highly lipophilic drugs (FNS) feature a good affinity with PLGA, leading to satisfactory encapsulation efficiency and great interest in the pharmaceutical industry [42, 43]. An in vitro release study demonstrated that the PLGA nanoparticles led to a prolonged release profile of FNS. Figure 2 showed that 100% of FNS was released after a period of 3 h. All FNS was released from PLGA nanoparticles.

Moreover, in this study, pH values, viscosity, and the organoleptic and tactile aspects of the developed formulations were evaluated and the results were present in Table 1.

Regarding the value of pH, the values obtained for the 3 formulations were compatible with human skin, which presents a pH within the range of 4.5 and 6.5 [44, 45]. Viscosity of all formulations was adequate. In case of shampoo, its viscosity allows the effective removal of the formulation and at the same time it must be easy to apply on the hair.

Fig. 3. Permeation profiles of the FNS in the different vehicles: lotion, solution, and shampoo.

Table 1. Parameters of the developed topical formulations (mean values $\pm$ SD)

Formulations	Organoleptic parameters	Viscosity, cps	pH values ($n = 3$)
Shampoo	Translucent Homogeneous Very good foaming ability	$2,650 \pm 15$	5.66 ± 0.01
Lotion	Colorless formulation Homogeneous	135 ± 5	5.31 ± 0.01
Solution	Colorless formulation Clear No particles in solution	<50	5.96 ± 0.02

During the in vitro skin permeation test, it was found that the higher diffusion rate of the FNS was observed with the shampoo and the lowest was observed in the solution (Fig. 3). It should be noted that all experimental variables, including the diffusion area (0.95 cm^2), cell volume ($4.09 \pm 0.16 \text{ mL}$), thickness (75 microns), and type of membrane (hydrophobic membrane of PDMS) were constant for all Franz cells. The steady state flows in the different formulations of FNS were also calculated using the slope obtained by the diffused amount of drug over time, after steady state was achieved. No significant differences were observed ($p = 0.4793$) (Table 2). In the case of the shampoo, a flux in steady state of $40.01 \pm 2.4 \mu\text{g}/\text{cm}^2 \cdot \text{h}^{-1}$ was obtained. Shampoo composition with sur-

Table 2. Permeation profiles of the tested formulations ($n = 5$)

Time, h	Mean cumulative diffused FNS $\pm$ SD, $\mu\text{g}/\text{cm}^2$		
	lotion	solution	shampoo
0.25	99.27 $\pm$ 17.38	61.11 $\pm$ 28.34	47.50 $\pm$ 18.77
0.50	141.48 $\pm$ 24.31	87.11 $\pm$ 25.72	82.41 $\pm$ 19.26
1.00	173.20 $\pm$ 20.75	110.66 $\pm$ 26.74	129.86 $\pm$ 19.23
2.00	209.93 $\pm$ 28.78	133.33 $\pm$ 26.12	179.53 $\pm$ 21.55
3.00	241.41 $\pm$ 31.96	158.20 $\pm$ 27.13	228.95 $\pm$ 24.29
4.00	270.55 $\pm$ 32.57	181.73 $\pm$ 26.43	284.51 $\pm$ 25.93
6.00	313.75 $\pm$ 55.77	213.12 $\pm$ 31.09	369.17 $\pm$ 21.45
8.00	354.13 $\pm$ 51.05	247.07 $\pm$ 33.99	448.99 $\pm$ 26.59
24.00	409.22 $\pm$ 64.86	300.22 $\pm$ 42.22	960.24 $\pm$ 56.87

Table 3. Comparison of the parameter a^* (erythema) and the TEWL of the different formulations ($n = 10$)

Formulations	Comparison of the parameter a^* , AU	Comparison of the TEWL, g/m^2
Lotion with NP	0.582	0.376
Lotions without NP	0.903	0.221
Solution with NP	0.153	0.107
Solution without NP	0.800	0.077
Shampoo with NP	0.372	0.329
Shampoo without NP	0.746	0.023
Negative control	0.898	0.623

TEWL, transepidermal water loss; NP, nanoparticles.

factants such as sodium lauryl ether sulfate may be responsible for those high flux values. Solution and the lotion showed similar values ($12.51 \pm 1.8 \mu\text{g}/\text{cm}^2 \cdot \text{h}^{-1}$ and $17.05 \pm 2.7 \mu\text{g}/\text{cm}^2 \cdot \text{h}^{-1}$, respectively). Those results can be explained with the particular characteristics of each formulations, such as the high water content and low viscosity of the lotion and solution, which influence the permeation capacity of the nanoparticles through the synthetic and hydrophobic membrane [46, 47].

The cytotoxicity profile of the FNS-loaded PLGA nanoparticles was characterized using the *S. cerevisiae* model. FNS was tested at different concentrations of 15, 30, and 45 $\mu\text{g}/\text{mL}$. Results showed a linear relationship between FNS concentrations and the magnitude of cytotoxic effects. The decrease in cell viability was probably related to an occlusive effect of the nanoparticles on *S. cerevisiae*. In fact, the cytotoxicity testing of nanoparticles on the *S. cerevisiae* model is limited by the amount of nanoparticles in suspension. Cytotoxicity profiles for nanosystems are not yet well understood. Other examples

of our group such as polycaprolactone nanoparticles with betamethasone-21-acetate confirmed the occlusive effect of nanocarriers [48]. In this case, betamethasone-21-acetate showed an IC_{50} of 168.8 $\mu\text{g}/\text{mL}$ and betamethasone-21-acetate-loaded nanoparticles showed an IC_{50} of 14.5 $\mu\text{g}/\text{mL}$. These empty nanoparticles also demonstrated a concentration-dependent toxic effect on the human keratinocyte HaCaT cell line [42, 49, 50]. In addition, our empty PLGA nanoparticles was already tested using the human keratinocyte HaCaT cell line [48] and corroborates with the present study.

In human safety testing, all excipients used in those formulations did not cause any erythema or other adverse reaction in any of the volunteers. All excipients are included in approved substance list of the European Commission and all are used in lower concentrations [15]. In this study, erythema effects were determined by comparing a^* values of the treated site with control sites. In this study, there were no significant differences between the values of the parameter a^* after application (Table 3) which means that PLGA or other reagents used in all formulations, such as surfactants used in shampoo or solvents, did not cause any change [51]. In this technique, the increase in skin redness was calculated by a difference between baseline skin redness and skin redness resulting from the effect of an irritant. This technique of reflectance spectrophotometry utilizes the fact that hemoglobin present in the blood vessels in the upper dermis is the main cutaneous chromophore of green light. Blood flow increases have been shown to correlate positively with increased erythema, TEWL, and skin thickness [15]. Surfactants like sodium lauryl ether sulfate generally increase cutaneous blood flow. Moreover, other surfactants have been shown to cause changes in skin temperature. Exposure of skin to surfactants may induce irritation, but in our case this fact was also not observed. The type and degree surfactant irritancy can be related to the type and concentration and also some other factors such as skin type, color, and age [1, 32, 51].

For TEWL analysis, experimental conditions were similar for all formulations. TEWL evaluation only showed that there were significant differences ($p < 0.05$) for solutions with nanoparticles and shampoo, probably due to the rheological properties of such formulations. High-viscosity values were observed after inclusion of nanoparticles in each vehicle [52]. The increase of TEWL values after application of the nanoparticles may be related to some destabilization of skin surface and follicular ducts, causing a modification on the stratum corneum as previously observed [46].

Conclusion

The success of topical products in alopecia therapy depends on the capability of the drug to penetrate through the skin in sufficient amounts to achieve the desired therapeutic effect. In this study, PLGA nanoparticles were chosen as carrier systems with the ability to locally incorporate FNS into hair follicles. The overall conclusion confirms that PLGA is a useful and safe polymer for drug delivery to the skin surface and for the potential application of nanotechnology in transdermal delivery systems. The resultant PLGA nanoparticles were shown to be an efficient encapsulation system for FNS with potential interest for pharmaceutical industry. No toxic effects of the FNS-loaded PLGA nanoparticles were observed in the *S. cerevisiae* model. Safety testing of the excipients using hu-

man volunteers demonstrated that formulation excipients were compatible with the skin. Future studies should include the study of the efficacy and safety of the previous FNS-loaded formulations.

Statement of Ethics

Volunteers were selected after informed consent, in full compliance with all ethical standards of the Declaration of Helsinki and its amendments.

Disclosure Statement

The authors declare no conflicts of interest or any sponsorship or funding arrangements relating to their research.

References

- 1 Sato A, Takeda A: Evaluation of efficacy and safety of finasteride 1 mg in 3,177 Japanese men with androgenetic alopecia. *J Dermatol* 2012;39:27–32.
- 2 Kumar R, Singh B, Bakshi G, Katore OP: Development of liposomal systems of finasteride for topical applications: design, characterization, and in vitro evaluation. *Pharm Dev Technol* 2007;12:591–601.
- 3 Libecco JF, Bergfeld WF: Finasteride in the treatment of alopecia. *Expert Opin Pharmacother* 2004;5:933–940.
- 4 Lademann J, Richter H, Teichmann A, Otberg N, Blume-Peytavi U, Luengo J, Weiss B, Schaefer UF, Lehr C-M, Wepf R: Nanoparticles – an efficient carrier for drug delivery into the hair follicles. *Eur J Pharm Biopharm* 2007;66:159–164.
- 5 Ahlin Grabnar P, Kristl J: The manufacturing techniques of drug-loaded polymeric nanoparticles from preformed polymers. *J Microencapsul* 2011;28:323–335.
- 6 Couvreur P, Barratt G, Fattal E, Legrand P, Vauthier C: Nanocapsule technology: a review. *Crit Rev Ther Drug Carrier Syst* 2002;19:99–134.
- 7 Reis CP, Roque LV, Baptista M, Rijo P: Innovative formulation of nystatin particulate systems in toothpaste for candidiasis treatment. *Pharm Dev Technol* 2016;21:282–287.
- 8 Madheswaran T, Baskaran R, Thapa RK, Rhyu JY, Choi HY, Kim JO, Yong CS, Yoo BK: Design and in vitro evaluation of finasteride-loaded liquid crystalline nanoparticles for topical delivery. *AAPS PharmSciTech* 2013;14:45–52.
- 9 Song SY: Future direction of nanomedicine in gastrointestinal cancer. *Korean J Gastroenterol* 2007;49:271–279.
- 10 Tanaka T, Decuzzi P, Cristofanilli M, Sakamoto JH, Tasciotti E, Robertson FM, Ferrari M: Nanotechnology for breast cancer therapy. *Biomed Microdevices* 2009;11:49–63.
- 11 Zhang H, Cui W, Bei J, Wang S: Preparation of poly(lactide-co-glycolide-co-caprolactone) nanoparticles and their degradation behaviour in aqueous solution. *Polym Degrad Stab* 2006;91:1929–1936.
- 12 Martinho N, Rosado C, Reis CP: Influência de duas técnicas diferentes no tamanho e potencial zeta de nanopartículas de poli (DL-láctico-co-glicólico). *Rev Lusófona Ciências Tecnol Saúde* 2011;8:329–338.
- 13 Jana U, Mohanty AK, Pal SL, Manna PK, Mohanta GP: Felodipine loaded PLGA nanoparticles: preparation, physicochemical characterization and in vivo toxicity study. *Nano Converg* 2014;1:31.
- 14 Peng D, Huang K, Liu Y, Liu S: Preparation of novel polymeric microspheres for controlled release of finasteride. *Int J Pharm* 2007;342:82–86.
- 15 Rowe RC, Sheskey PJ, Quinn ME (eds): *Handbook of Pharmaceutical Excipients*, ed 6. London, The Pharmaceutical Press, 2010, pp 637–638.
- 16 Trüeb RM: The value of hair cosmetics and pharmaceuticals. *Dermatology* 2001;202:275–282.
- 17 Noudeh GD, et al: Formulation of herbal conditioner shampoo by using extract of fenugreek seeds and evaluation of its physicochemical parameters. *African J Pharm Pharmacol* 2011;5:2420–2427.
- 18 Moldovan M, Părăuan S: Cosmetic evaluation of some commercial shampoos. *Farm Clujul Med* 2012;85:378–383.
- 19 Esmaeili F, Atyabi F, Dinarvand R: Preparation of PLGA nanoparticles using TPGS in the spontaneous emulsification solvent diffusion method. *J Exp Nanosci* 2007;2:183–192.
- 20 Murakami H, Kobayashi M, Takeuchi H, Kawashima Y: Further application of a modified spontaneous emulsification solvent diffusion method to various types of PLGA and PLA polymers for preparation of nanoparticles. *Powder Technol* 2000;107:137–143.
- 21 Murakami H, Kobayashi M, Takeuchi H, Kawashima Y: Preparation of poly(DL-lactide-co-glycolide) nanoparticles by modified spontaneous emulsification solvent diffusion method. *Int J Pharm* 1999;187:143–152.
- 22 Gomes A, Ascensão L, Rijo P, Baptista M, Candeias S, Martinho N, Fernandes A, Roberto A, Reis C: Evaluation of a new topical treatment for acne with azelaic acid-loaded nanoparticles. *Microsc Microanal* 2013;19:59–60.
- 23 van Tonder A, Joubert AM, Cromarty A: Limitations of the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay when compared to three commonly used cell enumeration assays. *BMC Res Notes* 2015;8:47.
- 24 Chauhan D, Ray A, Viktorsson K, Spira J, Pabba-Prada C, Munshi N, Richardson P, Lewensohn R, Anderson KC: In vitro and in vivo antitumor activity of a novel alkylating agent, melphalan-flufenamide, against multiple myeloma cells. *Clin Cancer Res* 2013;19:3019–3031.
- 25 Roberto A, Caetano P: A high-throughput screening method for general cytotoxicity. Part I: chemical toxicity. *Rev Lusófona Ciências Tecnol Saúde* 2005;2:95–100.

- 26 Marrot L, Labarussiat A, Perez P, Meunier JR: Use of the yeast *Saccharomyces cerevisiae* as a pre-screening approach for assessment of chemical-induced phototoxicity. *Toxicol Vitro* 2006;20:1040–1050.
- 27 Holder AL, Goth-Goldstein R, Lucas D, Koshland CP: Particle-induced artifacts in the MTT and LDH viability assays. *Chem Res Toxicol* 2012;25:1885–1892.
- 28 Löffler H, Happler R: Influence of climatic conditions on the irritant patch test with sodium lauryl sulphate. *Acta Derm Venereol* 2003;83:338–341.
- 29 Flannigan SA, Smith RE, McGovern JP: Intra-regional variation between contact Irritant patch test sites. *Contact Dermatitis* 1984;10:123–124.
- 30 York M, Griffiths HA, Whittle E, Basketter DA: Evaluation of a human patch test for the identification and classification of skin irritation potential. *Contact Dermatitis* 1996;34:204–212.
- 31 Basketter DA, York M, McFadden JP, Robinson MK: Determination of skin irritation potential in the human 4-h patch test. *Contact Dermatitis* 2004;51:1–4.
- 32 Hemminki A: Harmful impact of EU clinical trials directive. *BMJ* 2006;332:501–502.
- 33 Pich J, Carné X, Arnaiz J-A, Gómez B, Trilla A, Rodés J: Role of a research ethics committee in follow-up and publication of results. *Lancet* 2003;361:1015–1016.
- 34 Feng S-S: Nanoparticles of biodegradable polymers for new-concept chemotherapy. *Expert Rev Med Devices* 2004;1:115–125.
- 35 Vogt A, Rancan F, Ahlberg S, Nazemi B, Choe CS, Darvin ME, Hadam S, Blume-Peytavi U, Loza K, Diendorf J, Epple M, Graf C, Rühl E, Meinke MC, Lademann J: Interaction of dermatologically relevant nanoparticles with skin cells and skin. *Beilstein J Nanotechnol* 2014;5:2363–2373.
- 36 Idson B: Adsorption to skin and hair. *J Soc Cosmet Chem* 1967;103:91–103.
- 37 Dragicevic N, Maibach HI: Percutaneous penetration enhancers chemical methods in penetration enhancement; in: *Modification of the Stratum Corneum*. Berlin, Springer, 2016, pp 1–411.
- 38 Yilmaz E, Borchert HH: Design of a phytosphingosine-containing, positively-charged nanoemulsion as a colloidal carrier system for dermal application of ceramides. *Eur J Pharm Biopharm* 2005;60:91–98.
- 39 Hoeller S, Sperger A, Valenta C: Lecithin based nanoemulsions: a comparative study of the influence of non-ionic surfactants and the cationic phytosphingosine on physicochemical behaviour and skin permeation. *Int J Pharm* 2009;370:181–186.
- 40 Silva LAD, Taveira SF, Lima EM, Marreto RN: In vitro skin penetration of clobetasol from lipid nanoparticles: drug extraction and quantitation in different skin layers. *Braz J Pharm Sci* 2012;48:811–817.
- 41 Yang R, Shim W-S, Cui F-D, Cheng G, Han X, Jin Q-R, Kim D-D, Chung S-J, Shim C-K: Enhanced electrostatic interaction between chitosan-modified PLGA nanoparticle and tumor. *Int J Pharm* 2009;371:142–147.
- 42 Dinarvand R, Sepehri N, Manoochehri S, Rouhani H, Atyabi F: Polylactide-co-glycolide nanoparticles for controlled delivery of anticancer agents. *Int J Nanomedicine* 2011;6:877–895.
- 43 Hubbard AT: *Surfactants in Personal Care Products and Decorative Cosmetics: Angewandte Chemie International Edition*. Abingdon, Routledge Taylor & Francis Group, 2006, vol 40, p 9823.
- 44 Lambers H, Piessens S, Bloem A, Pronk H, Finkel P: Natural skin surface pH is on average below 5, which is beneficial for its resident flora. *Int J Cosmet Sci* 2006;28:359–370.
- 45 Morykwas MJ, Thornton JW, Bartlett RH: Zeta potential of synthetic and biological skin substitutes: effects on initial adherence. *Plast Reconstr Surg* 1987;79:732–739.
- 46 Parra A, Clares B, Rosselló A, Garduño-Ramírez ML, Abrego G, García ML, Calpena AC: Ex vivo permeation of carprofen from nanoparticles: a comprehensive study through human, porcine and bovine skin as anti-inflammatory agent. *Int J Pharm* 2016;501:10–17.
- 47 Pfaller MA, Diekema DJ: Epidemiology of invasive candidiasis: a persistent public health problem. *Clin Microbiol Rev* 2007;20:133–163.
- 48 Silva CO, Rijo P, Molpeceres J, Figueiredo IV, Ascensão L, Fernandes AS, Roberto A, Reis CP: Polymeric nanoparticles modified with fatty acids encapsulating betamethasone for anti-inflammatory treatment. *Int J Pharm* 2015;493:271–284.
- 49 Lobner D: Comparison of the LDH and MTT assays for quantifying cell death: validity for neuronal apoptosis? *J Neurosci Methods* 2000;96:147–152.
- 50 Tupker RA, Willis C, Berardksca E, Lee CH, Fartasch M, Atinrat T, Serup J: Guidelines on sodium lauryl sulfate (SLS) exposure tests. *Contact Dermatitis* 1997;37:53–69.
- 51 Fullerton A, Fischer T, Lahti A, Wilhelm K-P, Takiwaki H, Serup J: Guidelines for measurement skin colour and erythema. A report from the Standardization Group of the European Society of Contact Dermatitis. *Contact Dermatitis* 1996;35:1–10.
- 52 Bouchemal K, Briançon S, Perrier E, Fessi H: Nano-emulsion formulation using spontaneous emulsification: solvent, oil and surfactant optimisation. *Int J Pharm* 2004;280:241–251.