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Short Communication

Novel Beads Made of Alpha-cyclodextrin and Oil for Topical Delivery of a Lipophilic Drug

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Purpose. To investigate the potential of a novel lipid carrier, comprising beads of alpha-cyclodextrin and soybean oil, for topical drug delivery. Adapalene was chosen as a model drug to explore the ability of the beads to encapsulate and release a highly lipophilic compound.
Materials and Methods. Adapalene-loaded beads were prepared and characterised. Skin tolerance to unloaded beads was tested on human volunteers, while drug release and delivery into *stratum corneum*, was evaluated in pig skin *ex vivo*.
Results. The preparation and physical characteristics of the beads were not dependent on whether adapalene had been previously dissolved or dispersed in soybean oil. Drug encapsulation efficiency was high (>96%) and drug loading on the order of a therapeutic level could be achieved in freeze-dried beads prepared from an oily dispersion of adapalene. After application to human skin, unloaded beads induced no adverse reaction and were better tolerated than an alcoholic gel. Tape-stripping the *stratum corneum* from treated pig skin showed that adapalene release and penetration from the beads was comparable to that from gel and cream formulations available on the market.
Conclusion. These novel beads may offer a well-tolerated and efficient system for the encapsulation and topical delivery of lipophilic drugs.

KEY WORDS: adapalene; bead; cyclodextrin; lipid carrier; topical formulation.

INTRODUCTION

Due to its composition and structure, the skin exhibits excellent barrier function and limits the penetration of a wide range of therapeutic agents into the body. Indeed, only small, neutral and moderately lipophilic molecules can easily cross the skin. To improve the delivery of highly lipophilic drugs, several innovative formulations, such as microemulsions (1), liposomes (2) and solid lipid nanoparticles (3), have been developed in recent years.

To further extend the range of formulation strategies available for highly lipophilic therapeutic agents, a novel type of bead has been conceived. These carriers are prepared by a simple and original process involving a mixture of an aqueous solution of α-cyclodextrin with soybean oil, but without the use of any organic solvent or surface-active agent (4). The spherical beads have a diameter of 1.6±0.2 mm and consist in a partial crystalline matrix of cyclodextrin surrounding micro-domains of oil (4). Freeze-dried beads have high lipid content (80%) and a semi-solid consistency which facilitates their application to the skin.

Adapalene [6-(3-(1-adamantyl)-4-methoxyphenyl)-2-naphtoic acid] is a synthetic derivative of vitamin A (retinol), used for the treatment of acne. This drug is currently formulated as an aqueous gel or a cream, and is marketed as Differin®. Adapalene (412.52 g/mol, pK_a=4.23, logP=8.04, sparingly water-soluble from SciFinder database) was chosen as a model drug with which to explore the ability of the beads to encapsulate and deliver a highly lipophilic compound. The fluorescence properties of the molecule, and its inherent stability, were additional advantages that allowed its facile quantification and visualisation in formulations and in skin.

The present work focused on the preparation and the characterisation of adapalene-loaded beads. Skin tolerance to the unloaded beads was considered a prerequisite for further investigations and was therefore studied first. Subsequently, adapalene penetration into the outer layer of the skin was then quantified after application of the novel beads, and this uptake was compared to that from currently commercialised formulations.

MATERIALS AND METHODS

Materials

Alpha-cyclodextrin (α-CD) (Pharma grade) and Cropure® soybean oil were purchased from Wacker-Chemie (München, Germany) and Croda (Trappes, France), respectively. Butylated hydroxytoluene (BHT) was from Sigma

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Chemical Co. (St Louis, USA) and hydroxypropylcellusose pharmaceutical grade (Klucel M®) was a gift from Aqualon-Hercules Inc (Wilmington, USA). Adapalene was supplied by Sequoia Research Products (Oxford, UK). Differin® gel 0.1% (main excipients: carbomer 940, propylene glycol, poloxamer 182 and water) and Differin® cream 0.1% (main excipients: carbomer 934P, perhydrosqualene, cyclomethicone, glycerin and water) were purchased from Galderma (La Défense, France). Ethanol (96° and pure), tetrahydrofuran, acetonitrile, and trifluoroacetic acid (all HPLC grade) were obtained from Fisher Scientific (Loughborough, UK), while chloroform and methanol (analytical grade) were from Carlo Erba Reagenti (Val de Reuil, France).

Preparation of Adapalene-Loaded Beads

Adapalene was either solubilised (86 μg/ml) or dispersed (4.3 mg/ml) in soybean oil. Of this oily phase, 5.8 ml was added to 20 ml of an aqueous solution (8.1% w/v) of α-CD. The mixture was continuously shaken at 200 rpm (Salvis, Bioblock Scientific, Illkirch, France) at 28°C for several days. The resulting beads were then washed and freeze-dried during 48 h to eliminate water (Christ LDC-1 alpha1-4 freeze-dryer, Bioblock Scientific). Unloaded beads were prepared using the same protocol.

Characterisation of Beads

Bead diameter was determined after freeze-drying (n=50 beads) using an optical microscope (Leitz Diaplan microscope, Leica Microsystèmes, France) equipped with a Coolsnap ES camera (Roper Scientific, Germany). The physical state of adapalene in crushed beads was observed by fluorescence microscopy. Adapalene was excited with a mercury short arc lamp (HBO 50W, Osram, Germany) coupled with a filter (Leica Microsystèmes, France) having the following characteristics: excitation band-pass filter from 340 to 380 nm and emission long-pass filter >425 nm. Differin® gel and Differin® cream were submitted to the same examination.

Bead yield was calculated after freeze-drying from the relation:

$$Bead\ yield(\%) = \frac{weight\ of\ freeze-dried\ beads}{weight(\alpha - CD + oily\ phase)} \times 100$$

To extract adapalene from freeze-dried beads, 40 mg were precisely weighed, gently destroyed and then mixed with 5 ml of (2:1, v/v) chloroform/methanol. Insoluble bead material was eliminated by centrifugation for 5 min at 2,000×g (Capsule HF 120, Tomy Tokyo, Japan) and adapalene content in the supernatant was quantified by fluorescence (Luminescence Spectrometer 50B, Perkin Elmer, Bucks, UK).

Adapalene encapsulation efficiency and adapalene loading were calculated using the following equations:

$$Encapsulation\ efficiency(\%) = \frac{Amount\ of\ adapalene\ within\ beads}{Amount\ of\ adapalene\ soybean\ oil} \times 100$$

$$Drug\ loading\ (mg\ of\ adapalene/g\ of\ beads) = \frac{Amount\ of\ adapalene\ within\ beads}{Weight\ of\ beads}$$

Encapsulation efficiency and drug loading were expressed as mean±standard deviation (n=3 batches).

Skin Tolerance Study

Skin tolerance of unloaded, freeze-dried beads was assessed on seven healthy human volunteers (one male, six females, aged from 22 to 42 years). An adhesive template (Polyethylene foam tape 1772, 3 M™, St Paul, USA) was placed on the forearm to delimit the application site (diameter 2.0 cm). Taking into account the volumic mass of each formulation, 100 μl/cm^2 of the beads (0.2 g/ml) or of an alcoholic gel control (3.0% w/v hydroxypropylcellulose, 0.01%w/v butylated hydroxytoluene and 97% w/v ethanol; 0.9 g/ml) was applied under an occlusive cover comprising a double layer of adhesive (Book tape 845, 3 M™, St Paul, USA).

Another control site was untreated at all and simply occluded as a reference. Skin reaction was evaluated by observation and quantified by skin colour, using a chromameter (CM-2600d, Konica Minolta, Osaka, Japan), and by transepidermal water loss (TEWL) measurements (AquaFlux AF 102, BioX, London, UK). Values were obtained pre-application of formulations and at 24, 48 and 72 h post-treatment. The formulations were carefully removed immediately before each of the daily measurement, and then fresh vehicles were reapplied afterwards. Experiment was stopped in the case of intense cutaneous reaction on volunteer skin. The experimental protocol was approved by the Bath Local Research Ethics Committee.

Skin colour measurements were generated in a three-dimensional coordinate system and expressed as an admix-

Table I. Unloaded and Adapalene-Loaded Bead Characteristics (n=3 Different Batches; Mean±SD)

Oily Phase	Process Duration (days)	Fabrication Yield (%)	Bead Size (mm)	Encapsulation Efficiency (%)	Drug Loading (mg of drug/g of beads)
Soybean oil	2.5	84±4	1.6±0.2	–	–
Adapalene dissolved in soybean oil (86 μg/ml)	7	87±2	2.0±0.2	98±1	0.080±0.003
Adapalene dispersed in soybean oil (4.3 mg/ml)	7	90±4	2.1±0.2	96±4	3.4±0.2

ture of a^*, b^* and L^* values, where a^* represents the colour range from green to red, b^* the colour range from blue to yellow, and luminance (L^*) expresses the brightness. Attention was focused on the variation of the a^* value before (a_0^*) and after vehicle application and was corrected for a possible occlusion effect via the expression:

$$\Delta a^* = (a^* - a_0^*)_{\text{formulation}} - (a^* - a_0^*)_{\text{occlusion}}$$

Two-way ANOVA was used to evaluate the influence of the vehicle and of the duration of application on skin colour. p value less than 0.05 was considered statistically different.

TEWL variations were treated in a similar manner, using the value before formulation application ($TEWL_0$) as a reference, and correcting for an occlusion effect:

$$\Delta TEWL = (TEWL - TEWL_0)_{\text{formulation}} - (TEWL - TEWL_0)_{\text{occlusion}}$$

Results were calculated as mean±SD for test and control formulations and then the difference between the TEWL variations was determined:

$$\Delta\Delta TEWL_{\text{bead-gel}} = \Delta TEWL_{\text{bead}} - \Delta TEWL_{\text{gel}}$$

To compare the effects of the beads and the alcoholic gel, the Wilcoxon matched paired test (at $p<0.05$) was used. A Friedman test evaluated the influence of application time on TEWL variations (again, at $p<0.05$).

Adapalene Penetration in *Stratum Corneum*

Application of Adapalene Formulations

Abdominal skin from a single domestic pig (750 μm dermatomed) was the model used to assess adapalene absorption. After thawing the tissue, any obvious hair was trimmed with scissors, and four application sites (circular, 1.6 cm diameter) were delimited using an adhesive template (Scotch® Book Tape 845, 3 M, St. Paul, USA), taking care to avoid any skin abrasions or scars. Differin® gel 0.1%, Differin® cream 0.1%, and freeze-dried adapalene-loaded beads (corresponding to 0.01 mg of adapalene/cm^2) were each applied at one of the three identified positions. The skin was then placed in a 7 cm diameter Franz cell, the receptor compartment of which was maintained at 32°C and filled with phosphate-buffered saline (pH 7.4). The skin surface was not covered. Twenty-four hours later, the cell was dismantled and any remaining formulations were carefully removed using a wet tissue. Samples were then submitted either to a tape-stripping procedure or to fluorescent microscopic observation.

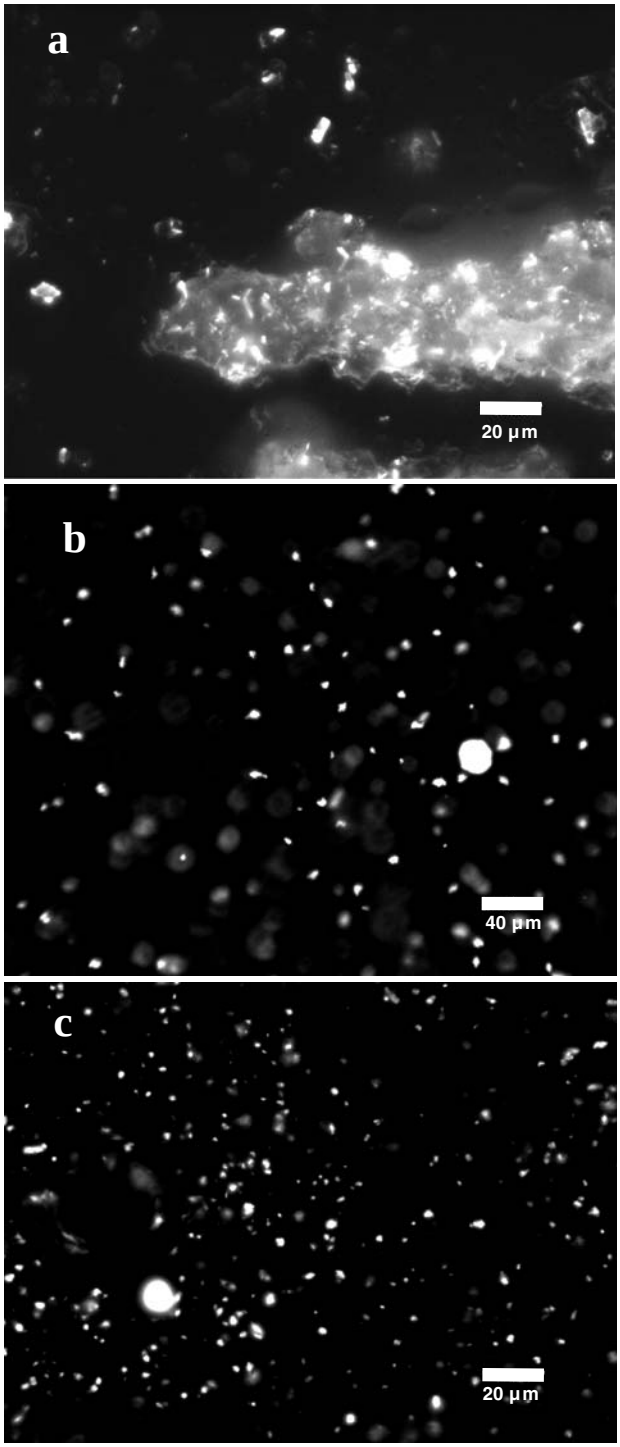

Fig. 1. Fluorescence micrographs of adapalene formulations (excitation band-pass filter from 340 to 380 nm and emission long-pass filter, λ>425 nm): **a** crushed beads, **b** Differin® gel, **c** Differin® cream.

Tape-Stripping Procedure

Skin sites were tape-stripped following previously published procedures (5). TEWL was recorded after every four strips until the value reached 80 g.m^{-2}.h^{-1} when it was assumed that most of the *stratum corneum* (SC) had been removed (6). Tapes were weighed using a high precision balance (Sartorius SE-2F, Goeltingen, Germany) before and after stripping (Δweight) to quantify the thickness of *stratum corneum* removed:

$$SC\ thickness\ removed = \frac{\Delta weight}{SC\ density \times stripped\ area}$$

assuming a mean *stratum corneum* density of 1 g.cm^{-3} (7). Adapalene was extracted from each tape-strip by overnight

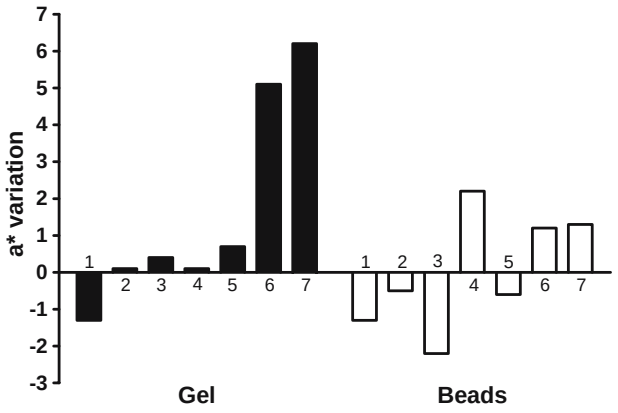

Fig. 2. Change in the *a** value after alcoholic gel (*full bars*) and unloaded bead (*open bars*) application (100 µl/cm^2) to human skin *in vivo*. The results were collected after 72 h of application for volunteers 1 to 6 and after 3 h for volunteer 7 (for whom the appearance of intense erythema led to an immediate termination of the study). Volunteer numbers are identified on each *bar*.

submersion into 1.5 ml of tetrahydrofuran/acetonitrile/water/trifluoroacetic acid (360/430/210/0.2 v/v), the solvent mixture subsequently used as the mobile phase in the chromatographic assay of the drug. The resulting samples were filtered and analysed by HPLC with fluorescence detection. The latter comprised a SOR-100 solvent rack, a P680 HPLC pump, an ASI-100 automated sample injector (injection volume of 20 µl), a thermostatted column compartment TCC-100 (25°C) and a RF 2000 Fluorescence detector (λ_{abs}=320 nm; λ_{em}=390 nm; Dionex, Surrey, UK). An Acclaim 120 column (5 µm, 4.6×150 mm, 120 Å [Dionex, Surrey, UK]) allowed facile adapalene separation at a flow rate of 1 ml/min using an isocratic method with the aforementioned mobile phase.

For each tape, the adapalene concentration in the *stratum corneum* was plotted as a function of the cumulative thickness removed. The total amount of adapalene taken up into *stratum corneum* was calculated as the sum of the drug amounts on the tapes collected at each application site (mean±SD ; n=5 for the three different vehicles).

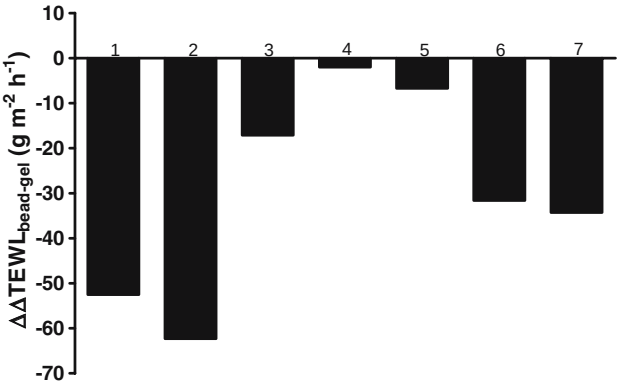

Fig. 3. Relative change in TEWL following application of unloaded beads and alcoholic gel (100 µl/cm^2) to human skin *in vivo* ($\Delta\Delta TEWL_{bead\text{-}gel}=\Delta TEWL_{bead}-\Delta TEWL_{gel}$). The results were collected after 72 h of application for volunteers 1 to 6 and after 3 h for volunteer 7 (for whom the appearance of intense erythema led to an immediate termination of the study). Volunteer numbers are identified on each *bar*.

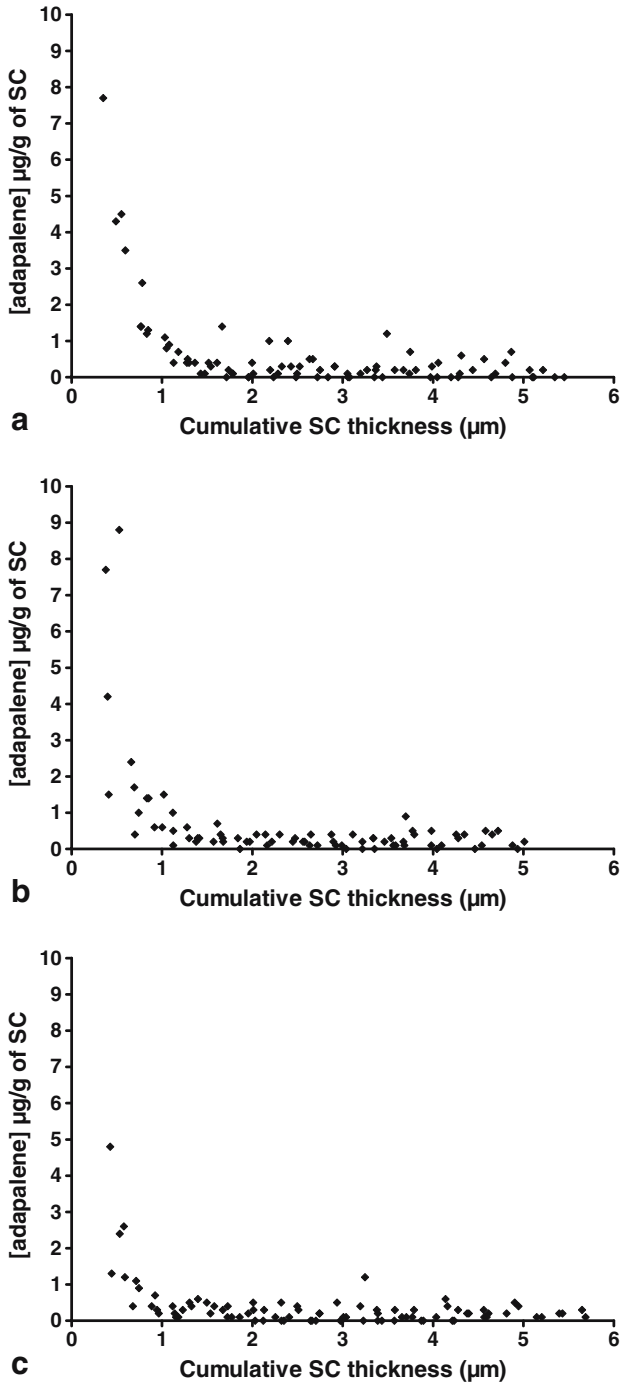

Fig. 4. Adapalene penetration into pig skin *stratum corneum ex vivo* after application of **a** adapalene-loaded beads, **b** Differin® gel 0.1%, **c** Differin® cream 0.1% (adapalene dose 0.01 mg/cm^2). All data from five separate experiments for each formulation are shown.

Fluorescence Microscopy

Adapalene was visualised in the skin by fluorescence microscopy (Leitz Diaplan microscope (Leica Microsystèmes, France), equipped with a Coolsnap ES camera (Roper Scientific, Germany). Adapalene was excited with a mercury short arc lamp as described above. Blue fluorescence from adapalene was clearly distinct from the autofluorescence of skin and hairs.

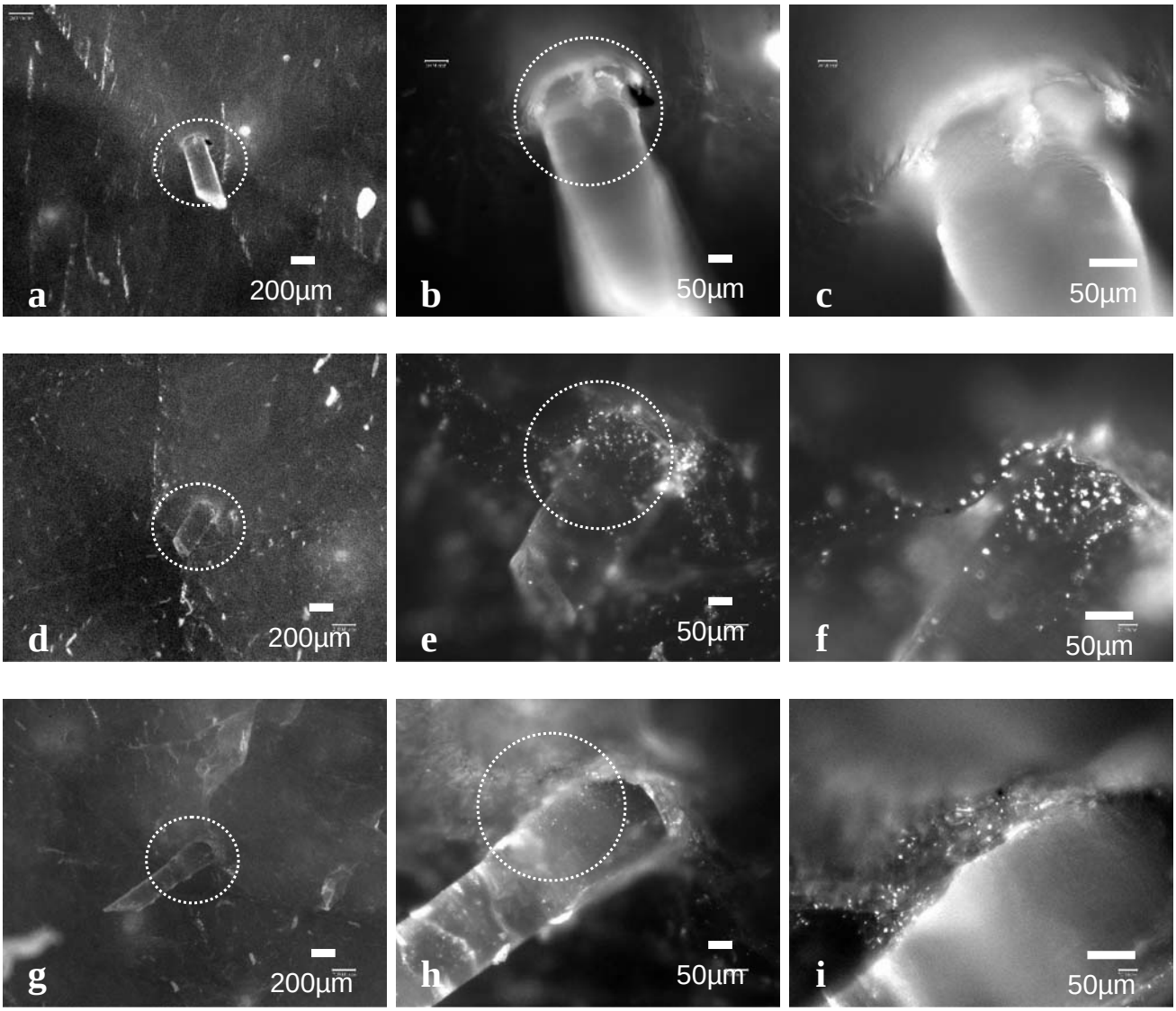

Fig. 5. Fluorescence micrographs of the openings of follicular ducts of pig skin *ex vivo* after topical application of adapalene-containing formulations for 24 h. **a–c** drug-loaded beads; **d–f** Differin® gel; **g–i** Differin® cream.

RESULTS AND DISCUSSION

Adapalene-Loaded Beads

The addition of adapalene previously solubilised, or dispersed in soybean oil, did not affect bead formation. Bead fabrication yield was independent of drug presence and was >84%. These results clearly demonstrated that the method developed and previously optimised (4) is suitable for adapalene (Table I). Indeed, encapsulation efficiency of this compound was >96% regardless of its physical state in soybean oil. Self-evidently, the greater affinity of this lipophilic drug for the oily phase, relative to the aqueous phase, meant that it was almost completely entrapped within oil micro-domains in the beads (4). Drug loading was directly dependent on adapalene concentration in soybean oil, and was about 40-times higher when adapalene was dispersed in oil (Table I).

The bead preparation time was longer, and the bead diameter was greater, when the drug was included in the formulation (Table I). It is possible that adapalene interferes in interactions essential to bead formation between cyclodextrin and the triglycerides at the oil/water interface (4).

The physical state of adapalene in crushed beads and in currently commercialised products was examined by fluorescence microscopy (Fig. 1). A homogeneous distribution of fluorescence was seen in beads formulated with solubilised drug (data not shown), whereas needle-shaped, inhomogenously-sized crystals (from 1 to 20 µm) were apparent when adapalene was initially dispersed in soybean oil (Fig. 1a). Differin® gel has been described as a homogeneous suspension of adapalene microcrystals (8); no information has been published, however, about the physical state of the drug in Differin® cream. Figures 1b and c show that both products contain mostly spherical adapelene microparticles ranging in size from 1 to 5 µm.

As a result of these findings, further experiments were undertaken only with the freeze-dried beads prepared from the oily suspension, in which the adapalene content was similar to that in the Differin® formulations (3.4±0.2 mg/g).

Skin Tolerance to Beads

Skin reaction to drug-free beads was evaluated by three different methods and compared to that provoked by an alcoholic gel. The latter was chosen as an irritant reference and to validate the experimental approach. Indeed, ethanol is known to extract lipids from the *stratum corneum* under certain conditions (9). The alcoholic gel was prepared according to the composition of a currently commercialised product (but excluding the drug, of course).

No clinical reaction (erythema or desquamation) was observed after unloaded bead application for 72 h. In contrast, all volunteers exhibited visible desquamation after the continuous application of alcoholic gel. Moreover, on two volunteers (numbers 6 and 7) erythema was clearly visible post-alcoholic gel application, in one case to such an extent that the experiment had to be terminated after only 3 h. Cutaneous erythema was quantified via measurements of skin redness. The changes in a^* values (Fig. 2) were influenced neither by the duration of application (p=0.75) nor by the formulations (p=0.085). Clearly, for volunteers 6 and 7, high and positive a^* value changes were observed after alcoholic gel application (Δa^*=+5.1 and +6.2, respectively), consistent with the erythema observed in these same subjects.

Similar to the erythema results, the duration of formulation application did not have any influence on transepidermal water loss (TEWL) measurements (p=0.5). TEWL changes were significantly lower with unloaded beads than those provoked by the alcoholic gel (p=0.0003). Indeed, for each volunteer, $\Delta\Delta TEWL_{unloaded\ beads/gel}$ was negative (Fig. 3). Moreover, the unloaded beads caused TEWL to decrease indicating an occlusive effect on the skin.

The unloaded beads were composed of α-CD (20% w/w) and soybean oil (80% w/w) (4). Cyclodextrins have been reported to be non-irritating when applied to skin but no investigation has ever been conducted with concentrations greater than 10% w/v (10–12). The present work demonstrates that a high cyclodextrin content in beads is not irritant *in vivo*. Moreover, the oil content of the beads was sufficient to provide an occlusive effect, as shown by the TEWL results. It should be emphasised that the application conditions used in this study, involving a large amount of vehicle administered continuously for 72 h under occlusion, are 'extreme' in relation to the expected conditions of use for a dermatological product. It may be reasonably concluded, therefore, that no bead-associated adverse effects would be observed under the clinical use of such a formulation.

Adapalene Penetration Study

Adapalene penetration into, and its concentration profile across, the *stratum corneum* were determined using the tape-stripping procedure. Adapalene was released from the novel beads and penetrated to the same extent into the *stratum corneum* as that achieved when the drug was applied in formulations already on the market (Fig. 4). The amounts of adapalene delivered into the skin from Differin® gel, Differin® cream and adapalene-loaded beads were, respectively, 3.6±1.6, 2.3±1.6, and 3.4±0.7% of the applied dose. These results confirm the relatively poor percutaneous absorption of adapalene. Despite the different excipients used in the three formulations tested (α-CD and soybean oil in the beads, principally carbomer 940 and water in Differin® gel, and perhydrosqualene, cyclomethicone and water in Differin® cream), drug delivery was not significantly different suggesting that adapalene was present in each vehicle at a similar thermodynamic activity. This is consistent with the very poor solubility of this drug in almost all solvents and its presence in the form of a suspension in the three formulations tested.

Finally, adapalene localisation after skin application was evaluated by fluorescence microscopy. For all three formulations, adapalene was clearly visualised at the openings of follicular ducts (Fig. 5), believed to be the preferred target sites for this drug (8, 13).

CONCLUSIONS

This work describes a new strategy for lipophilic drug encapsulation and topical delivery. The bead preparation process is simple and advantageously avoids the use of organic solvent, surface-active agents or elevated temperature. The beads are well-tolerated by skin; micro-domains of oil present within the bead inner structure favour high adapalene loading and confer an occlusive property. The beads release adapalene as efficiently as currently commercialised medicines.

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