# Site-Specific Drug Delivery to Pilosebaceous Structures Using Polymeric Microspheres

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In order to improve the therapeutic index of adapalene, a new drug under development for the treatment of acne, site-specific delivery to the hair follicles using 50:50 poly(DL-lactic-co-glycolic acid) microspheres as particulate carriers was investigated *in vitro* and *in vivo*. The percutaneous penetration pathway of the microspheres was shown to be dependent on their mean diameter. Thus, after topical application onto hairless rat or human skin, adapalene-loaded microspheres (5-μm diameter) were specifically targeted to the follicular ducts and did not penetrate via the stratum corneum. The *in vitro* release of adapalene from the microspheres into artificial sebum at 37°C was controlled and faster than the *in vivo* sebum excretion in humans. Aiming to reduce either the applied dose of drug or the frequency of administration, different formulations of adapalene-loaded microspheres were evaluated *in vivo* in the rhino mouse model. A dose-related comedolytic activity of topical formulations of adapalene-loaded microspheres was observed in this model. Furthermore, by applying a site-specific drug delivery system (0.1% adapalene) every other day or by administering a 10-fold less concentrated targeted formulation (0.01%) every day, a pharmacological activity equivalent to a daily application of an aqueous gel containing drug crystals (0.1% adapalene) was observed. Since an aqueous gel containing 10% adapalene-loaded microspheres was not irritating in a rabbit skin irritancy test, this formulation was applied onto forearms of human volunteers. Site-specific drug delivery was further evidenced by follicular biopsy. These results support the view that follicular drug targeting using 5-μm polymeric microspheres may represent a promising therapeutic approach for the treatment of pathologies associated with pilosebaceous units.

KEY WORDS: site-specific drug delivery; transfollicular route; adapalene; *in vitro* cutaneous penetration; follicular targeting; poly(lactide-co-glycolide) microspheres; rhino mouse model; *in vivo* cutaneous distribution.

## INTRODUCTION

Following topical application of a formulation onto the skin, percutaneous drug absorption may follow two routes: the transepidermal and the transfollicular (or "shunt") pathways. The role of skin appendages in the penetration process is often neglected. Indeed, the surface occupied by pilosebaceous duct orifices represents only about 0.1% of the total skin surface. However, there is increasing evidence that the transfollicular pathway plays an important role in the percutaneous absorption of some active compounds (1–5).

Keister *et al.* (2) investigated the transport pathways of ibuprofen through excised human skin using mathematical analysis of transient diffusion data. They demonstrated that ibuprofen penetrated the skin more rapidly via the transfollicular pathway (mean lag time = 8.6 min) than via the transepidermal pathway (mean lag time = 92 min). The contribution of the shunt pathway was relatively important, approximately 25% at steady state.

Wepierre *et al.* (3) and Illel *et al.* (4) developed experimental models for drug penetration through the follicular pathway. The role of follicles in the *in vitro* percutaneous absorption of drugs such as hydrocortisone, niflumic acid, caffeine, and *p*-aminobenzoic acid was estimated in appendage-free skin relative to normal hairless rat skin. For all of these compounds, the steady-state flux and the amounts diffusing in 24 or 48 hr were two to four times lower in the absence of appendages than in their presence. These results were further confirmed by application of hydrocortisone to 5-day postnatal rat skin (normal skin) as compared to 1-day-old rat skin without follicles. The steady-state flux and cumulative amount of drug diffusing in 24 hr were fivefold lower in appendage-free skin. Hueber *et al.* (5) compared the *in vivo* diffusion of two steroids, hydrocortisone and testosterone, through skin with and without appendages. Their investigations showed that the reservoir function of scar skin is more important than that of normal skin. Moreover, sebaceous glands contributed significantly to the promotion of skin penetration of these two steroids.

For the treatment of pathologies [e.g., acne, alopecia, and other sebaceous gland dysfunctions (6)] associated with pilosebaceous structures, it may be important to increase the distribution of certain drugs in the hair follicles. Although some drugs may passively diffuse into pilosebaceous units as reported above, most of the drugs generally enter the skin via the transepidermal pathway. Therefore, we investigated the possibility of targeting drugs to the hair follicles using specifically designed particulate carriers (7). There are several objectives of follicular targeting of drugs.

- Reducing or bypassing the transepidermal pathway.
- Decreasing the drug systemic toxicity.
- Increasing the drug concentration within pilosebaceous units.
- Increasing the therapeutic index of certain drugs.
- Possibly reducing the applied dose of a drug and/or the frequency of its administration.

For drug follicular targeting, microspheres were envisaged mainly as a site-specific drug delivery system since they present several advantages:

- Good stability of the microspheres when applied on the skin.
- Easy preparation of microspheres with a defined mean size in a narrow size distribution.
- Protection of the drug incorporated into the microspheres against degradation in the formulation (oxidation, hydrolysis) or premature inactivation on the skin surface.
- Controlled release of the drug in the hair follicles from the microspheres.
- Possibility of incorporating either lipophilic or hydrophilic drugs into the microspheres.

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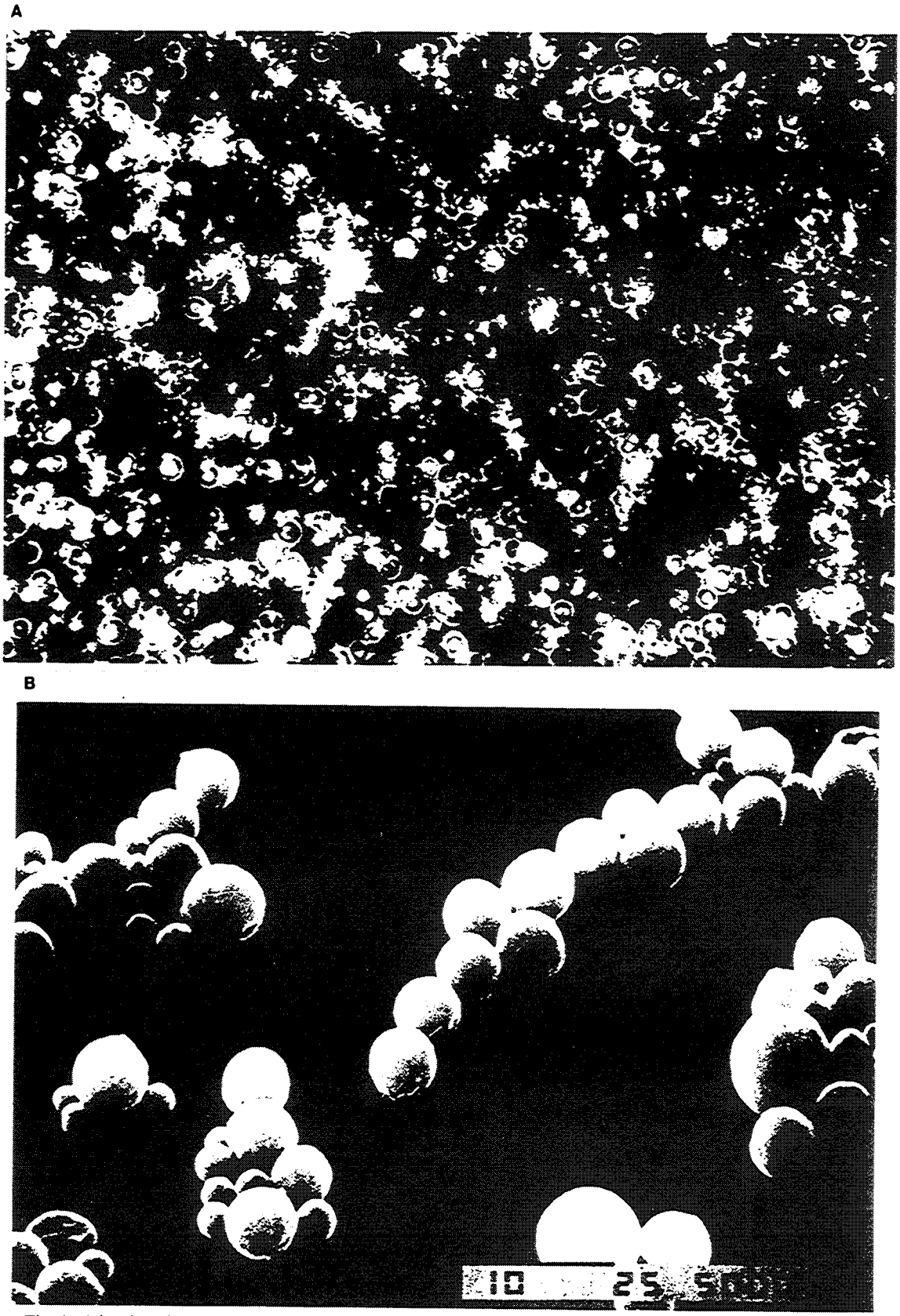


Fig. 1. Adapalene-loaded PLGA microspheres. (A) Fluorescence microscopy; (B) scanning electron microscopy.

Earlier studies demonstrated that microparticles with a diameter ranging from 3 to 10 μm selectively penetrate the follicular ducts, whereas particles larger than 10 μm remain on the skin surface and microspheres smaller than 3 μm are randomly distributed into the hair follicles and stratum corneum (8). Therefore, in the present work, in order to improve the therapeutic index of adapalene, a new naphthoic acid derivative under development for the treatment of acne (9), site-specific drug delivery to the hair follicles was investigated *in vitro* and *in vivo* using poly(lactide-co-glycolide) microspheres as particulate carriers.

## MATERIALS AND METHODS

### Chemicals

Adapalene[6-(3-(1-adamantyl)4-methoxyphenyl)-2-naphthoic acid] was synthesized at Cird Galderma (10). Medisorb 5050 DL [50:50 poly(DL-lactic-co-glycolic acid)] was purchased from Dupont (Medisorb Technologies Int. L.P., Cincinnati, OH). All solvents were of analytical grade. Aberel gel (Tretinoin 0.025%) was commercially available from Cilag Laboratories.

### Preparation of Adapalene-Loaded Microspheres

PLGA microspheres containing 1% adapalene were prepared by a solvent evaporation technique (11). Medisorb 5050 DL and 1% (w/w) adapalene were dissolved in methylene chloride and the organic phase was then added to a 1% poly(vinyl alcohol) aqueous solution, emulsified with either a mechanical stirrer or an ultrasound probe, and stirred for 4 hr at room temperature. After complete evaporation of the solvent, solid microspheres were collected, washed three times with distilled water, and freeze-dried. Depending on the stirring rate and emulsification procedure, three batches of adapalene-loaded PLGA microspheres with different mean diameters were obtained, i.e., 1, 5, and 20 μm. Drug content in the microspheres was analyzed by UV spectrophotometry (320 nm). Since adapalene is a fluorescent drug, microspheres were observed by fluorescence microscopy, then by scanning electron microscopy (SEM). The drug-loaded microspheres were finally dispersed in an aqueous gel (0.1%, w/w, adapalene final concentration).

### Skin

Female hairless rats (strain ICO, 7–8 weeks old) were purchased from Iffa Credo (France) and human skin was obtained from surgery.

### *In Vitro* Cutaneous Penetration of Microspheres

After carefully removing subcutaneous fat from human skin and excised ventral skin of female hairless rats, circular biopsies of full-thickness skin were placed in static diffusion cells. The dermal side was in contact with the 8-mL aqueous receptor phase (0.9% NaCl, 0.2% Tween 80), continuously stirred at 600 rpm, and thermostated at 37°C. The skin surface was thus maintained throughout the experiment at a constant temperature of 32°C. The formulations (50 mg/cm$^2$) were applied onto the skin surface (1.2 cm$^2$) and massaged for 3 min with a glass spatula. After 35 and 300 min the excess of formulation was wiped off, the diffusion cells were dismantled, and the skin samples were frozen at −80°C.

Frozen skin specimens (1 × 0.5 cm$^2$) were imbedded in Tissue Tek at −20°C and longitudinal sections realized with a microtome were then observed by fluorescence microscopy (excitation wavelength, 313 nm; emission wavelength, 400 nm) after hydration with glycerol or by scanning electron microscopy after dehydration and gold sputter-coating.

### *In Vitro* Release of Adapalene

The release of adapalene from the PLGA microspheres into artificial sebum was evaluated *in vitro*. Adapalene-loaded microspheres, (10%, w/w; 5 μm) were incubated at 37°C in artificial sebum. At various time intervals, sebum aliquots were sampled and centrifuged. The supernatant was then analyzed using a UV spectrophotometer at 320 nm.

### Animals

Male and female rhino mice (5–6 weeks) were purchased from CNRS (Orléans, France).

### Rhino Mouse Test

*PLGA Microspheres (5 μm) Containing 0.1, 0.5, and 1% Adapalene*

Aqueous gels containing 10% adapalene-loaded micro-

Fig. 2. Follicular localization of 5-μm adapalene-loaded microspheres 300 min after *in vitro* topical application to human skin (fluorescence microscopy).

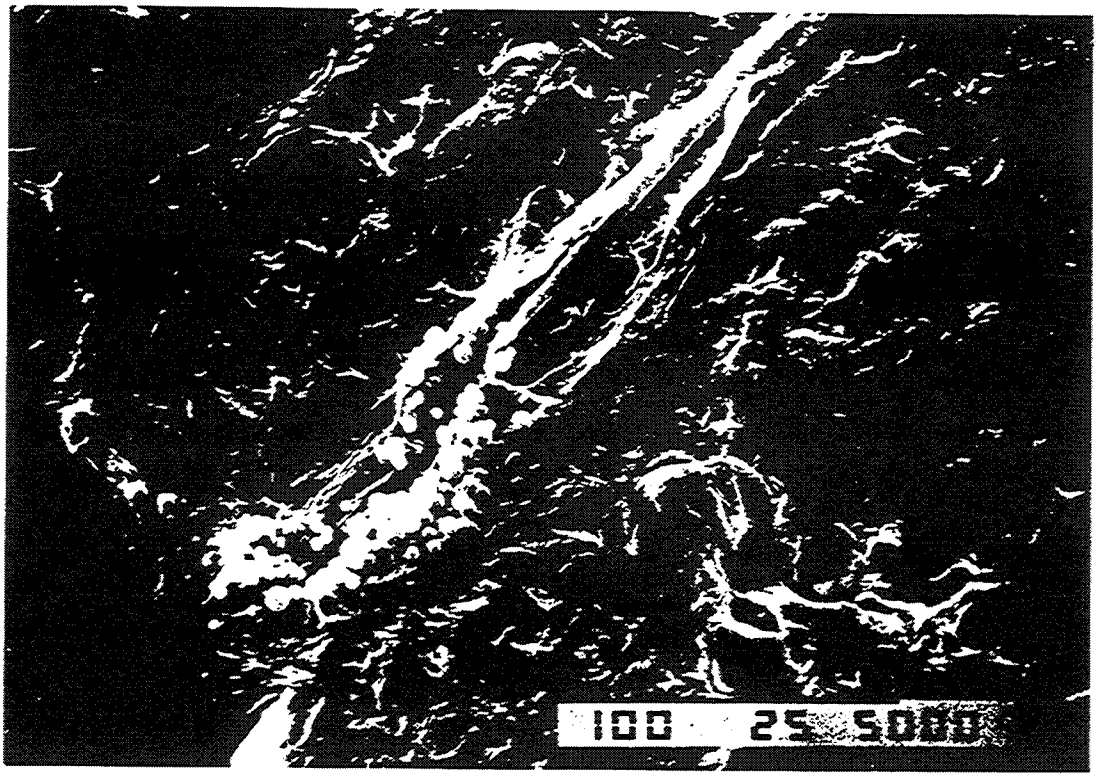

Fig. 3. Follicular localization of 5-μm adapalene-loaded microspheres 35 min after *in vitro* topical application to human skin (scanning electron microscopy).

spheres [0.1, 0.5, and 1% (w/w) adapalene] corresponding to drug final concentrations of 0.01, 0.05, and 0.1% (w/w) were administered topically for 3 weeks, on a 5 days per week basis, to rhino mice. Comedolytic activity of the three microsphere formulations was compared to that produced by topical application of an aqueous gel containing 0.1% (w/w) adapalene crystals.

At the end of the treatment, skin biopsies were taken from the treated dorsal area of the rhino mice. The histological preparations were observed with a microscope coupled to a videocamera and different parameters were measured using a semiautomatic MOP-Videoplan (Kontron). The computer calculated the total number of epidermal comedones per centimeter of stratum corneum length, the epidermal thickness, and the comedone profile ($r = d/D$), $d$ being the width of the surface orifice and $D$ the largest width of the utricle or width at half-depth (12).

### *PLGA Microspheres (5 μm) Containing 1% Adapalene*

Aqueous gels containing 1 and 10% adapalene-loaded microspheres [1% (w/w) adapalene], corresponding to drug final concentrations of 0.01 (a) and 0.1% (b) (w/w) were administered topically for 3 weeks, at 5 days per week (a) or at 3 days per week (b; every-other-day application), to rhino mice. Comedolytic activity of the three microsphere formulations was compared to that produced by topical application of an aqueous gel containing 0.1% (w/w) adapalene crystals using the technique described previously.

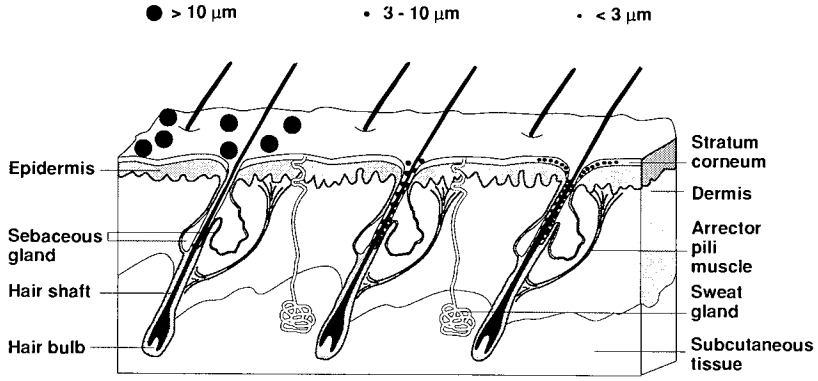


Fig. 4. Schematic representation of the influence of microsphere size on cutaneous penetration pathways.

### Rabbit Skin Irritancy Test

Aqueous gels containing either 10% adapalene-loaded microspheres or 10% empty microspheres were applied daily onto rabbit skin for 4 days. The irritancy potency of these formulations was compared to that produced by topical application of the vehicle (aqueous gel). Visual scores were measured 5 and 8 days after the first topical application.

### Follicular Biopsy

The formulation was applied *in vivo* to forearms of human volunteers. Following a 3-min massage, the excess formulation was wiped off and follicular biopsy was achieved by hair shaft removal with tweezers. The inner parts of the hair shafts, corresponding to a depth of approximately 200–400 μm, were observed by fluorescence microscopy and SEM.

## RESULTS AND DISCUSSION

The adapalene-loaded PLGA were homogeneously fluorescent and nonaggregated, with a narrow size distribution (Figs. 1A and B). By SEM, the microspheres appeared spherical, with a smooth surface, no adapalene crystal being visible on the microparticule surface.

After *in vitro* topical application of aqueous gels containing 10% adapalene-loaded 5-μm PLGA microspheres (i.e., 0.1%, w/w, adapalene final concentration) to full-thickness hairless rat or human skin for 35 and 300 min, fluorescence was observed exclusively in the hair follicles (Fig. 2). The microspheres were clearly visualized in the follicular ducts on the scanning electron micrographs (Fig. 3). The penetration depth of the microparticles into pilosebaceous structures seemed to be related to the application time: a deeper follicular penetration was observed after 300 min than after 35 min. This specific drug localization suggests that site-specific delivery of adapalene to pilosebaceous units can be achieved using 5-μm microspheres.

The 1-μm microspheres randomly distributed into the stratum corneum and hair follicles. However, the main penetration pathway of these drug-loaded microspheres was the transepidermal route since the outer surface of follicular orifice represents only 0.1% of the total skin surface. The largest microparticles (20 μm) did not penetrate the skin and remained on the stratum corneum surface. Adapalene specific delivery to pilosebaceous units using poly(lactide-co-glycolide) microspheres of defined size is illustrated in Fig. 4.

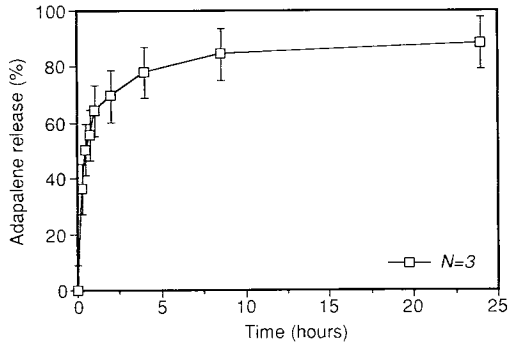


Fig. 5. *In vitro* release of adapalene from PLGA microspheres into artificial sebum.

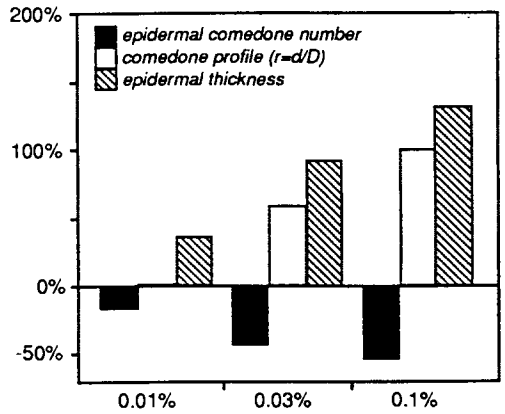


Fig. 6. Dose-related comedolytic and epidermal hyperplasic activities of topical gel containing adapalene-loaded microspheres in the rhino mouse model. Values (%) relative to control (acetone).

Although PLGA microspheres are biodegradable in an aqueous environment, they are quite stable in the follicular ducts, which contain mainly lipophilic material. Therefore, to be available for producing its pharmacological effect, the drug has to be released from the polymeric microspheres by diffusion. Besides, as the PLGA microspheres, after entering the hair follicles, are eliminated by sebum excretion, drug release from the microparticles has to be much faster than 8 days, which is the sebum excretion time in humans (13).

Adapalene release from the PLGA microspheres was therefore evaluated *in vitro* in artificial sebum. As shown in Fig. 5, adapalene release from the microspheres into sebum was rapid, with a half-life of approximately 60 min. A plateau of drug release was reached after 4 hr. Based on the *in vitro* release kinetics of adapalene from microspheres, one may assume that *in vivo* the drug will be released in the hair follicles in a much shorter period than the time corresponding to sebum excretion.

Adult rhino mice are hairless mutants which carry the *rhino* gene, a recessive allele of the hairless gene ($hr^{rh}/hr^{rh}$).

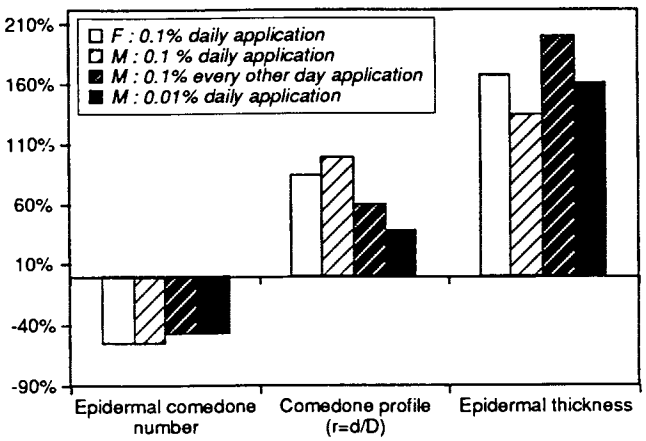


Fig. 7. Effect of topically applied aqueous gel of free adapalene (F) or adapalene-loaded microspheres (M) in the rhino mouse model. Values (%) relative to control (acetone).

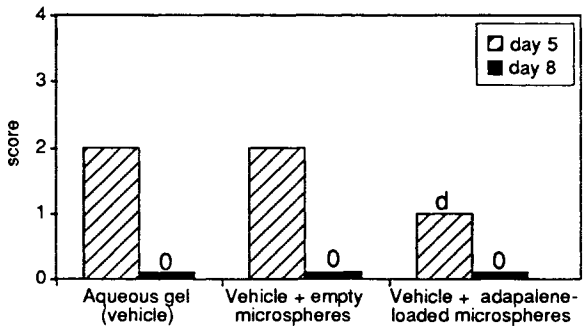


Fig. 8. Irritation studies in rabbits after 4 days of repeated topical application of aqueous gels containing either 10% of adapalene-loaded microspheres or 10% of empty microspheres. Erythema, 0 to 5 (5 as maximum); desquamation, d.

Prior to the end of their first hair growth cycle, a defect in catagen results in irreversible hair loss. Consequently, the follicular ducts widen, accumulate keratin, and undergo a transformation into horn-filled utriculi which resemble human noninflamed microcomedones. At Cird Galderma, standardized histological and image analysis methods were set up to characterize and quantify the morphological effects of retinoids in rhino mouse skin.

The comedolytic activity of an aqueous gel containing 10% adapalene-loaded microspheres [0.1, 0.5, and 1% (w/w) adapalene] corresponding to drug final concentrations of 0.01, 0.05, and 0.1% (w/w) was thus evaluated in the rhino mouse model.

A dose-related comedolytic activity of topical formulations of adapalene-loaded microspheres was observed in the rhino mouse model, the 0.05 and 0.1% adapalene formulations producing a highly significant reduction in the epidermal comedone number. At 0.01% a slight but statistically significant reduction was observed for epidermal comedones. Little effect on epidermal thickness was observed in animals treated with 0.01% adapalene (Fig. 6).

In order to increase the topical comedolytic and epidermal hyperplasic activities of the 0.01% adapalene formulation, an aqueous gel corresponding to the same drug final concentration (0.01%) was prepared with 1% PLGA microspheres containing 1% adapalene (enhanced concentration of adapalene in the microspheres). This formulation was administered topically over 3 weeks at 5 days per week, to rhino mice of both sexes. As shown in Fig. 7, adapalene-loaded microspheres present comedolytic and epidermal hyperplastic activities equivalent to those of the 0.1% free adapalene-containing aqueous gel.

The comedolytic activity of 0.1% adapalene loaded-microspheres administered topically for 3 weeks, at 5 days per week, was compared to that produced by a topical application of the same formulation for 3 weeks, at 3 days per week. As shown in Fig. 7, adapalene-loaded microspheres (0.1%) administered topically every other day for 3 weeks had an equal comedolytic activity and a greater hyperplastic activity than those observed with a daily application of the same formulation. Moreover, the activities (i.e., comedolysis and epidermal thickening) obtained with 0.1% adapalene-loaded microspheres at 3 days per week were comparable with those observed with 0.01% adapalene at 5 days per week. These data suggest that follicular targeting of ada-

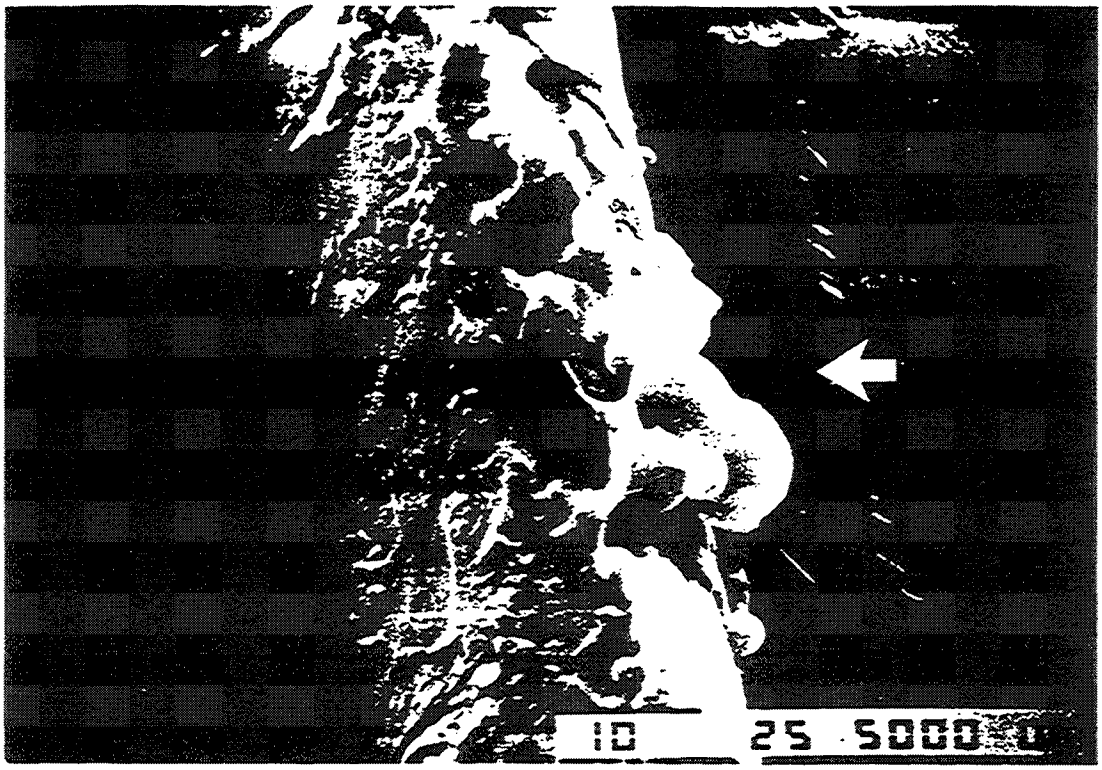

Fig. 9. *In vivo* localization of adapalene-loaded microspheres on the inner part of hair shafts after application to forearms of human volunteers (scanning electron microscopy).

palene by daily application for 3 weeks of polymeric microspheres seems to saturate the follicular ducts.

Since the aqueous gel containing 10% adapalene-loaded PLGA microspheres (5 μm) was not irritating in a rabbit skin irritancy test (Fig. 8), this formulation was applied *in vivo* to forearms of human volunteers. The observed adapalene-loaded microspheres associated with the hair shafts clearly demonstrate the possibility of targeting drugs to pilosebaceous units by using particulate carriers of defined size (Fig. 9).

## CONCLUSION

Using 5-μm polymeric microspheres, specific delivery and controlled release of adapalene into the hair follicles were evident *in vitro* and *in vivo*. With site-specific drug delivery systems such as 5-μm microspheres, a reduction of either the applied dose or the frequency of administration was shown to give pharmacological results in the rhino mouse model comparable to a daily administration of 0.1% free adapalene-containing aqueous gel. Since clinical data obtained with different formulations of retinoids, in particular adapalene, indicated a good correlation between clinical efficacy in acne vulgaris and comedolytic activity in the rhino mouse, a significant therapeutic efficacy of adapalene-loaded microspheres in acne vulgaris may be anticipated. A clinical study will have to be performed in order to compare the efficacy and safety of different formulations of adapalene-loaded microspheres to classical topical drug delivery systems. Drug follicular targeting using polymeric microspheres may represent a promising and valuable therapeutic approach for pathologies associated with pilosebaceous structures.

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