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Research paper

# Stability of polylactic acid particles and release of fluorochromes upon topical application on human skin explants

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ABSTRACT

Particle-based drug delivery systems allow the controlled and targeted release of incorporated active compounds to the skin and are promising tools to improve the efficacy of topical therapies. In this study we investigated the stability and release properties of biodegradable polylactic acid (PLA) particles upon topical application on human skin explants. PLA particles loaded with the hydrophilic fluorochrome 4-Di-2-Asp (DiAsp-PLA) were compared to PLA particles loaded with the lipophilic fluorochrome Bodipy 630/650 (BP-PLA). Changes of the particle morphology after their incubation on skin surface were investigated by means of electron microscopy while fluorescence microscopy and flow cytometry were used to evaluate particle penetration in hair follicles and fluorochrome release. We found that BP-PLA particles released rapidly the loaded fluorochrome and lost the particulate morphology within a few hours after application on skin surface. On the contrary, DiAsp-PLA particles maintained the particulate morphology, accumulated in hair follicles, and allowed a constant release of the incorporated fluorochrome for up to 16 h. These results show that, once applied to skin surface, PLA particles release the incorporated fluorochromes in a time-dependent manner and suggest the perspective to modulate particle stability and release properties by incorporating excipients with different degree of lipophilicity.



## 1. Introduction

During the last years, carrier systems have proved to be a matter of intense medical and pharmaceutical research due to the need for innovative, non-invasive, low-risk, as well as selective and more effective diagnostic tools and therapeutic approaches. Providing enhanced bioavailability and limited side effects of incorporated substances through novel administration methods and penetration pathways [1], nanoparticles are going to find broad application in both biotechnology and medicine fields [2–7]. Microspheres and nanoparticles have already been investigated for use in diagnostic and treatment of cancer, in cardiovascular medicine, pneumology, neurology, ophthalmology, immunology, and dermatology [8–15]. Based on their preferential penetration and accumulation in hair follicles (HFs) [16,17] along with the perspective to release the incorporated substances selectively to epidermis, dermis, or sebaceous glands [2,6,15], particles represent promising carrier systems for local drug delivery in dermatology. A variety of particle types and preparation methods have been introduced so far [18–28] in order to influence particle biodistribution. Multiple studies suggest that selective skin penetration and specific targeting effects can be achieved by modifying particle size, lipophilicity, surface charge, and stability [18–28].

Biodegradable particles represent a highly interesting option in the development of carrier systems for skin targeting strategies. In fact, reports on the possible translocation of nanoparticles across the skin barrier have fostered safety concerns for the use of micro- and nanoparticles in dermatological applications [29]. Since polylactic acid (PLA) is a biodegradable polymer with low toxicity, having already found medical use in the reconstructive medicine

*Abbreviations:* PLA, polylactic acid; DiAsp, 4-(4-diethylaminostyryl)-N-methylpyridinium iodide; BP, bodipy 630/650 methyl bromide; EM, electron microscopy; SEM, scanning electron microscopy; TEM, transmission electron microscopy; HFs, hair follicles; MFI, mean fluorescence intensity.

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(e.g., stents, joint replacement), PLA particles seem to be a promising carrier system for topical as well as transdermal therapy strategies.

In a model of barrier-disrupted skin, we have found that polystyrene (PS) particles (Fluospheres, Molecular Probes, 40 nm) applied on skin surface accumulated in HFs could translocate to the viable epidermis and, as a consequence, were internalized by Langerhans cells [30]. On the contrary, in a successive study we found that PLA particles were not associated with Langerhans cells after topical application on barrier-disrupted skin. Particle accumulation in HFs was followed by particle aggregation and destabilization along with the diffusion of the incorporated fluorochrome to the skin [15]. Interestingly, such destabilization only occurred after PLA particle contact with the skin surface or a lipophilic solvent but not when particles were dispersed in aqueous solvents. We therefore hypothesized that the physicochemical nature of the constituting polymer influences particle stability in the different environments. Nevertheless, those incorporated substances (e.g., fluorochromes or drugs) intercalated between the polymer strands, having affinity for hydrophobic environments and being free to diffuse out of the particles, might also influence PLA particle stability on skin surface. In our previous work, we used PLA particles loaded with high lipophilic dyes (nile red and coumarin-6) having high affinity to cell membranes and lipophilic skin areas. In this study, we investigated whether the incorporation of a moderately lipophilic fluorochrome (Bodipy 630/650, BP, $\log P = -1.109$[2]) or a hydrophilic one (4-Di-2-Asp, DiAsp, $\log P = 0.755$[2]) can improve the stability of PLA particles on skin surface. The two PLA particle formulations, DiAsp-PLA and BP-PLA, were therefore compared with regard to skin penetration and fluorochromes release kinetics. Furthermore, electron microscopy was used to detect particle distribution and morphology after topical application on human skin explants.

## 2. Material and methods

### 2.1. Preparation of PLA particles

Two PLA particle formulations were investigated. BP-PLA particles were loaded with 0.2% (w/w) 8-bromomethyl-4,4-difluoro-3,5-bis-(2-thienyl)-4-bora-3a,4a-diaza-*s*-indacene (Bodipy 630/650 methyl bromide, Invitrogen, France), and DiAsp-PLA particles were loaded with 0.2% (w/w) (4-(4-diethylaminostyryl)-N-methylpyridinium iodide (4-Di-2-Asp, Invitrogen, France). Poly (D,L-lactic acid) 50,000 g/mol was purchased from Phusis (Grenoble, France). Particles were synthesized as described in Lamalle-Bernard et al. [31]. This method allows the preparation of anionic PLA nanoparticles without addition of any surfactant. Briefly, the polymer was dissolved in acetone at a concentration of 2% w/w together with the fluorescent dyes. This solution was added to an aqueous solution under moderate stirring and the mass transfer of acetone into the continuous aqueous phase resulted in the formation of PLA nanoparticles by precipitation. The solvents were then evaporated under reduced pressure at room temperature. No residual solvent was detected by proton NMR spectra from dissolved particle samples. Nanoparticle size distribution was determined by quasi-elastic light scattering at 25 °C, at a scattering angle of 90°, using a Zeta sizer 3000HS (Malvern instruments, UK). Particle average size was 207 nm (polydispersity index 0.067) for Di-Asp-PLA particles and 164 nm (polydispersity index 0.045) for BP-PLA particles. Zeta potential of $-50 \pm 3$ mV and $-55 \pm 5$ mV was measured for Di-Asp-PLA and BP-PLA particles, respectively.

[2] Calculated with Molinspiration Property Calculator: http://www.molinspiration.com/cgi-bin/properties.

The final PLA concentration was between 60 and 70 mg/ml depending on the batch and was precisely measured by weighing the wet and dried materials. Solutions were protected from light throughout the experimental procedure. Particles were stored after synthesis at 4 °C.

### 2.2. Scanning electron microscopy (SEM)

PLA and PS (200 nm carboxylate-modified microspheres, Fluospheres®, Invitrogen, USA) particle dispersions in phosphate buffer were dried on gold electron microscopy (EM) grids coated by a ~300 nm polyvinyl formal film (Formvar, Polysciences Inc., MW 24,000–40,000 g/mol). SEM images were recorded immediately after drying and after 72 h with a Hitachi S-4000 SEM using an acceleration voltage of 20 kV. The images were analyzed with the Digital Image Processing System from Point Electronic GmbH.

### 2.3. Tissue samples and topical particle application

Human skin (retroauricular region, breast, and abdomen) was obtained within 24 h after surgical excision from healthy volunteers undergoing plastic surgery. Volunteers had signed an informed consent approved by the Institutional Ethics Committee of the Medical Faculty of the Charité-Universitätsmedizin Berlin and in accordance with the ethical rules stated in the Declaration of Helsinki Principles. Particle application on skin explants was performed according to established procedures [15]. Prior to the application of particles, cyanoacrylate skin surface stripping (CSSS) was performed once as described previously [17] using superglue (UHU GmbH, Buehl/Baden, Germany). Experiments were done on retroauricular skin samples with size of $2 \times 2$ cm = 4 cm$^2$ for the evaluation of skin penetration and on breast and abdomen skin with size of $4 \times 4$ cm = 16 cm$^2$ for the flow cytometry analyses of isolated epidermal cells. Shortly before the transcutaneous application of particles, dispersions in PBS (pH = 7.4) were prepared by dilution of the stock solutions and vigorous vortexing. The particle dispersions were applied on the surface of each skin sample (20 µl/cm$^2$) at a concentration of 0.1% w/v, which corresponded to 3.47 E+11 particles per ml in case of BP-PLA particles and 1.72 E+11 particles per ml in case of DiAsp-PLA. Particle suspensions were applied centrally on each skin sample, leaving safety margins of 0.5 cm to the border of the tissue in order to avoid sideways non-specific penetration of tested particles into the tissue. Samples were then placed in a humidified chamber and incubated at 37 °C, 5% $CO_2$, 100% humidity over 2, 4, 8, 12, and 16 h. In follicular penetration experiments, skin samples from three different donors were used for each incubation time. For epidermal cell isolation, abdominal or breast skin samples from five different donors were used.

### 2.4. Transmission electron microscopy (TEM)

After PLA particle application on human skin explants and incubation (2, 4, 8, and 16 h) in humidified chambers (37 °C, 5% $CO_2$, 100% humidity), samples of particles were re-collected from skin by placing 400 nm copper TEM grids coated by a ~15 nm carbon film (Quantifoil) on the skin and pressing them slightly against the skin using a cover slip. Transmission electron microscopy (TEM) images were recorded with a Zeiss EM 10 CR. with an acceleration voltage of 80 kV. The images were analyzed with the software Simple PCI from C-Images.

### 2.5. Adhesive tape stripping

After particles incubation (2, 4, 8, and 16 h) on excised human skin, adhesive tape stripping was performed five times on the