International Journal of Pharmaceutics 524 (2017) 290–303

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Dermal absorption behavior of fluorescent molecules in nanoparticles on human and porcine skin models

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ARTICLE INFO

Article history:
Received 13 January 2017
Received in revised form 30 March 2017
Accepted 31 March 2017
Available online 2 April 2017

Keywords:
Human
Porcine
Skin
Penetration
Nanospheres
Nanocapsules

ABSTRACT

The percutaneous passage of poorly skin absorbed molecules can be improved using nanocarriers, particularly biodegradable polymeric nanospheres (NSs) or nanocapsules (NCs). However, penetration of the encapsulated molecules may be affected by other factors than the nanocarrier properties. To gain insight information on the skin absorption of two fluorescent cargos, $DiIC_{18}(5)$ and coumarin-6 were incorporated in NSs or NCs and topically applied on various human and porcine skin samples. 3D imaging techniques suggest that NSs and NCs enhanced deep dermal penetration of both probes similarly, when applied on excised human skin irrespective of the nature of the cargo. However, when *ex vivo* pig skin was utilized, the cutaneous absorption of $DiIC_{18}(5)$ was more pronounced by means of PLGA NCs than NSs. In contrast, PLGA NSs noticeably improved the porcine skin penetration of coumarin-6, as compared to the NCs. Furthermore, the porcine skin results were reproducible when triplicated whereas from various human skin samples, as expected, the results were not sufficiently reproducible and large deviations were observed. The overall findings from this comprehensive comparison emphasize the potential of PLGA NCs or NSs to promote cutaneous bioavailability of encapsulated drugs, exhibiting different physicochemical properties but depending on the nature of the skin.

1. Introduction

Local skin diseases are conventionally treated using topical dermal formulations which mainly include creams, foams, gels and ointments, the physical characteristics of which vary widely (Convention, 2011). The external compartment of the skin, the corneal layer, is an efficient partition that limits the percutaneous transport of chemical compounds applied topically (Bouwstra et al., 2003). The 500 Da molecular weight concept postulates that the stratum corneum barrier permits the passage of small compounds, while bulky molecules cannot penetrate into the living basal layer of the epidermis and the dermis (Bos and Meinardi, 2000; Lim et al., 2007; Billich et al., 2005). This rule excludes circumstances of inflamed skin that causes a defective barrier thus preventing absorption of molecules slightly larger than 800 Da, such as tacrolimus and ascomycin topically administered to atopic dermatitis patients (Bos and Meinardi, 2000). Additionally, the hydrophobic character of the horny layer allows the absorption of moderately lipophilic rather than hydrophilic molecules, while the hydrophilic domains in the stratum corneum restrict highly lipophilic compounds (Hadgraft and Pugh, 1998). In general, percutaneous absorption refers to the passage of molecules across the skin, including first *penetration* (the entry of a compound into a particular skin layer), followed by *permeation* (the passage of a compound through one layer into another) and finally *resorption* (the uptake of a compound into the circulatory system, Bolzinger et al., 2012).

Different strategies to promote percutaneous drug transport of poorly-absorbed active ingredients through the skin protective

Abbreviations: AUC, area under curve; CLSM, confocal laser scanning microscope; Cryo-TEM, cryo-transmission electron microscopy; DHEA, dehydroepiandrosterone; MCT, mid-chain triglyceride; NC, nanocapsule; NP, nanoparticle; NS, nanosphere; PDI, polydispersity index; PLC, poly(ε-caprolactone); PLGA, poly (lactic-co-glycolic acid); PTA, phosphotungstic acid; Rhod B, rhodamine B; TEM, transmission electron microscopy; TEWL, transepidermal water loss.

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http://dx.doi.org/10.1016/j.ijpharm.2017.03.091
0378-5173/

barrier involve either the use of chemical penetration enhancers, various physical enhancement methods, or nanocarriers (Prausnitz and Langer, 2008; Lane, 2013; Trommer and Neubert, 2006). In this context, biodegradable polymeric nanoparticles (NPs), including nanospheres (NSs) and nanocapsules (NCs), are extensively investigated as a considerable means to improve cutaneous bioavailability of compounds exhibiting diverse physicochemical qualities (Zhang et al., 2013; Cosco et al., 2008). We have previously reported that the endogenous hormone dehydroepiandrosterone (DHEA) levels were significantly increased in the viable skin and the receptor compartment, when dissolved in the oil core of poly(lactic-co-glycolic acid) (PLGA) NCs, as compared to the non-particulated form using an *ex vivo* pig skin model. Moreover, these 170 nm NCs elicited *in vitro* collagen synthesis in the dermis (Badihi et al., 2014). Traditionally, the reports in the literature emphasize that the dermal absorption of nanocarriers has been dependent on the size and rigidity of the particles. Indeed, increased flexibility and reduced mean diameter size of the NPs promote enhanced skin penetration of the incorporated molecule (Alvarez-Roman et al., 2004; Zhang et al., 2010; de Brum et al., 2015). However, other reports have shown that different polymeric NPs (NSs *vs.* NCs), ranging from 170 to 320 nm, can improve the dermal absorption of the incorporated drugs, mostly *via* hair follicular delivery irrespective of the size and flexibility (Badihi et al., 2014; Glowka et al., 2014; Lademann et al., 2007; Ozcan et al., 2013; Parra et al., 2016). For example, macrolide antibiotic roxithromycin loaded-biodegradable poly(ε-caprolactone) (PCL) NSs successfully delivered the drug into hair follicles of human scalp skin specimens *via* rigid nanocarriers exhibiting a mean size of 300 nm (Glowka et al., 2014). When biodegradable PLGA NSs with a mean diameter of 320 nm were evaluated using an *ex vivo* pig skin model, it was found that the encapsulated fluorescein dye penetrated much deeper into the hair follicles than the free dye if a massage had been applied (Lademann et al., 2007). Moreover, different skin models exhibited similar results: a rat abdominal skin *ex vivo* model showed a significant deposition within the dermis and epidermis of the steroid betamethasone valerate by means of PLGA NSs (280 nm) in comparison to the commercial cream (Ozcan et al., 2013), while *ex vivo* mouse ear skin retention studies exhibited that PLGA NSs (190 nm) retained the largest amount of carprofen as compared to the solution of this non-steroidal anti-inflammatory drug (Parra et al., 2016). In contrast, coumarin-6-loaded PLGA NSs with a mean diameter of 200 nm exhibited poor penetration of the fluorescent molecules when evaluated on human abdominal skin specimens (Zhang et al., 2010). Furthermore, particularly small PEGylated PCL NSs with a mean size of 60 nm did not show permeation properties of the lipophilic agent, Zn(II)-phthalocyanine, utilizing a porcine ear skin model (Conte et al., 2015). Finally, PCL NCs with a mean size of 255 nm retained octylmethoxycinnamate in the stratum corneum of pig ear skin, limiting the dermal absorption of this sunscreen agent, even when a more flexible nanocarrier than NSs was used. It appears that despite the publication of several investigations involving biodegradable dermal application of polymeric nanocarriers the skin biofate of the loaded drugs has still not been completely elucidated. Other factors than the size and rigidity of the nanoparticulate delivery systems may considerably influence the dermal absorption of cargo molecules mediated by biodegradable NPs. On these premises, two sets of PLGA nanocarriers were prepared and evaluated, while each of these PLGA NSs or NCs were loaded with two different fluorescent probes: $DiIC_{18}(5)$ and coumarin-6. These nanocarriers were topically applied on human and porcine skin models in an attempt to gain an insight into the deposition beyond the stratum corneum barrier of the two cargo molecules as a function of the polymeric vehicle nature.

2. Materials and methods

2.1. Materials

1-Hydroxybenzotriazole (HOBT), 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT), chloroform-d ($CDCl_3$), coumarin-6, DIC *N,N′*-diisopropylcarbodiimide, polysorbate 80 (Tween® 80) and rhodamine-B were acquired from Sigma-Aldrich (Rehovot, Israel). Dimethyl sulfoxide (DMSO) was purchased from Fluka (Steinheim, Switzerland). Dry *N,N*-dimethylformamide (DMF) and triethanolamine (TEA) were obtained from Acros Organics (Geel, Belgium). Silica gel 60 column was acquired from Merck (Darmstadt, Germany). PLGA (molecular weight 4000 Da) was purchased from SurModics Pharmaceuticals (Birmingham, AL, USA). 0.2 μm fluorescent latex NSs (Fluoresbrite® YO Carboxylate Microspheres) were obtained from Polyscience, Inc (Warrington, PA, USA). DiD' oil; $DiIC_{18}(5)$ oil (1,1′-dioctadecyl-3,3,3′,3′-tetramethylindodicarbocyanine perchlorate) was purchased from Invitrogen (Carlsbad, CA, USA). Macrogol 15 hydroxystearate (Solutol HS 15) was a generous gift from BASF (Ludwigshafen, Germany). Middle chain triglyceride (MCT) was kindly provided by Societé des Oleagineux (Bougival, France). HPLC grade organic solvents were acquired from J.T. Baker (Deventer, Holland) and tissue culture media were obtained from Biological Industries Ltd (Beit Ha Emek, Israel).

2.2. Human skin tissue treatment

Human skin was obtained from patients undergoing elective cosmetic surgery (abdominoplasty), and approved of by the Hadassah University Hospital Ethics Committee (approval number 0071-09-HMO). Full-thickness human skin organ culture was performed as described previously (Badihi et al., 2014). The skin was kept at 37 °C in an atmosphere containing 5% CO_2 in serum-free DMEM containing 2 mM L-glutamine, 100 IU/mL penicillin and 100 μg/ml streptomycin.

2.3. Preparation of porcine skin specimens

Fresh pig ears were treated according to a procedure previously described (Badihi et al., 2014). In brief, about 750 μm thick, trimmed, porcine ear skin was purchased from Lahav Animal Research Institute (Kibbutz Lahav, Israel). The skin was cleaned carefully and the dermatomed skin was either treated or frozen and stored at −20 °C for up to a maximum of one month before use. Skin integrity was measured by transepidermal water loss (TEWL) using a VapoMeter device (Delfin Technologies, Finland). Skin samples with TEWL values $\leq 15\,g\,h^{-1}\,m^{-2}$ were used in the experiments (Weiss-Angeli et al., 2010).

2.4. Synthesis and characterization of the fluorescent- labeled polymer rhod B-PLGA

Fluorescent labeling of the polymer PLGA to the fluorescent probe rhodamine B (Rhod B-PLGA) was performed by covalent conjugation using carbodiimide chemistry. A detailed description of the fluorescent polymer synthesis including comprehensive characterization is provided in the Supplementary content: Appendix A.

2.5. Preparation of blank or fluorescent NPs

The well-established solvent displacement preparation method (Fessi et al., 1989) of polymeric NSs and NCs is detailed elsewhere (Badihi et al., 2014). Briefly, PLGA 4K at 50:50 blend of LA:GA was dissolved in acetone containing 0.2% w/v Tween® 80, at a