European Journal of Pharmaceutics and Biopharmaceutics 84 (2013) 355–364

Contents lists available at SciVerse ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

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

Research paper

Controlled intra- and transdermal protein delivery using a minimally invasive Erbium:YAG fractional laser ablation technology

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ARTICLE INFO

Article history:
Available online 30 November 2012

Keywords:
Transdermal drug delivery
Fractional laser ablation
Protein delivery
Erbium:YAG
Follicle stimulating hormone (FSH)
Human growth hormone

ABSTRACT

The aim of the study was (i) to investigate the feasibility of using fractional laser ablation to create micropore arrays in order to deliver proteins into and across the skin and (ii) to demonstrate how transport rates could be controlled by variation of poration and formulation conditions. Four proteins with very different structures and properties were investigated – equine heart cytochrome c (Cyt c; 12.4 kDa), recombinant human growth hormone expressed in *Escherichia coli* (hGH; 22 kDa), urinary follicle stimulating hormone (FSH; 30 kDa) and FITC-labelled bovine serum albumin (FITC-BSA; 70 kDa). The transport experiments were performed using a scanning Er:YAG diode pumped laser (P.L.E.A.S.E.®; Precise Laser Epidermal System). The distribution of FITC-BSA in the micropores following P.L.E.A.S.E.® poration was visualised by using confocal laser scanning microscopy (CLSM). Porcine skin was used for the device parameter and CLSM studies; its validity as a model was confirmed by subsequent comparison with transport of Cyt c and FITC-BSA across P.L.E.A.S.E.® porated human skin. No protein transport (deposition or permeation) was observed across intact skin; however, P.L.E.A.S.E.® poration enabled total delivery after 24 h of 48.2 ± 8.9, 8.1 ± 4.2, 0.2 ± 0.1 and 273.3 ± 30.6 μg/cm^2 for Cyt c, hGH, FSH and FITC-BSA, respectively, using 900 pores/135.9 cm^2. Calculation of permeability coefficients showed that there was no linear dependence of transport on molecular weight ((1.6 ± 0.3), (0.1 ± 0.05), (0.08 ± 0.03) and (0.9 ± 0.1) × 10^{-3} cm/h, for Cyt c, hGH, FSH and FITC-BSA, respectively); indeed, a U-shaped curve was observed. This suggested that molecular weight was not a sufficiently sensitive descriptor and that transport was more likely to be determined by the surface properties of the respective proteins since these would govern interactions with the local microenvironment. Increasing pore density (i.e. the number of micropores per unit area) had a statistically significant effect on the cumulative permeation of both Cyt c (at 100, 150, 300 and 600 pores/cm^2, permeation was 11.2 ± 2.4, 15.3 ± 11.8, 33.8 ± 10.5 and 51.2 ± 15.8 4 μg/cm^2, respectively) and FITC-BSA (at 50, 100, 150 and 300 pores/cm^2, it was 58.5 ± 15.3, 132.6 ± 40.0, 192.7 ± 24.4, 293.3 ± 76.5 μg/cm^2, respectively). Linear relationships were established in both cases. However, only the delivery of FITC-BSA was improved upon increasing fluence (53.3 ± 22.5, 293.3 ± 76.5, 329.6 ± 11.5 and 222.1 ± 29.4 μg/cm^2 at 22.65, 45.3, 90.6 and 135.9 J/cm^2, respectively). The impact of fluence – and hence pore depth – on transport will depend on the relative diffusivities of the protein in the micropore and in the 'bulk' epidermis/dermis. Experiments with Cyt c and FSH confirmed that delivery was dependent upon concentration, and it was shown that therapeutic delivery of the latter was feasible. Cumulative permeation of Cyt c and FITC-BSA was also shown to be statistically equivalent across porcine and human skin. In conclusion, it was demonstrated that laser microporation enabled protein delivery into and across the skin and that this could be modulated via the poration parameters and was also dependent upon the concentration gradient in the pore. However, the role of protein physicochemical properties and their influence on transport rates remains to be elucidated and will be explored in future studies.

1. Introduction

Pharmacological potency, biological specificity and a concomitant decrease in the risk of side-effects make peptides and proteins extremely interesting leads for the development of new therapeutics [1]. Their physicochemical properties and stability requirements mean that they are almost always administered

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http://dx.doi.org/10.1016/j.ejpb.2012.11.018

parenterally – most frequently by subcutaneous or intramuscular injection [2]. Although tolerable, this is non-ideal and new approaches to the controlled intra-epidermal, intra-dermal or transdermal delivery of these agents may open the door to alternative, less invasive strategies for effective targeted local delivery or systemic administration of 'biotech' therapeutics.

However, the stratum corneum – the uppermost layer of the epidermis – represents a formidable barrier against the transport of even small hydrophilic molecules into the body [3]. Hence, it is unrealistic to envisage that passive diffusion of peptides and proteins across intact and healthy skin will be sufficient to enable therapeutic amounts to be delivered in reasonable timeframes. As a result, several 'minimally invasive' techniques have been developed to reversibly compromise skin barrier function and so facilitate molecular transport while provoking minimal irritation at the application site [4,5]. These methods involve the creation of transport channels in the skin either by mechanical perforation (e.g. via the use of microneedles) or by the application of physical energy (e.g. radiofrequency) [6–8].

The P.L.E.A.S.E.® (Precise Laser Epidermal System) device contains a scanning Er:YAG laser that emits short duration pulses of radiation at 2.94 μm, a principal excitation wavelength for water molecules [9–17]. Application of laser energy to the skin results in their excitation and subsequent explosive evaporation from the epidermis, which in turn leads to the formation of micropores. Since the duration of the energy pulse is shorter than the thermal relaxation time of water, there is negligible heat transfer – and hence thermal damage – to the surrounding tissues. In contrast to laser devices that have frequently been used in earlier studies to enhance drug delivery with large beam spot diameters (3–7 mm) and which removed appreciable areas of contiguous skin [18–21]; the P.L.E.A.S.E.® device is a scanning fractional laser ablation system with a much narrower beam that creates smaller pores (typical diameter 150–200 μm) [9,10]. The major advantage of this 'fractional ablation' of the skin is that the micropores are surrounded by healthy tissue which facilitates skin recovery; typically, only 5–15% of the skin surface is removed [22–24]. A significant advantage of the P.L.E.A.S.E.® technology is the control afforded over delivery kinetics by modulation of the number of micropores created per cm^2 (i.e. pore density) and their depth. The latter is controlled by varying the amount of laser energy applied per unit area (or fluence; J/cm^2) to create each micropore [10,12–15]. Furthermore, since it is a scanning system, the full energy of the laser beam is applied to create each micropore in the array instead of the need for a fractionation of the beam [25]. Previous studies with the P.L.E.A.S.E.® system have demonstrated the controlled delivery of lidocaine, diclofenac and prednisone across porcine and human skin [10,12,14]. Preliminary studies have also shown the feasibility of using this technique to deliver a structurally intact peptide (exenatide [13]) and a protein (cytochrome c [11]). More recently, the first report into the use of fractional laser ablation for the 'needle-less' controlled delivery of structurally intact and biologically active therapeutic antibodies into and across the skin has also been published [15]. However, to-date, there is no information concerning the effect of permeant physicochemical properties – of low and (obviously) high molecular weight species – on transport through laser porated skin. The aim of this study was to investigate the effect of P.L.E.A.S.E.® poration and formulation parameters on the delivery kinetics of four proteins of different sizes and molecular properties through laser treated skin: namely, equine heart cytochrome c (Cyt c; 12.4 kDa) [26], recombinant human growth hormone expressed in *Escherichia coli* (rhGH; 22 kDa) [27], urinary follicle stimulating hormone – a post-translationally modified heterodimer, where two Asn residues are glycosylated in both the alpha and beta chains (FSH; 30 kDa) [28] and FITC-labelled bovine serum albumin (FITC-BSA; 70 kDa) [29] (Fig. 1). There is an approximately 6-fold difference in molecular weight, and the proteins have different secondary structure elements and as mentioned above, FSH is also glycosylated; these are all factors which may influence transport or affect the types of interactions with the transport pathway.

The specific objectives of the present investigation were (i) to demonstrate feasibility of protein delivery and to identify any common trends and (ii) to investigate the effect of micropore density (i.e. the number of micropores per cm^2 and hence the fractional ablated area) and of fluence (and hence pore depth) on protein transport through the skin and how these parameters could be used to control delivery, and (iii) to study the influence of protein concentration on transport. In addition, the presence of the FITC label conjugated to BSA meant that it could be visualised in the skin by confocal laser scanning microscopy.

2. Materials and methods

2.1. Chemicals

Equine heart cytochrome c, FITC and FITC-BSA were purchased from Fluka (Buchs, Switzerland). Follicle stimulating hormone

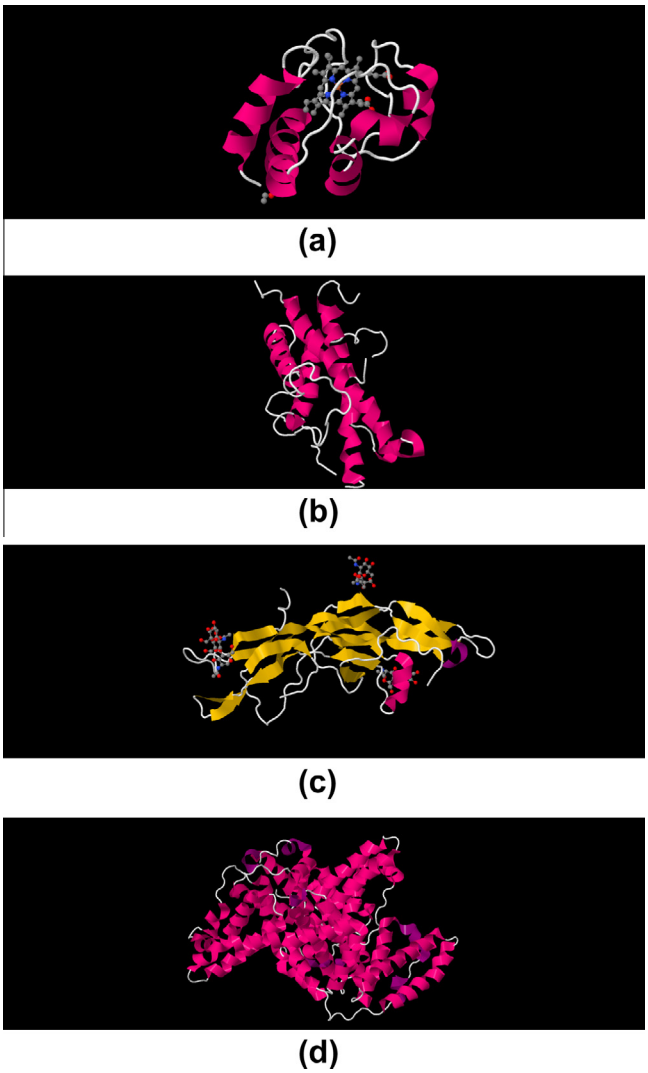

Fig. 1. Three dimensional representations of (a) Cyt c (using 1HRC.pdb), (b) hGH (using 1HGU.pdb), (c) FSH (using 1FL7.pdb) and (d) BSA (using (3VO3.pdb) illustrating the different secondary structure elements. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)