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Research Paper

Diffusion profile of macromolecules within and between human skin layers for (trans)dermal drug delivery

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ABSTRACT

Delivering a drug into and through the skin is of interest as the skin can act as an alternative drug administration route for oral delivery. The development of new delivery methods, such as microneedles, makes it possible to not only deliver small molecules into the skin, which are able to pass the outer layer of the skin in therapeutic amounts, but also macromolecules. To provide insight into the administration of these molecules into the skin, the aim of this study was to assess the transport of macromolecules within and between its various layers. The diffusion coefficients in the epidermis and several locations in the papillary and reticular dermis were determined for fluorescein dextran of 40 and 500 kDa using a combination of fluorescent recovery after photobleaching experiments and finite element analysis. The diffusion coefficient was significantly higher for 40 kDa than 500 kDa dextran, with median values of 23 and 9 $\mu m^2/s$ in the dermis, respectively. The values only marginally varied within and between papillary and reticular dermis. For the 40 kDa dextran, the diffusion coefficient in the epidermis was twice as low as in the dermis layers. The adopted method may be used for other macromolecules, which are of interest for dermal and transdermal drug delivery. The knowledge about diffusion in the skin is useful to optimize (trans)dermal drug delivery systems to target specific layers or cells in the human skin.

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1. Introduction

(Trans)dermal administration is of interest for the delivery of drugs that cannot be administered orally, due to e.g. a strong first-pass effect, or have the skin as the target. The administration of vaccines is an example for which the dermal route may offer several advantages. By delivering a vaccine into the skin, the required dose to achieve a sufficient high immune response may be reduced when compared to conventional methods (Chen et al., 2011, 2010; Fernando et al., 2010; Gelinck et al., 2009; Quan et al., 2010). Multiple techniques to deliver antigens or other macromolecular drugs into the skin are currently in development, such as microneedles or microjet systems (Arora et al., 2008; Van der Maaden et al., 2012). These techniques are necessary to overcome the skin barrier provided by the stratum corneum, the upper layer of the epidermis. However, to target specific layers of the skin, it is important to obtain insight in the transport of these molecules in the skin layers. This knowledge can be used to improve the design of delivery systems and to optimize delivery strategies.

The delivery and transport of fluorescent molecules in the skin have been previously visualized (Bal et al., 2010; Chen et al., 2012; Grams et al., 2004; Prow et al., 2010; Raphael et al., 2010), although the diffusion coefficient was not quantitatively determined. This parameter is essential to describe the passive transport of a molecule in a material and is dependent on both the properties of the diffusing molecule and the material through which it diffuses. In some previous studies, the diffusion coefficient of various model compounds in skin of different species was determined (Anissimov et al., 2012; Cornelissen et al., 2008; Hanh et al., 2001; Liu et al., 2013; Raphael et al., 2013; Wonglertnirant et al., 2010; Xing et al., 2009). Most of these studies report a single value of the diffusion coefficient for a single skin layer. For example, Cornelissen et al. (2008) measured the diffusion in human epidermis and Anissimov et al. (2012) in the stratum corneum. However, the diffusion coefficient may not only vary between skin layers, but also within the layers of the skin. This was shown in porcine dermis for a small molecule which has the property to extend the imaging depth using micro-Raman spectroscopy (Liu et al., 2013), and for a macromolecule in murine skin layers using confocal microscopy in combination with computational analyses (Raphael et al., 2013). However, for macromolecules and species with a thicker skin, like humans, these methods are not feasible due to the limitations in the imaging depth. To facilitate the development of (trans)dermal delivery systems, information on the specific diffusion properties of the human skin layers is essential. Indeed previous studies have reported differences in skin properties between species, including permeability and structure (Pasparakis et al., 2014; Scott et al., 1991), suggesting that diffusion coefficients in the various skin layers may be significantly different from those measured in murine skin. To our knowledge, the variation in diffusion coefficient within and between human skin layers is currently unknown.

A possible method to determine the diffusion coefficient within the human skin is fluorescent recovery after photobleaching (FRAP, also referred to by video-FRAP or scanning microphotolysis) (Deschout et al., 2014). With FRAP, the entire tissue is made fluorescent. Subsequently, a region in the tissue is bleached using a high laser power. After bleaching, the bleached and unbleached fluorescent molecules will diffuse through the tissue. By recording the fluorescent intensity with a confocal microscope, the diffusion of the molecules can be determined by either analytical analysis (Braeckmans et al., 2003; Hauser et al., 2008; Soumpasis, 1983), Fourier analysis (Travascio et al., 2009; Tsay and Jacobson, 1991), or finite element analysis (Irrechukwu and Levenston, 2009; Sniekers and van Donkelaar, 2005). Numerical analyses have been previously presented that could account for both inhomogeneity of the tissue (Sniekers and van Donkelaar, 2005) and anisotropic diffusion (Travascio et al., 2009; Tsay and Jacobson, 1991). These methods can be adapted for human skin.

To improve targeted drug delivery, the aim of present study was to determine the diffusion coefficient of two model compounds in human skin throughout its different layers. This was achieved using FRAP experiments in combination with finite element analysis. The diffusion of fluorescent dextran molecules with two different molecular weights was examined.

2. Methods

2.1. *Preparation of human skin samples*

Human skin with subcutaneous fat of 3 female patients, aged between 40 and 58 years, who had undergone abdominoplasty, was obtained from the Catharina Hospital, Eindhoven, The Netherlands, according to Dutch guidelines of secondary used materials. Within 4 h post-surgery, the skin was transported to the host laboratory and processed. The surface of the skin was cleaned with ethanol and samples with an approximate surface area of 8 mm by 8 mm and a thickness of approximately 4 mm, including the epidermis, dermis and part of the subcutaneous fat, were prepared using a scalpel, surgical scissors and a punch. These samples were cut perpendicular to the skin surface using a cutting device designed and described by Grams et al. (2004), creating a flat cutting plane. After removal from the cutting device, the samples were immerged in 0.8 mg/ml 40 kDa or 0.8–1.2 mg/ml 500 kDa fluorescein dextran (Molecular Probes) in Hank's balanced salt solution with 20 μg/ml Hoechst 33342 (Molecular Probes) and stored at 4 °C for at least 20 h.

Before the FRAP experiments, samples were allowed to equilibrate to room temperature for at least 30 min. Each sample was patted dry with gauze and placed on a glass cover slip with the cutting plane facing downwards. A border of polydimethylsiloxaan (PDMS) of 5 mm in height was placed on the cover slip around the sample and covered with a moistened gauze to prevent dehydration of the skin.

2.2. *FRAP protocol*

FRAP experiments were performed at 8 different depths measured from the surface of the skin. The depths