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Cutaneous gene expression of plasmid DNA in excised human skin following delivery via microchannels created by radio frequency ablation

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Abstract

The skin is a valuable organ for the development and exploitation of gene medicines. Delivering genes to skin is restricted however by the physico-chemical properties of DNA and the stratum corneum (SC) barrier. In this study, we demonstrate the utility of an innovative technology that creates transient microconduits in human skin, allowing DNA delivery and resultant gene expression within the epidermis and dermis layers. The radio frequency (RF)-generated microchannels were of sufficient morphology and depth to permit the epidermal delivery of 100 nm diameter nanoparticles. Model fluorescent nanoparticles were used to confirm the capacity of the channels for augmenting diffusion of macromolecules through the SC. An ex vivo human organ culture model was used to establish the gene expression efficiency of a β-galactosidase reporter plasmid DNA applied to ViaDerm™ treated skin. Skin treated with ViaDerm™ using 50 μm electrode arrays promoted intense levels of gene expression in the viable epidermis. The intensity and extent of gene expression was superior when ViaDerm™ was used following a prior surface application of the DNA formulation. In conclusion, the RF-microchannel generator (ViaDerm™) creates microchannels amenable for delivery of nanoparticles and gene therapy vectors to the viable region of skin.

Keywords: Radiofrequency-microchannels; Radiofrequency ablation; Plasmid DNA; Skin; Gene therapy

1. Introduction

The ability to target genes directly to the skin provides a strategy for the treatment of certain localised heritable genetic skin diseases (Greenhalgh et al., 1994; Ehrlich et al., 1995), various forms of malignancies (Hart and Vile, 1994) and cutaneous wounds (Byrnes et al., 2004; Lee et al., 2004). Furthermore, 'genetic immunisation' via the skin provides a method of vaccinating patients by introducing DNA into cells, leading to expression of foreign antigen and the subsequent induction of an immune response (Fynan et al., 1993; Raz et al., 1994; Shi et al., 1999). Intra-cutaneous DNA vaccines utilise the highly competent antigen-presenting capabilities of epidermal Langerhans cells in eliciting a systemic immune response, leading to more proficient and cost-efficient vaccination compared with conventional vaccines (Lin et al., 2000). As the immune response is induced by a single gene rather than an entire organism, this approach is also considered to be safer than using live attenuated vaccines (Durrant, 1997).

The challenge of delivering genes to the viable region of skin is a product of the physico-chemical properties of the large hydrophilic DNA molecule, with or without an additional carrier vehicle, and the significant barrier properties of cutaneous tissue. Superficially the skin is regarded as a valuable organ for the development and clinical administration of gene medicines as it is readily accessed, well characterized and easily monitored (Hengge et al., 1996). However, if cutaneous gene therapy is to translate from the laboratory to clinical practice then approaches

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must be developed to efficiently and reproducibly transport the delivered transgene to the target cell population. The primary role of the skin however, is to serve as a physical barrier to the invasion of foreign material. In humans, the epidermis, which constitutes the uppermost layer of the skin, is approximately 50–150 μm thick with the non-viable SC layer, approximately 15–20 μm in thickness, representing the principal barrier to penetration and permeation of substances through the skin (Birchall, 2004). Therefore, in order to deliver therapeutic compounds to the epidermis, the underlying dermis or the systemic circulation, delivery strategies must overcome the physical barrier created by the nature of the tightly packed dead cells of the SC. Traditional transdermal formulation strategies aim to enhance the delivery of small therapeutic molecules, less than 500 molecular weight, across the SC by paracellular, transcellular or intracellular routes. However, in order to deliver DNA and proteins, more innovative and radical methods of drug delivery are required. To date, the physico-chemical methods employed to promote therapeutic drug or gene transfer to the skin include the use of direct DNA injection (Hengge et al., 1995, 1996; Chesnoy and Huang, 2002) chemical enhancers (Barry, 1987; Pillai and Panchagnula, 2003), iontophoresis (Green, 1996; Préat and Dujardin, 2001), biolistic particle bombardment (Cheng et al., 1993; Heiser, 1994; Udvardi et al., 1999), electroporation (Prausnitz et al., 1993; Dujardin et al., 2001; Zhang et al., 2002), sonophoresis (Lavon and Kost, 2004), laser ablation (Nelson et al., 1991), microseeding (Eriksson et al., 1998), skin tattooing (Bins et al., 2005) and the recent use of microfabricated microneedles (Henry et al., 1998; McAllister et al., 2000, 2003; Chabri et al., 2004).

Recently, we have developed an innovative technology, coined ViaDerm™, which creates transient microchannels across the SC thereby enabling a more direct and controlled passage of molecules to the underlying viable epidermis and dermis. ViaDerm™ has an intimately spaced array of microelectrodes which are placed against the surface of skin to individually conduct an applied alternating electrical current at radio frequency (RF). Application of this rf electrical current (100–500 kHz) to the tissue elicits a vibration in motion of ions with localized frictional heating of tissue resulting in a rapid obliteration of cells close to the energy source. The intimate and orderly spacing of the microelectrodes therefore drives the orderly generation of functional microchannels. The passage of the electric current through cells in the upper skin strata generates localised ionic vibrations, heating, evaporation and cell ablation to create microchannels.

Previously, we have reported that RF-generated microchannels reside in the epidermis and dermis and are amenable to the effective transdermal delivery of small molecules (Sintov et al., 2003) and proteins (Levin et al., 2005) into the systemic circulation. Furthermore, the microchannels did not impinge on underlying blood vessels and nerve endings thus minimizing skin trauma, bleeding and neural sensations (Sintov et al., 2003). Clearly, the use of electricity for augmenting transcutaneous drug delivery also applies to some of the other aforementioned physical delivery methods, e.g. iontophoresis, electroporation. Unlike these examples however, the technology described in this study leads to the creation of an orderly array of defined microchannels by cell ablation at specific locations (Levin et al., 2005).

The purpose of the present study using the ViaDerm™ technology was two-fold. Firstly, to extensively characterize ViaDerm™-generated microchannels within ex vivo human skin. Secondly, to assess the feasibility of ViaDerm™ in supporting the transdermal delivery of a mammalian expression plasmid with subsequent reporter expression within the target region of the skin.

2. Materials and methods

2.1. Materials

The 7.2 kb pCMVβ plasmid construct containing the β-galactosidase reporter gene and the pEGFP-N1 (4.7 kb) plasmid containing the green fluorescent protein reporter gene were propagated and purified as detailed previously (Birchall et al., 1999). Fluorescein isothiocyante (FITC)-labelled polystyrene nanospheres (L-1280) were obtained from Sigma Chemicals (Poole, UK). OCT embedding medium and Histobond® microscope slides were from RA Lamb Ltd. (Eastbourne, UK). One percent aqueous eosin solution and Harris' haematoxylin solution were from BDH Laboratory Supplies (Dorset, UK). One percent aqueous toludine blue solution was from TAAB Laboratories Equipment Ltd. (Berkshire, UK). Cell culture plastics were obtained from Corning-Costar (High Wycombe, UK). MEM (EAGLES) 25 mM HEPES, Dulbecco's Modified Eagle's Medium (DMEM 25 mM HEPES), foetal bovine serum, penicillin-streptomycin solution and trypsin-EDTA solution 1× were obtained from In-Vitrogen Corporation, Paisley, UK. All other reagents were of analytical grade and purchased from Fisher Scientific UK (Loughborough, UK).

2.2. ViaDerm™ treatment of human skin

Full-thickness human breast skin was obtained from mastectomy or breast reduction with ethical committee approval and informed patient consent. Skin was collected from a variety of donors ranging from 45 to 65 years of age. Matched samples were used for each individual experiment. To maintain structural and cellular viability the skin tissue was transported on ice in MEM (EAGLES) 25 mM HEPES growth media and used within 3 h of excision. All excess adipose tissue was removed by blunt dissection.

The components and operating conditions of the RF-microchannel generator (ViaDerm™, TransPharma Medical, Israel) have been described previously (Sintov et al., 2003). Briefly the ViaDerm™ device comprises an electronic controller unit and a disposable array of stainless steel electrodes (100 or 50 μm in length) at a density of 100 electrodes/cm^2 in a total area of 1.4 cm^2. Thus, application of an RF-activated array (1.2 cm × 1.2 cm) resulted in the generation of 144 microchannels over the 1.4 cm^2 area. Studies were performed using the electrodes at device parameter settings resulting in one, two or five bursts of 700 μs burst length at an applied voltage of 290 or 330 V and an RF frequency of 100 kHz. Control experiments

involved equivalent pressure application of the ViaDerm™ device to human skin in the absence of the RF-generating power source.

2.3. Electron microscopy of full thickness skin

ViaDerm™ treated (100 μm electrode, density of 200 microchannels/cm^2) full thickness human skin samples were fixed with 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4) for 60 min at room temperature and washed for 10 min (2 × 5 min) in the same buffer. The samples were post-fixed in 1% osmium tetroxide in 0.1 M cacodylate buffer for 1 h at 4 °C and then dehydrated with a graded series of ethanol concentrations as follows (70% for 10 min at 4 °C, 100% for 10 min at 4 °C, 100% for 10 min at 4 °C, 100% for 10 min at 4 °C). The samples were subsequently transferred to a critical point drier (Samdri 780, Maryland, USA) for 12 h. The samples were mounted on metal stubs and gold sputter coated, using an Edward sputter coater, prior to examination in a Philips XL-20 scanning electron microscope.

2.4. Electron microscopy of epidermal sheets

Following ViaDerm™ treatment (100 μm electrode) of full thickness human skin, epidermal sheets were isolated by a heat separation technique (Christophers and Kligman, 1963). The resulting epidermal sheets were placed in cold distilled water and then gently lifted from the water onto a metal stub. The mounted epidermal sheet was allowed to dry, gold sputter coated and the samples were examined using a scanning electron microscope (Philips Xl-200 SEM) (Electron Microscopy Unit, Cardiff School of Biosciences, Cardiff University, Cardiff, UK).

2.5. Visualisation of microchannels en face

ViaDerm™ treated (100 μm electrode) skin was incubated in media (MEM (EAGLES), 25 mM HEPES) for 24 h at 37 °C. Following two washes in phosphate buffered saline (PBS) the skin was fixed in 0.5% gluataraldehyde for 2 h on ice. Methylene blue staining involved a 5 min surface application of five drops of methylene blue solution on the ViaDerm™ treated skin followed by removal of excessive stain with a brief PBS rinse and an ethanol surface swab. Tissue stained with methylene blue was visualised using an Olympus BX50 microscope and a Schott KL1500 electronic light source.

2.6. Histology of ViaDerm™ treated tissue

Skin was treated with ViaDerm™ using either 50 or 100 μm electrode arrays. Following treatment the skin was washed with PBS and fixed for 4 h in 0.5% glutaraldehyde on ice. Fixed tissue was embedded in OCT and sectioned using a Leica CM3050S Cryostat. Sections were collected on Histobond® microscope slides and stained with either—(i) eosin: 1% aqueous eosin solution for 5 s, (ii) haematoxylin and eosin (H&E): Harris' haematoxylin solution for 5 min followed by 1% aqueous eosin solution for 5 s or (iii) toludine blue: 1% aqueous toludine blue solution for 5 min.

2.7. Diffusion of fluorescent nanoparticles through RF-microchannels™

Non-treated and ViaDerm™ treated (50 and 100 μm electrodes) full thickness human skin was heat separated in order to isolate the epidermal membranes which were subsequently mounted between the donor and receptor compartments of static Franz-type glass diffusion cells. The receptor phase of each cell was filled with phosphate buffered saline (PBS; pH 7.4). The receptor arm was sealed with a foil cap and the donor chamber occluded with NESCO® film to prevent sample evaporation. The cells were placed on a stirring plate in a water-bath maintained at 37 °C, to provide continuous agitation and a skin surface temperature of 32 °C. Prior to addition of the test formulations to the donor chamber, cells were allowed to equilibrate for at least 30 min and the integrity of epidermal membranes was visually inspected.

Fluorescently (FITC) labelled polystyrene nanospheres (100 nm diameter) were used as a size-representative model for the delivery of non-viral gene therapy vectors (Chabri et al., 2004). A volume of 500 μl of a 50 μl/ml dilution of the fluorescent nanosphere stock suspension, concentration 4.57^{10} μl^{-1}, was applied to the surface of ViaDerm™ treated epidermal membranes. Control cells consisted of untreated epidermal membrane with either the nanosphere suspension or PBS applied to the donor phase. At each timepoint 200 μl samples were removed from the receptor arm at regular intervals and replaced with PBS. On completion of the experiment, samples were analysed using a fluorescence spectrophotometer (BMG Fluostar, Aylesbury, UK) with excitation and emission wavelengths set at 485 and 520 nm, respectively. A calibration curve was performed using standard dilutions of the suspension of fluorescent nanoparticles.

2.8. Localised delivery of fluorescent nanoparticles in ViaDerm™ treated human skin

ViaDerm™ treated (100 μm electrode) skin was placed in a six-well cell culture plate and maintained in 1.5 ml MEM (EAGLES) 25 mM HEPES. Fifty microliters of a concentrated (4.57^{10} μl^{-1}) stock of fluorescent red polystyrene nanospheres was applied to the treated skin surface and the sample incubated for 6 h at 37 °C. At 6 h a further 2 ml of media was added the submerged skin was incubated for a further 18 h. Following two washes in PBS the skin was fixed in 0.5% gluataraldehyde for 1 h on ice and embedded in OCT medium prior to tissue sectioning using a Leica CM3050S Cryostat. Sections were either visualised unstained under blue fluorescence or stained with haematoxylin and eosin (H&E) (Olympus BX50 microscope).

2.9. Gene expression in ViaDerm™ treated human skin

Human skin was pre-treated with the ViaDerm™ device, 50 μm electrode arrays, prior to the topical application of 50 μl

of pCMVβ plasmid DNA solution (1 mg/ml) to the skin surface. This area of skin was thereafter post-treated with the ViaDerm™ device at the identical skin location as the first ViaDerm™ application. The treated human skin was placed on lens tissue supported by metal gauze in a six-well cell culture plate containing 7.5 ml media (DMEM 25 mM HEPES supplemented with 5% foetal bovine serum and 1% penicillin/streptomycin) per well. This organ culture maintained the skin at an air–liquid interface for 24 h at 37 °C. Following one wash in PBS/$MgCl_2$ (30 min) the tissue was fixed for 2 h in 2% glutaraldehyde/$MgCl_2$ at 4 °C. Subsequently the tissue was rinsed in a series of PBS/$MgCl_2$ solutions for 2, 3 h and 30 min. The tissue was stained for β-galactosidase expression over 20 h using X-Gal staining solution [X-Gal (5% (v/v) of a 40 mg/ml solution in dimethylformamide), potassium ferricyanide (0.84% (v/v) of a 0.6 M solution), potassium ferrocyanide (0.84% (v/v) of a 0.6 M solution), magnesium chloride (0.2% (v/v) of a 1 M solution), Tris–HCl buffer pH 8.5 (50% (v/v) of a 0.2 M solution), deionised water to 100%]. Tissue was visualised en face using either a Zeiss Stemi 2000C Stereomicroscope with a 2.0× attachment or an Olympus BX50 microscope, both with a Schott KL1500 electronic light source.

For sectioning, the samples were embedded in OCT and sectioned using a Leica CM3050S Cryostat. Tissue sections were collected onto Histobond® microscope slides and stained with H&E.

3. Results and discussion

The surface morphology of the microchannels created in full-thickness breast skin following application of ViaDerm™ was initially investigated using scanning electron microscopy (SEM). Fig. 1 shows a channel created using the 100 μm electrode appearing as a deep invagination into the surface of the skin tissue. Further SEM characterisation of the heat-separated epidermal membrane, comprising of SC and viable epidermis, treated with ViaDerm™ is shown in Fig. 2. These data show that the RF-microchannels either totally or partially penetrate the epidermal membrane. Although the depth of the microchannels was variable, possibly due to variation in thickness of the separated epidermal sheet (Eriksson et al., 1998), the diameter of the microchannels (∼50 μm), was reproducible and consistent

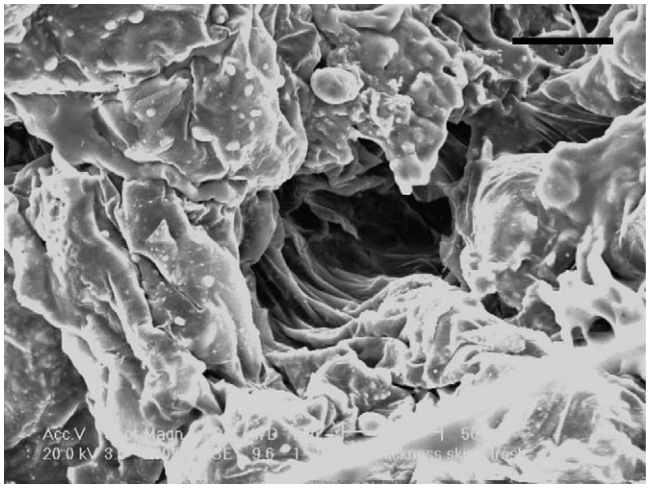

Fig. 1. Scanning electron microscopy of an RF-microchannel in intact human skin. Bar = 50 μm.

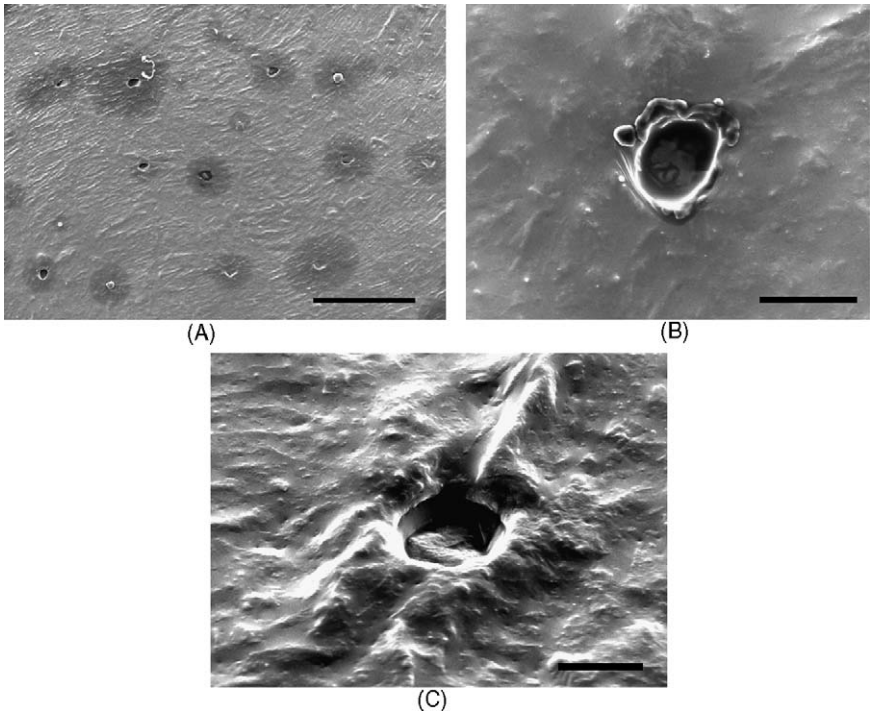

Fig. 2. Scanning electron microscopy of RF-microchannels in heat-separated epidermal membrane. (A) Low magnification showing distribution pattern of channels following two applications of ViaDerm™, bar = 1 mm; (B) high magnification showing dimensions of microchannels, bar = 100 μm; (C) visualisation of microchannel depth using an angled electron beam, bar = 50 μm.

with the microchannel dimensions observed in full-thickness skin (Fig. 1). More accurate determinations of the depth and structural morphology of the microchannels are provided in the histological tissue sections.

The quantity and distribution pattern of microchannels created in ViaDerm™ treated skin is shown in Fig. 3. The distribution pattern of the channels can be visualised through their ability to uptake and retain a low molecular weight marker, i.e. methylene blue (Fig. 3A). At higher magnification the dye appears to diffuse to the periphery of the microchannel (Fig. 3B). The application and considerable potential of this technology for the cutaneous delivery of low molecular weight medicaments has previously been reported (Sintov et al., 2003).

The structural dimension of microchannels created in human breast skin following application of ViaDerm™ was assessed using transverse sectioning. The photomicrographs are representative of the entire population of channels observed. Fig. 4 illustrates the dimensions of RF-microchannels that are created in human breast skin following application of ViaDerm™ with 50 μm electrode arrays at different parameter settings. In the majority of processed skin sections ($n > 100$), the channels are approximately 50 μm in length and 30–50 μm at their widest aperture, extending only to the viable epidermis.

In line with the data depicted in Fig. 4, doubling the electrode length to 100 μm resulted in further penetration through the human epidermis and impingement into the superficial dermal layer (Fig. 5). Representative sections ($n > 100$) show that microchannels were approximately 100 μm in length and 30–50 μm at their widest aperture. Consequently, using isolated human breast skin, the 100 μm electrode arrays can create a microchannel of sufficient length to permit specific cell targeting for localised cutaneous gene therapy applications (Greenhalgh et al., 1994; Sawamura et al., 2002) and genetic vaccination (Dean et al., 2003). Clearly, the exploitation of different electrode lengths for creating microchannels of varying depths underscores the flexibility of ViaDerm™ for permitting controlled delivery of therapeutics to different target cell populations.

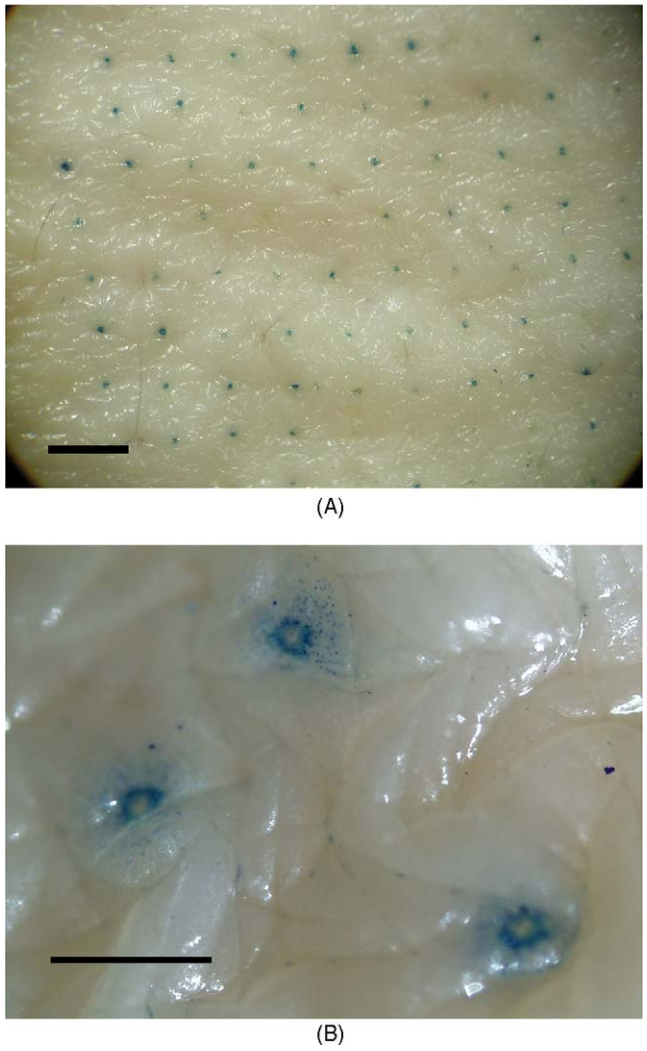

Fig. 3. Light microscopy of methylene blue stained skin following ViaDerm™ treatment. (A) Low magnification, bar = 1 mm; (B) high magnification, original magnification = 40×, bar = 500 μm.

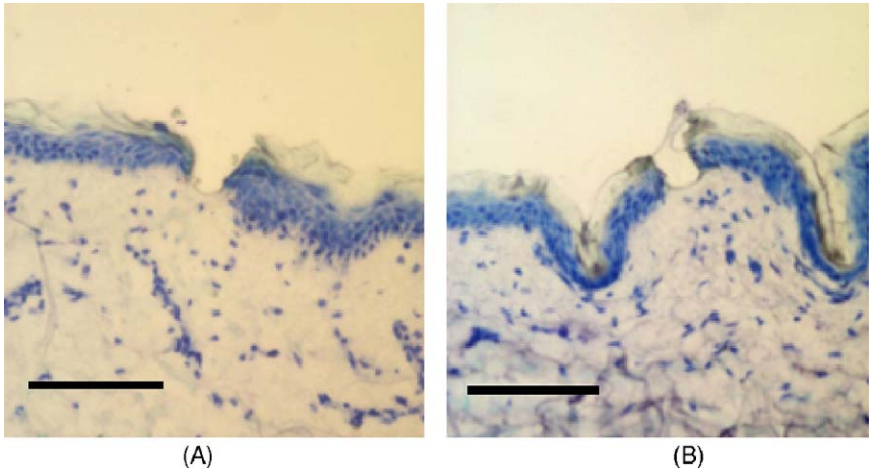

Fig. 4. Light microscopy of human breast skin treated with ViaDerm™ 50 μm electrode arrays. (A) One burst of 700 μs burst length, toludine blue stained; (B) two bursts of 700 μs burst length, toludine blue stained. Original magnification = 200×, bar = 100 μm.

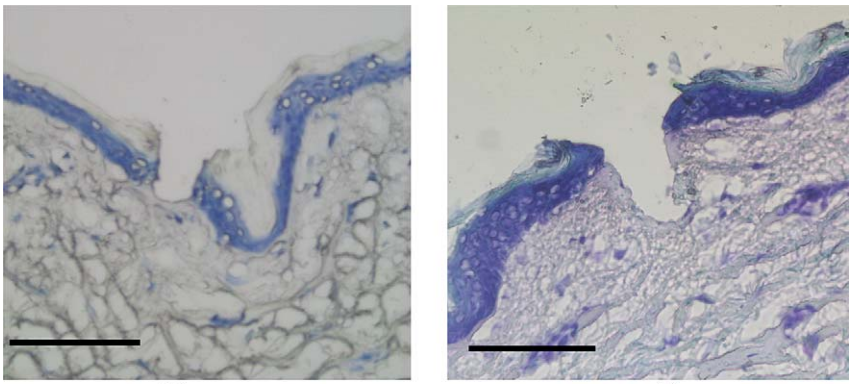

Fig. 5. Light microscopy of human breast skin treated with ViaDerm™ 100 μm electrode arrays. (A) One burst of 700 μs burst length, toludine blue stained; (B) three bursts of 700 μs burst length, toludine blue stained. Original magnification = 200×, bar = 100 μm.

Previously, from ex vivo studies employing a permeation methodology, we have demonstrated the total inability of the ViaDerm™ device to generate microchannels when disconnected from a power source as evidenced by both negative visualization and lack of drug permeation (Sintov et al., 2003). Such findings were totally substantiated in follow up in vivo studies whereby application of drugs at a ViaDerm™ treated skin site in the absence of a power supply resulted in no transdermal drug delivery as compared to robust drug deliveries with a functional power supply (Sintov et al., 2003; Levin et al., 2005). In our histological studies, and subsequent gene delivery experiments, we confirm the previously published ex vivo and in vivo observations (Sintov et al., 2003; Levin et al., 2005) of the total absence of microchannels on the surface skin following the placement of the ViaDerm™ device disconnected from a functional power source.

Following confirmation of the ability of ViaDerm™ to create microchannels in human skin, further experiments were

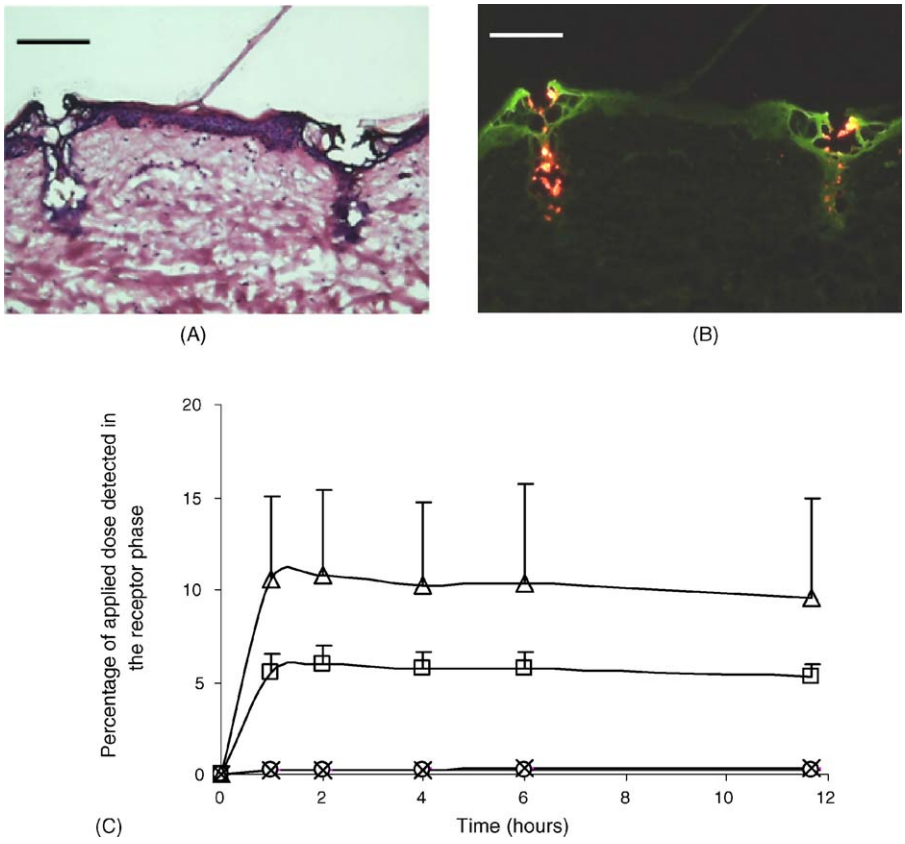

Fig. 6. Light (A) and fluorescent (B) photomicrographs of RF-microchannels containing fluorescent nanoparticles. Original magnification = 100×, bar = 100 μm; (C) diffusion of fluorescent nanoparticles through ViaDerm™ treated epidermal membranes. Data presented as percentage of topical nanoparticle dose detected in the receptor phase of Franz cells over a 12 h period. (◯) Untreated skin—PBS donor phase, (×) Untreated skin—topical nanoparticles, (□) 50 μm array ViaDerm™ treated skin—topical nanoparticles, (△) 100 μm array ViaDerm™ treated skin—topical nanoparticles ($N = 3 \pm$ S.D.).

performed to demonstrate the capability of these microchannels to permit cutaneous delivery of macromolecules or nanoparticulates. To that end, 100 nm fluorescent nanoparticles were selected as an easily detectable and size-representative model nanoparticle delivery system. Indeed, we have previously reported their application as an experimental tool for lipid:polycation:pDNA (LPD) non-viral gene delivery particle studies (Chabri et al., 2004). Fig. 6 confirms that the RF-microchannels created in skin following application of ViaDerm™ are of sufficient dimensions to uptake, entrap and permit the diffusion of 100 nm fluorescent nanoparticles. The channels shown in Fig. 6A and B appear to be larger than those observed in Fig. 5, possibly due to changes in the tissue sample over the incubation period (24 h compared with 0 h). These micrographs imply that the RF-microchannels generated can be considered to be of appropriate dimensions for the cutaneous delivery of macromolecules and non-viral gene therapy vectors.

Fig. 6C shows the data from a Franz-type diffusion experiment designed to determine the transit of the 100 nm nanoparticles through ViaDerm™ treated and control epidermal

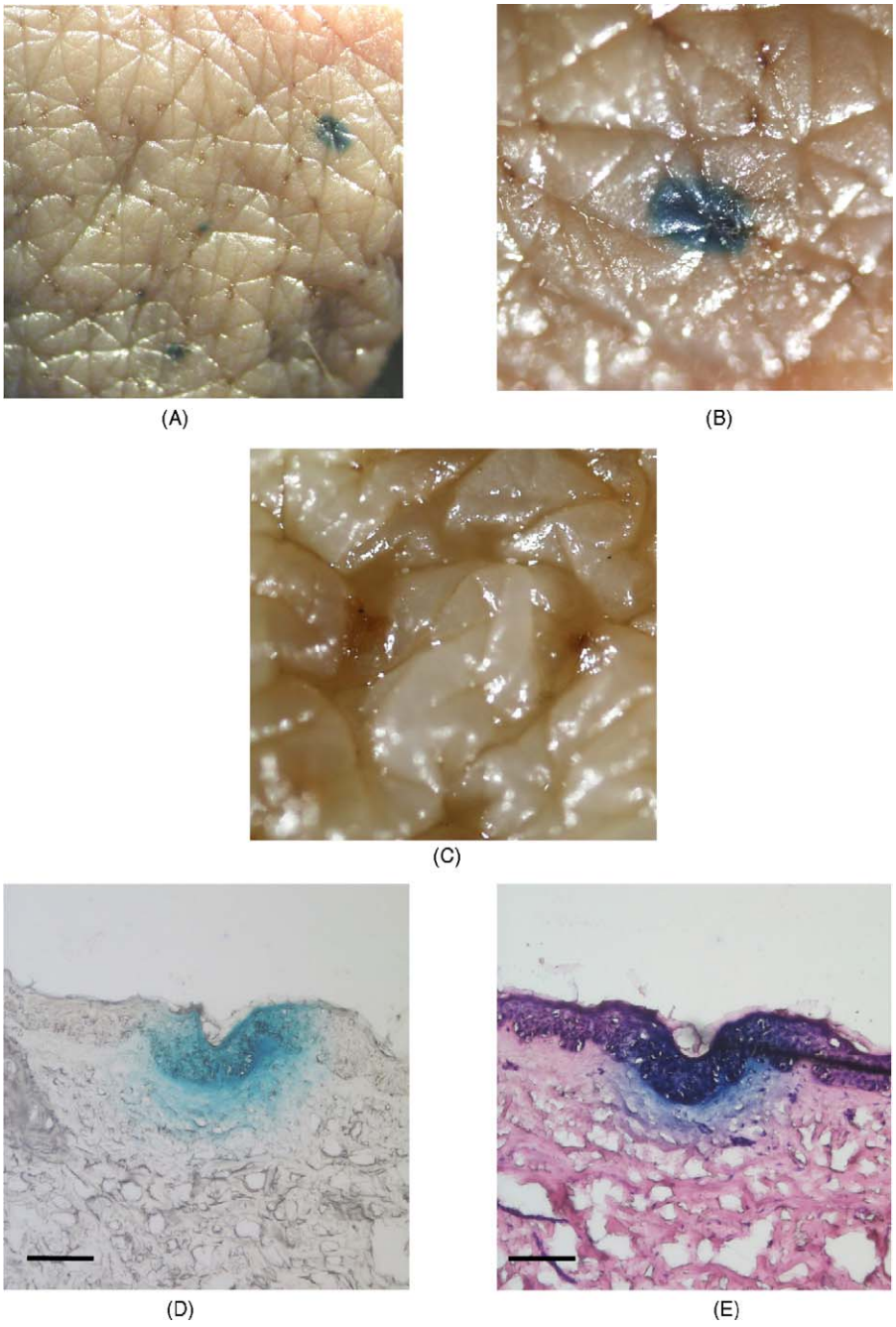

Fig. 7. Photomicrographs of ViaDerm™ treated human skin stained for β-galactosidase expression (50 μm arrays). (A) En face stereomicroscopy; (B) en face light microscopy, original magnification = 40×; (C) en face stereomicroscopy of ViaDerm™ treated human skin treated with the pEGFP-N1 plasmid; (D) unstained cryosection, original magnification = 100×; (E) H&E stained cryosection, original magnification = 100×, bar = 100 μm.

membranes. The non-treated epidermal membranes demonstrate the significant barrier function of this membrane to 100 nm nanoparticles, with undetectable penetration observed following 12 h incubation. Following ViaDerm™ treatment the epidermal membranes demonstrated a significantly enhanced ($p > 0.05$, one-way analysis of variance) permeability to the nanoparticles. Interestingly, whilst application of the 50 μm electrode arrays mediated reproducible permeation of the membrane to facilitate the diffusion of approximately 5% of the surface-applied nanoparticles, appliance of the 100 μm electrode arrays led to enhanced, though more variable, disruption of the membrane, as evidenced by an increase in mean penetration of the 100 nm nanoparticles. A possible mechanism for the more variable permeation of nanoparticles following ViaDerm™ treatment using the 100 μm electrodes is provided by the SEM images in Fig. 2. When the skin is treated with ViaDerm™ using the 50 μm electrodes and the epidermal membrane is subsequently removed by heat separation, it is not guaranteed that the entire membrane, i.e. stratum corneum and viable epidermis, will be punctured although disruption of the outer 15–30 μm will be sufficient to overcome the primary diffusive barrier, the stratum corneum. The observed increase in nanoparticle permeation therefore results from particle transit through the ablated SC channels and subsequent diffusion through the underlying epidermis. As shown in Fig. 2, skin treatment with ViaDerm™ using the 100 μm electrodes can occasionally effect complete penetration through the heat-separated epidermal sheet. Variability will therefore arise from the proportion of complete punctures, which in turn will depend on the thickness of the epidermal membrane following heat separation.

The delivery and expression of plasmid DNA in viable human skin via RF-microchannels has been initially demonstrated using the 50 μm electrode arrays. In these experiments the plasmid was used alone, i.e. without any non-viral carrier system, as numerous studies have shown the ability of naked DNA to undergo efficient expression in vivo (Hengge et al., 1995, 1996; Chesnoy and Huang, 2002). Fig. 7A and B clearly show the presence of intense blue staining, relating to substantial reporter gene expression with no expression evident in skin treated with ViaDerm™ and probed with the pEGFP-N1 plasmid (control; Fig. 7C). The expression is primarily localised in the viable epidermal cells surrounding the RF-microchannel (Fig. 7D and E). Interestingly, when a solution of DNA is applied topically to an area of ViaDerm™ treated skin the resulting epidermal gene expression is relatively low (data not shown). When the skin is treated with ViaDerm™ both prior to and following a topical application of the DNA solution the extent and level of gene expression is demonstrably greater. Consequently, it is reasonable to suggest that the ViaDerm™ might be used not only to create microchannels in the skin but also to enhance the intracellular uptake of the delivered DNA via a mechanism analogous to electroporation (Titomirov et al., 1991; Zhang et al., 2002).

In conclusion, we have demonstrated that the channels created in human breast skin following application of the RF-microchannel generator (ViaDerm™) are of appropriate dimensions, and enhance skin permeability to such a degree, as to permit the delivery of macromolecules and gene therapy vectors to the skin. The ViaDerm™ device represents a significant breakthrough in the challenge of delivering high molecular weight medicaments through the SC barrier. In particular, the ability to facilitate minimally invasive, targeted and controlled delivery of genes to the viable epidermis further supports the experimental and clinical evaluation of this novel transdermal drug delivery technology.

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