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Enhancing DNA delivery into the skin with a motorized microneedle device

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ABSTRACT

The purpose of this study was to evaluate a motorized microneedle device in delivery of DNA into skin for gene expression. A plasmid DNA encoding both luciferase (Luc) and enhanced green fluorescent protein (EGFP) was delivered into rat skin by puncturing the skin with the microneedle device. Puncturing rat skin with a pre-applied DNA solution on the skin showed much higher luciferase gene expression than that with the procedure of puncturing the skin first then applied the DNA solution. The microneedle puncturing method was more efficient than intradermal injection method in generating high gene expression in the skin. There was no significant difference in the skin gene expression when rat skin was punctured with the microneedle device of different microneedle lengths (0.25 mm, 0.5 mm or 0.75 mm). On the other hand, there was a significant difference in the skin gene expression between the short (10 s) and the long puncturing durations (30 or 60 s), with longer puncturing duration showed higher gene expression. Puncturing the skin with longer needles (0.75 mm) caused some skin damage, while puncturing the skin with shorter microneedle length (0.25 mm) caused only minimal skin damage. The EGFP gene expression was observed predominately in the epidermis layer of the skin from the puncturing method in delivery of DNA into the skin. In summary, the motorized microneedle device could have great potential in skin gene delivery.

1. Introduction

Delivery of DNA into the skin could provide treatment for a variety of skin diseases such as genetic skin disorders (Therrien et al., 2008), skin cancers (Heller and Coppola, 2002), psoriasis (Zibert et al., 2011), and diabetic neuropathy (Ropper et al., 2009). However, it is challenging to deliver macromolecules like DNA into the skin because of the barrier effect from stratum corneum. Intradermal injection of naked DNA is safe, but only can generate relatively low level of gene expression (Chesnoy and Huang, 2002). In addition, the injection procedure is painful and multiple injections are needed for large skin area. Applying electroporation on the skin surface after intradermal injection of naked DNA can significantly improve the gene expression level (Heller and Heller, 2006; Heller et al., 2008), but applying high voltage during the electroporation will cause shocking sensation and possibly tissue damage. Jet injection (Ren et al., 2002) and gene gun (Yang et al., 2001) can generate relatively high level of gene expression in the skin, but both procedures are limited to small skin surface area and the pain sensations are similar to needle injections (Jackson et al., 2001).

Puncturing skin with a tattooing device was attempted for delivering DNA into the skin and stronger gene expression than that from intradermal injection method was observed (Ciernik et al., 1996). More recently, this tattooing method was investigated for intradermal DNA vaccination (Quaak et al., 2009; Van Den Berg et al., 2009) and strong immune responses were observed (Pokorna et al., 2008). However, tattooing procedure was an invasive procedure that led to epidermal necrosis, epidermal inflammation, and dermal hemorrhage after tattooing (Gopee et al., 2005).

Puncturing skin with microneedles can create micrometer size pathways across the stratum corneum into the skin and usually cause little pain sensation (Haq et al., 2009). By evaluating the transepidermal water loss (TEWL), it was demonstrated that skin integrity recovered within 24 h after microneedle array puncturing on hairless rat skin with needle lengths ranged from 100 μm to 1.2 mm (Yan et al., 2010). Delivering DNA into skin was attempted by coating DNA on the surface of the microneedles and then piercing the coated microneedles into the skin (Gonzalez-Gonzalez et al., 2011). This coating and piercing approach was investigated for immunization purpose (Prausnitz et al., 2009). One drawback of this method is that the microneedle arrays are usually small and can only cover limited skin surface. Applying microneedle arrays to deliver DNA to large skin area would require a large number

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of coated microneedle arrays, which would be economically and practically impossible.

Stainless steel microneedles such as derma rollers and derma stamps with needle length from 0.2 mm to 2 mm are commercially available for large skin area cosmetic procedures (Doddaballapur, 2009), such as anti-wrinkle therapy and scar removal. More recently, motorized microneedle devices such as Dermapen with adjustable microneedle length and puncturing speed have also been used for those cosmetic procedures in treating large skin area. Motorized microneedle device and dermal roller showed similar safety profile on puncturing hairless mouse skin in a recent study (Park et al., 2013). Motorized microneedle device may be more advantageous than the manually operated dermal roller because of a more precisely controlled puncturing force by the device. The purpose of this study was to evaluate the feasibility of utilizing a motorized microneedle device in delivering DNA into the skin with a focus on how the microneedle length and puncturing duration affected the gene expression in the skin.

2. Material and methods

2.1. Materials

Plasmid DNA that encoding enhanced green fluorescent protein and firefly luciferase (pEGFP-Luc) was a gift from Professor Mulero at the University of Murcia (Alcaraz-Perez et al., 2008). The pEGFP-Luc was propagated with E-Coli competent cell from Hardy Diagnostics (Santa Maria, CA) and purified with a Qiagen plasmid mega kit. Male Sprague Dawley rats around 8 weeks old were obtained from Harlan Laboratories. Luciferase assay kit was obtained from Promega. Pierce BCA protein assay kit and Dulbecco's modified eagle medium (DMEM) were obtained from Thermo Scientific (Rockford, IL). Lipofectamine 2000 was obtained from Invitrogen. Branched polyethyleneimine (PEI, 25 kD) was obtained from Sigma. Isoflurane was obtained from Med-Vet International (Mettawa, IL).

The motorized microneedle device used in this study was the My-M microneedle device manufactured by Bomtech Electronics Co. (Seoul, Korea). It has adjustable microneedle length from 0.25 mm to 2.0 mm by adjusting the stamp needle array position from the guard ring around it (see Fig. 1). When the device is powered on, the stamp needle array is moving like a piston in the guard ring. The needle length is the part of the microneedles exposed out from the guard ring during the reciprocal movement of the stamp needle array in the guard ring. It also has five adjustable puncturing speed settings by controlling the stamp needle array reciprocal movement speed which is ranging from 20 to 90 turns/s. The speed setting 2 was used in this study. We estimated it would have a speed of 40 turns/s. There are 12 stainless steel microneedles (diameter around 200 μm) on the device. For each turn, the microneedle device will provide 12 needle punctures. Thus an application time of 10 s, 30 s, or 60 s roughly provides 4800, 14,400, or 28,800 needle punctures from the device, respectively.

2.2. Preparation of rats for the study

The Sprague Dawley rats were anesthetized with isoflurane gas. The hair on the back skin was carefully shaved off with an electric clipper without any visible damage to the skin. Then the skin was sanitized with swapping of 70% Isopropyl alcohol. After that, the back skin was marked with six 2-cm by 2-cm squares with three squares on each side: two on each side of the rib cage area and one on each side of the buttock area.

2.3. Evaluation of different methods in delivery of DNA into the skin

2.3.1. Passive diffusion method in delivery of DNA solution or DNA lipoplexes into skin

10 μg of pEGFP-Luc in 100 μl of plain DMEM solution was applied on a marked skin square and then gently rubbed on the skin with a finger tip for 1 min. In a separate condition, the DNA first formed lipoplexes with Lipofectamine 2000 by mixing 10 μl of 1 mg/ml pEGFP-Luc solution and 20 μl of Lipofectamine solution with vortexing for 5 s, and then diluted to 100 μl with plain DMEM solution. Then the 100 μl lipoplexes solution was applied onto the marked skin and then gently rubbed on the skin with a finger tip for 1 min. Luciferase gene expression in each marked skin was determined two days after the application.

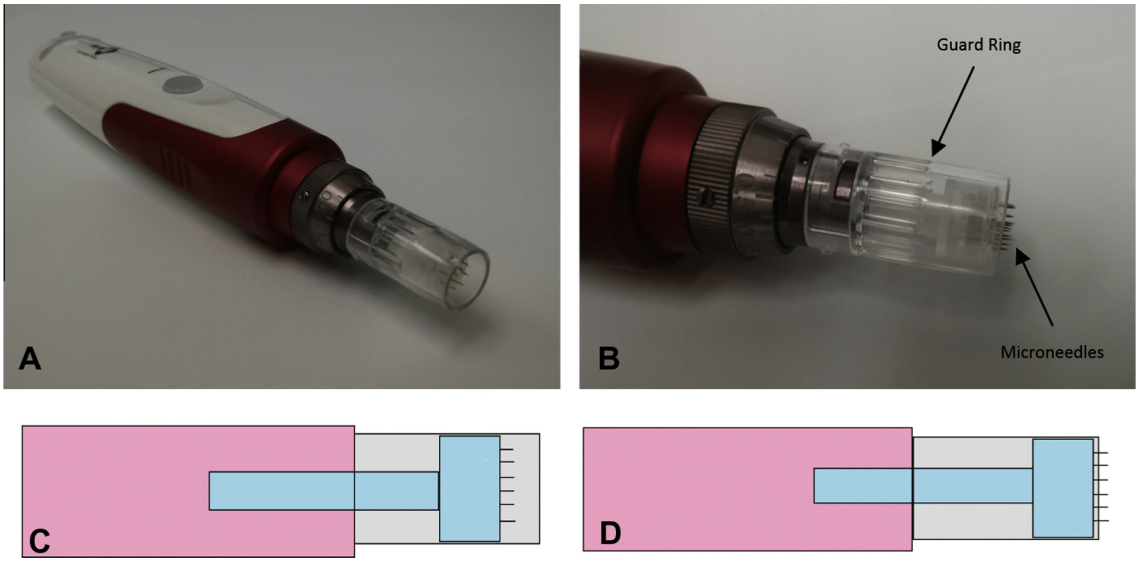

Fig. 1. (A) A picture of the motorized microneedle device used in this study; (B) A picture of motorized microneedle device with the microneedles exposed out of the guard ring; (C) The schematic diagram of the microneedle device in Picture A in which the microneedles on the piston shape stamp were retracted back into the guard ring; (D) The schematic diagram of the microneedle device in Picture B in which the microneedles on the piston shape stamp were protruded out of the guard ring.