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A proposed model membrane and test method for microneedle insertion studies

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ABSTRACT

A commercial polymeric film (Parafilm M®, a blend of a hydrocarbon wax and a polyolefin) was evaluated as a model membrane for microneedle (MN) insertion studies. Polymeric MN arrays were inserted into Parafilm M® (PF) and also into excised neonatal porcine skin. Parafilm M® was folded before the insertions to closely approximate thickness of the excised skin. Insertion depths were evaluated using optical coherence tomography (OCT) using either a force applied by a Texture Analyser or by a group of human volunteers. The obtained insertion depths were, in general, slightly lower, especially for higher forces, for PF than for skin. However, this difference was not a large, being less than the 10% of the needle length. Therefore, all these data indicate that this model membrane could be a good alternative to biological tissue for MN insertion studies. As an alternative method to OCT, light microscopy was used to evaluate the insertion depths of MN in the model membrane. This provided a rapid, simple method to compare different MN formulations. The use of Parafilm M®, in conjunction with a standardised force/time profile applied by a Texture Analyser, could provide the basis for a rapid MN quality control test suitable for in-process use. It could also be used as a comparative test of insertion efficiency between candidate MN formulations.

1. Introduction

Microneedle (MN) devices are composed of an array of micron-size needles. These systems are currently attracting great interest in transdermal drug delivery research (Chandrasekhar et al., 2013; Henry et al., 1998; Kim et al., 2012; Tuan-Mahmood et al., 2013). MN has the ability to pierce the outermost layer of the skin, the stratum corneum (SC) and create micro-conduits that can deliver drugs to the deeper layers of the skin from where they can be absorbed directly into the systemic circulation (Prausnitz, 2004).

Several key physical factors affect MN performance. These are: type of material, needle height, tip-radius, base diameter, needle geometry and needle density. The penetration depth and the fracture force of MN are determined by all these factors (Davis et al., 2004). Clearly, effective penetration of MN arrays into the skin is the primary pre-requisite for effective drug delivery. However, when developing and testing MN systems, it is apparent that there are limited techniques to evaluate this aspect. Most are based on the measurement of transepidermal water loss (TEWL) (Badran et al., 2009; Bal et al., 2008) or in the visualization of the micropores created after the application of a dye to the skin surface (Oh et al., 2008; Park et al., 2005; Verbaan et al., 2008; Wang et al., 2006). An alternative to these techniques is to take a biopsy of the MN pierced tissue and section it using histological techniques (Badran et al., 2009; Wang et al., 2006; Widera et al., 2006). In this latter case, the subsequent treatment of the skin could change the structure of the micropores. Previously, optical coherence tomography (OCT) has been demonstrated as a good option to evaluate the insertion of MN (Coulman et al., 2011; Donnelly et al., 2010). It is a non-invasive technique and, in addition to pore diameter, the penetration depth of the MN can be readily obtained.

MN insertion studies have typically been performed in biological tissue and this can present some disadvantages, in that tissue samples are often heterogeneous, unstable and difficult to obtain. In addition, the use of biological materials sometimes presents legal issues. Importantly, many of the reported methods, although valuable during the product development phase, are too complex to be suitable as a standard, routine quality control (QC) method for MN. Thus, for QC applications, it is desirable to overcome these limitations by using an artificial material in place

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of skin. Critically, this will allow the speed and repeatability of experiments to be improved. There are many reports on the use of artificial membranes for drug diffusion studies generally (Ng et al., 2012) and, specifically, for MN mediated transdermal drug delivery (Donnelly et al., 2009; Garland et al., 2012; Zhang et al., 2014). Artificial membranes are widely used for hypodermic needle mechanical testing and bench tests have been developed and standardised for this purpose (Vedrine et al., 2003). However, to the best of our knowledge, studies on artificial membranes for MN insertion or mechanical characterization are scarce (Hamilton, 2011; Koelmans et al., 2013; Muthu, 2007). The implementation of an artificial membrane method for insertion studies can also provide valuable and important comparison tool between different types of MN arrays.

In this work, we propose the use of a polymeric film as a model for MN insertion studies. A comparative study between the insertion of MN into this material and excised neonatal porcine skin was carried out. OCT was used as a tool to evaluate the insertion of MN inside the tissue, taking into account aspects such as the insertion force. Additionally, the force that patients use to apply MN arrays to their skin was evaluated. To the best of our knowledge, there are no studies relating test conditions to actual real life use of MN, in the context of skin insertion by patients, a key factor in designing a reliable QC test method.

2. Materials and methods

2.1. Materials

Gantrez® S-97 ($M_w = 1.2 \times 10^6$), a copolymer obtained from the free acid of methyl vinyl ether and maleic anhydride polymers, was provided by Ashland (Tadworth, Surrey, UK). Poly(ethyleneglycol) (PEG) 10,000 Da was obtained from Sigma–Aldrich (Poole, Dorset, UK). Parafilm M®, a flexible thermoplastic sheet (127 μm thickness) made of olefin-type material, was used as skin simulant for insertion studies, was obtained from BRAND GMBH (Wertheim, Germany). Deka® poly(urethane) needle testing foil was provided by Melab GmbH (Leonberg, Germany).

2.2. Methods

2.2.1. Preparation of MN arrays

To fabricate MN, aqueous blends containing Gantrez® S-97 (20% w/w) and PEG 10,000 (7.5% w/w) were micromoulded in laser-engineered silicone micromould templates, as previously described (Donnelly et al., 2010, 2011; Migalska et al., 2011; Garland et al., 2011; Singh et al., 2009, 2010). Three different MN geometries were used (Table 1). Light microscope images of the two main MN arrays used in this work can be seen in Fig. 1A. Alternatively, two different formulations were used to prepare either brittle (prepared by adding sodium carbonate 3.5% w/w to the original formulation) or flexible (prepared by replacing PEG in the original formulation with 10% w/w glycerine) MN arrays. In the preparation of brittle and flexible formulations the MN was not crosslinked (Donnelly et al., 2012a).

Table 1
Physical properties of the different MN arrays.

MN per array	Height (μm)	Width at base (μm)	Interspacing at base (μm)
11 × 11	600	300	300
11 × 11	900	300	300
19 × 19	600	300	50

2.2.2. Human manual force measurements

The forces that 20 volunteers applied using their thumbs were measured using a TA.XTPlus Texture Analyser (Stable Micro Systems, Surrey, UK). The selected volunteers were 10 males and 10 females aged between 20 and 35 years. The volunteers were asked to apply the same force they would use to push an elevator button or to press a stamp onto an envelope, using their right thumb and a 30 s application period, as shown in Fig. 1B. The Texture Analyser was used in tension mode to register the force curves. Three different parameters were determined from these curves: the maximum, minimum and average forces applied during this time interval (Fig. 1C).

2.2.3. Insertion of MN arrays

Full thickness neonatal porcine skin can be considered a good model for human skin in terms of hair sparseness and physical properties (Meyer, 1996). It was obtained from stillborn piglets and excised <24.0 h after birth. Full thickness skin (≈0.5 mm) was then stored in aluminium foil at −20.0 °C until further use. Two sections of skin were placed together, with the dermal side contacting each other, such that the stratum corneum surface was exposed at either side, giving a total skin thickness of about 1 mm. This was then utilised for the OCT assessment of MN penetration.

As an alternative to neonatal porcine skin, Parafilm M® (PF) film and a needle testing polyurethane film were used as skin simulants. A sheet of Parafilm was folded to get an eight-layer film (≈1 mm thickness) and a poly(urethane) needle testing film (Deka®) was used as received (0.4 mm thickness). The skin/Parafilm® was then placed onto a sheet of expanded poly (ethylene) for support.

Two insertion methods were carried out: manual and Texture Analyser insertion. For manual insertion, different volunteers were recruited to apply the MN arrays following the same instructions as in the force measurement experiment. The Texture Analyser insertion was performed using a TA.XTPlus Texture Analyser (Stable Micro Systems, Surrey, UK) in compression mode. MN arrays were placed on the surface of the skin/artificial membrane and sticky tape (Office Depot, Boca Raton, USA) was carefully applied on the upper surface without applying force (Fig. 1D). The probe was lowered onto the skin/artificial membrane at a speed of 0.5 mm s^{-1} until the required force was exerted. Forces were held for 30 s and varied from 10 N to 50 N per array. Once the target force was reached, the probe was moved upwards at a speed of 0.5 mm s^{-1}.

2.2.4. Optical coherence tomography

Inserted MN arrays were immediately viewed using an EX1301 OCT Microscope (Michelson Diagnostics Ltd., Kent, UK). The swept-source Fourier domain OCT system has a laser centre wavelength of 1305.0 ± 15.0 nm, facilitating real-time high-resolution imaging of the upper skin layers (7.5 μm lateral and 10.0 μm vertical resolution). The skin was scanned at a frame rate of up to 15 B-scans (2D cross-sectional scans) per second (scan width = 2.0 mm). The 2D images were analysed using the imaging software ImageJ® (National Institutes of Health, Bethesda, USA). The scale of the image files obtained was 1.0 pixel = 4.2 μm, thus allowing accurate measurements of the depth of MN penetration and the width of pore created. Three replicates were performed, and the insertion depths of 25 MN were measured in total.

2.2.5. MN insertion testing using light microscopy

MN arrays were inserted using a Texture Analyser, as described above, into eight-layer folded PF sheets. In these cases, sticky tape was not used. After the insertion, the MN arrays were removed from the polymeric sheet. The PF was unfolded and the number of holes in each layer was evaluated using a Leica EZ4 D digital

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