International Journal of Pharmaceutics 496 (2015) 351–359

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

Ex vivo evaluation of a microneedle array device for transdermal application

Sunaina Indermun[a], Yahya E. Choonara[a], Pradeep Kumar[a], Lisa C. du Toit[a], Girish Modi[b], Sandy van Vuuren[a], Regina Luttge[c,d,e], Viness Pillay[a,*]

[a] *Wits Advanced Drug Delivery Platform Research Unit, Department of Pharmacy and Pharmacology, School of Therapeutic Sciences, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, 7 York Road, Parktown, 2193, South Africa*
[b] *Department of Neurology, Division of Neurosciences, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, 7 York Road, Parktown, 2193, South Africa*
[c] *MESA + Institute for Nanotechnology, University of Twente, 7500AE Enschede, The Netherlands*
[d] *MyLife Technologies BV, Enschede 7500AE, The Netherlands*
[e] *Department of Mechanical Engineering, Microsystems Group and Institute for Complex Molecular Systems (ICMS), Eindhoven University of Technology, Eindhoven Q2 5600MB, The Netherlands*

ARTICLE INFO

Article history:
Received 3 June 2015
Received in revised form 29 September 2015
Accepted 30 September 2015
Available online 21 October 2015

Keywords:
Electro-modulated hydrogel
Microneedles
Micro-organisms
Transdermal drug delivery
Indomethacin

ABSTRACT

A new approach of transdermal drug delivery is the use of microneedles. This promising technique offers the potential to be broadly used for drug administration as it enables the dramatic increase in permeation of medicaments across the stratum corneum. The potential of microneedles has evolved to spawn a plethora of potential transdermal applications. In order to advance the microneedle capabilities and possibly revolutionize advanced drug delivery, this study introduces a novel transdermal electro-modulated hydrogel–microneedle array (EMH–MNA) device composed of a nano-porous, embeddable ceramic microneedle array as well as an optimized EMH for the electro-responsive delivery of indomethacin through the skin. The *ex vivo* permeation as well as drug release experiments were performed on porcine skin tissue to ascertain the electro-responsive capabilities of the device. In addition, the microbial permeation ability of the microneedles across the viable epidermis in both microneedle-punctured skin as well as hypodermic needle-punctured skin was determined. *Ex vivo* evaluation of the EMH–MNA device across porcine skin demonstrated that without electro-stimulation, significantly less drug release was obtained (±0.4540 mg) as compared to electro-stimulation (±2.93 mg).

1. Introduction

Although not a relatively new concept, drug delivery research has focused on the transdermal route of administration due to limitations in oral drug delivery. Furthermore, there are additional negative connotations associated with conventional injections such as needle phobias, accidental needle-sticks and pain.

The consortium of microneedle array (MNA) technology is a promising technique that combines the advantages of hypodermic syringes and transdermal patches by using microscopic projections to facilitate drug transport (Chiarello, 2004). MNA technology has been proposed as a hybrid to overcome the individual limitations of both injections and patches (Lee et al., 2011). The dimensions of the MNAs are within the micron range, consequently allowing, upon topical application, restricted penetration to the most superficial layers of the tissue *i.e.*, the viable epidermis and papillary dermis thereby preventing penetration into the subcutaneous tissue containing nerve endings (Coulman et al., 2011). Microneedles are therefore a pain-free and patient-friendly means of delivering a host of low molecular weight and macromolecular therapeutics into the skin and/or systemic circulation (Pearton et al., 2012).

In order to further advance MNA technology, a novel patented electro-modulated hydrogel (EMH) was developed to be combined with the MNAs (Indermun et al., 2014). Under normal conditions the drug compound remains entrapped in the hydrogel, but upon the actuation using an electro-stimulus, the drug is released into the skin. When the electro-stimulus is removed, the change is reversed and drug release ceases.

* Corresponding author. Fax: +27 11 642 4355/86 553 4733.
E-mail address: viness.pillay@wits.ac.za (V. Pillay).

http://dx.doi.org/10.1016/j.ijpharm.2015.09.070
0378-5173/

In addition to protecting the body from the external environment, the stratum corneum prevents microbial access to the skin and thus pathogenic disease. Moreover, it is a very effective barrier to the permeation of drug substances. Depending on their physicochemical properties, certain drug substances achieve a therapeutic effect solely through passive diffusion, while other substances require additional permeation enhancement methods such as the use of microneedles. Although studies have been conducted on the ability of microneedles to effectively breach the stratum corneum, few reports exist on the needles causing any skin or systemic infection (Prausnitz, 2004; Donnelly et al., 2009), nor is there any available literature of such a device being developed. Thus, preliminary *ex vivo* tests were performed demonstrating the feasibility of the EMH–MNA device for transdermal drug delivery *in vivo*. The studies allowed for the potential safety/toxicity profile to be determined and more importantly, an evaluation of the efficacy of the device prior to undertaking *in vivo* studies. In order to assess the microbial permeation through the skin after the application of a microneedle, three micro-organisms where selected correlating to the commensal inhabitants of human skin viz. *Staphylococcus epidermidis*, *Pseudomonas aeruginosa* and *Candida albicans.*

The aerobic, Gram-positive cocci cluster of *S. epidermidis* is thought to comprise more than 90% of the aerobic resident flora of the skin (Cogen et al., 2008). Often resistant to antibiotics, *S. epidermidis* has the ability to form biofilms on plastic devices which contributes towards the major virulence factor for the micro-organism. *S. epidermidis*i is thus one of the primary causes of implanted medical-device related infections (McCann et al., 2008). *P. aeruginosa* is a Gram-negative, rod-shaped bacterium and is an opportunistic pathogen causing disease mainly in patients with poor immunity (Sharma et al., 2014). Commonly the cause of nosocomial infections, the pathogen is distinguished from other Gram-negative bacteria by its ability to produce fluorescent molecules such as pyocyanin, pyoverdin or fluorescein, and pyorubin (Cogen et al., 2008). The commensal, *C. albicans* is found on the mucosal surfaces of the gastrointestinal and genitourinary tracts, becoming pathogenic only when the body is immunocompromised and can infect a broad range of body sites (Hube, 2004; Kumamoto, 2011).

The most relevant membrane to be employed in *ex vivo* studies is human skin. However, due to ethical reasons, a number of animal models such as porcine, rat, mouse, primates, and guinea pig models, have been suggested as suitable replacements for the evaluation of percutaneous permeation studies (Godin and Touitou, 2007). The most relevant animal model for human skin is the pig as both its biochemical and histological properties have been shown to repeatedly be similar to that of human skin (Gray and Yardley, 1975; Dick and Scott, 1992; Jacobi et al., 2007). In addition, the follicular structure resembles that of humans, with hairs and infundibula extending deeply into the dermis (Godin and Touitou, 2007), with averages of 20 hairs/cm^2 present on porcine skin as compared to 14–32 hairs, except the forehead area, in humans (Jacobi et al., 2007). Pig skin and human skin also share similar microbiological colonization (Baird-Parker, 1962), dermal collagen fiber arrangement and vascular anatomy. The contents of stratum corneum ceramides and glycosphingolipids are also found to be similar in the pig and human. Of importance, skin resistance in the pig model is also similar (1.18 kΩ/cm^2) to that of humans (3.94 kΩ/cm^2) as compared to the rat (0.98 kΩ/ cm^2), rabbit (0.35 kΩ/ cm^2) and guinea pig (1.97 kΩ/ cm^2) (Davies et al., 2004).

To date, a transdermal drug delivery device of this nature has not been developed. The safety and success of this device has been investigated in *in vitro* studies (Indermun et al., 2014). Consequently, to be well accepted, *ex vivo* drug release studies have to be undertaken and are thus detailed herein.

2. Materials and methods

2.1. Materials

Poly(ethyleneimine) solution (M_w 750,000), 1-vinylimidazole (≥99%), Indomethacin (≥99%), Poly(vinyl alcohol) (M_w 89,000-98,000, 99 + % hydrolyzed), Acrylic acid (anhydrous, 99%), *N*,*N*′-Methylenebisacrylamide (≥99.5%) and Potassium Persulfate (≥99.0%) were all purchased from Sigma–Aldrich® (St. Louis, USA). All other ingredients were of analytic grade and were used as received. Stock cultures of *Staphylococcus epidermis* (ATCC 2223), *Candida albicans* (ATCC 10231) and *Pseudomonas aeruginosa* (ATCC 27853), were obtained from Davies Diagnostics, Randburg, South Africa.

2.2. Preparation of the electro-modulated hydrogel-microneedle device

For the electro-responsive release hydrogel, a 6% PVA-1 M sodium hydroxide solution was prepared, to which poly(ethyleneimine) solution and 1-vinylimidazole was added. Subsequently, the therapeutic agent and acrylic acid was added to the mixture. *N*, *N*′-Methylenebisacrylamide was added as a cross-linking agent to facilitate the formation of a semi-interpenetrating hydrogel network, instituting vinyl addition polymerization to increase the interconnectivity of the matrix. Response optimization was carried out utilizing statistical software (Minitab®, V14, Minitab Inc®, PA, USA).

2.3. Preparation of the electro-conductive hydrogel-microneedle array device

A MNA (Fig. 1) was made using a mold that was prepared as described by Bystrova and Luttge (2011) using photolithographic techniques on a low-pressure chemical vapor deposition (LPCVD)-silicon nitride covered 4-inch wafer of silicon (100). For ceramic casting, a slurry made of 43 wt.% alumina (AKP 30, Sumitomo) suspended in 46 wt.% ethanol containing 6.4 wt.% poly(vinyl

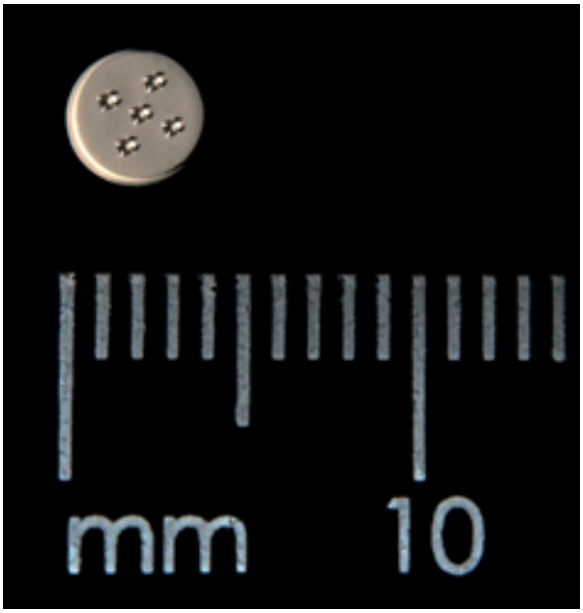

Fig. 1. Image depicting the array containing microneedles.