Intradermal delivery of imiquimod using polymeric microneedles for basal cell carcinoma

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Abstract

Despite being one of the most efficacious drugs used in the treatment of basal cell carcinoma (BCC), imiquimod has limited cutaneous permeation. The current work presents the development of polyvinylpyrrolidone-co-vinyl acetate (PVPVA) microneedles loaded with imiquimod for improving intradermal delivery of imiquimod for the treatment of nodular BCC. In vitro permeation studies, using full thickness ex vivo porcine skin were used to evaluate the effectiveness of these imiquimod loaded polymeric microneedles in comparison to the topical application of commercial Aldara™ cream. HPLC analysis demonstrated similar intradermal permeation of imiquimod from Aldara™ cream and imiquimod-loaded microneedles despite the microneedle having a six-fold lower drug loading than the clinical dose of Aldara™ used for BCC management. In addition, ToF-SIMS analysis of skin cross sections demonstrated intradermal localisation of imiquimod following microneedle-based delivery while the Aldara™ treated skin showed the drug localised predominantly within the stratum corneum. ToF-SIMS analysis also demonstrated intradermal co-localisation of the PVPVA polymer, used in fabricating the microneedle, with imiquimod within the microneedle channels in a label-free manner. This study demonstrates that a polymeric microneedle system may be a viable approach to improving the intradermal delivery of imiquimod for the treatment of nodular BCC with lower drug loading.

KEYWORDS: Microneedles, imiquimod, basal cell carcinoma, time-of-flight secondary ion mass spectrometry, polyvinylpyrrolidone-co-vinyl acetate
1. Introduction

Basal cell carcinoma (BCC) is the most prevalent type of skin cancer, with the number of individuals affected by the disease escalating worldwide. For instance, a large US sex-stratified cohort study by Wu et al. highlighted that the incidence rate of BCC in both men and women have doubled over the past 20 years (Wu et al., 2013). In addition, similar trends have been reported in Canada, Asia, Australia and Europe (Cameron et al., 2019). Furthermore, it is anticipated that the incident rate will continue to escalate due to the rise in an aging population coupled with historical UV exposure (Diffey and Langtry, 2005). Given that UV exposure is a risk factor in developing BCC, it therefore quite common that BCC typically manifests on sun exposed regions of the body such as the face, arms and necks (Suppa et al., 2015). The two most common BCC subtypes are superficial and nodular (Kuijpers et al., 2002). Superficial BCC generally manifests as flat red patches on the skin on the skin surface and proliferates parallel to the epidermis (Colver, 2002; Crowson, 2006). On the other hand, nodular BCC manifests as a translucent pearly nodule that resides within dermis at approximately 400 µm below the skin surface (Sabri et al., 2020; Williams et al., 2017). In addition, it has been reported that at least one-third of nodular BCC coexists with superficial BCC (Goldenberg et al., 2010).

Despite displaying low metastasis rates of 0.0028 %, this form of skin cancer can lead to considerable local tissue destruction (nose, ears and eyelids) leading to poor cosmetic outcomes and disfigurement (Mackiewicz-Wysocka et al., 2013; Mehta et al., 2012). The high incidence rate of the disease also forms a considerable proportion of a dermatologist’s workload (Wu et al., 2015). Therefore, there is a need to develop a simple and efficacious treatment strategy, which patients could simply administer by themselves in the management of BCC. Although surgical intervention is effective in treating BCC, such treatment is both time-consuming and technical (Nijssen et al., 2002). In addition, some patients may opt for non-surgical alternatives that offer lower overall treatment costs and improved cosmetic outcomes (Lien and Sondak, 2011; Tinelli et al., 2012). One of the most effective non-surgical interventions is the topical application of the immunomodulator, imiquimod (Jansen et al., 2017).
Imiquimod is a potent immune response modifier that induces its immunomodulation through Toll-like receptors located on antigen presenting cells. Upon binding to these receptors, imiquimod induces the release of pro-inflammatory cytokines which culminate in its antitumoral activity (Lacarrubba et al., 2008). Imiquimod is marketed as Aldara™ cream (5% w/w) by 3M Pharmaceuticals for the treatment of anogenital warts, actinic keratosis and superficial BCC. Despite its potency, the drug has limited dermal permeation thus reducing its therapeutic value to superficial BCC (Ma et al., 2015).

However, in the case of nodular BCC, it has been found that topical therapy with imiquimod typically has a lower cure rate than surgical intervention (Williams et al., 2017). Such a finding is attributed to the efficient barrier function of the stratum corneum which limits the permeation of imiquimod into the skin (Al-Mayahy et al., 2019). In addition, the location of nodular BCC that resides deep within the aqueous dermis presents another barrier for imiquimod delivery. This is because imiquimod is poorly water soluble and thereby has limited permeation within the dermis (Sauder, 2000; Yang et al., 2012).

The poor permeation properties of imiquimod deeper into the skin is attributed to the various physiochemical properties of the drug. It is suggested some of the ideal characteristics for a drug to permeate deeper into the skin include a Mw < 600 Da, a Log P 1.0 to 3.0, a low melting point, and ≤2 hydrogen bonding group(s) (Sabri et al., 2019). Although imiquimod meets some of these criteria, the drug has a high melting point of 292 °C and a total of 4 hydrogen bonding groups. The presence of such hydrogen bonding groups, especially the primary amine that may interact with the anionic components of the stratum corneum, contribute to the poor permeation profile of imiquimod deeper into the skin (Al-Mayahy et al., 2019). Furthermore, the drug has poor aqueous solubility which precludes the drug from permeating deeper into the aqueous and water rich dermal layers (Sabri et al., 2020). The combination of these factors presents a challenge in delivering imiquimod in a concentrated and localised fashion into the dermis for the treatment of nodular BCC.

It has been reported by patients that topical treatment such as creams are often unfavorable as this drug delivery vehicle typically has poor cosmetic feel upon administration as well as exuding an unpleasant scent once applied. In addition, the restriction in daily activities post application imposed
After application (e.g. not being able to shower and bathe after application) may result in poor compliance (Devaux et al., 2012). Also, the likelihood of the cream spreading onto clothes and healthy skin also raises the issue of unwanted side effects.

One of the drug delivery platforms that could be utilised to improve the delivery of imiquimod into the dermis for the management of basal cell carcinoma are microneedles. Microneedles consist of arrays of fine micro-projections that generate transient channels when applied to the skin. The accepted view is that microneedles breach the outermost layer of the skin, *stratum corneum*, generating channels which promote the entry of molecules into and across the skin (Prausnitz, 2004).

Microneedles have been widely investigated as a drug delivery platform for skin cancer therapy, as such systems provide more localised delivery of therapeutics. In the context of skin tumours, microneedle-based drug delivery systems can locally mediate the release of drugs and increase their permeation into deeper tumour regions within the skin (Sabri et al., 2019). Several researchers have investigated the utility of using solid microneedles, as a skin pre-treatment via the patch-and-poke strategy, to improve the utility of delivering anticancer compounds into the skin for the treatment of skin cancer. Naguib *et al.* demonstrated, via a murine model, the feasibility of using solid stainless-steel microneedles to enhance the intradermal delivery of 5-fluorouracil, 5-FU to treat skin tumours.

In their *in vitro* work, the group demonstrated that 5-FU flux increased by 4.5-fold when 5-FU cream was applied on microneedle perforated murine skin compared to cream application on intact skin (Naguib *et al.*, 2014). More recently, Al-Mayahy *et al.* showed that using a two-step application process involving pre-treating the skin with solid stainless-steel microneedles followed by Aldara™ cream application, they were able to enhance the permeation of imiquimod deeper into the skin (Al-Mayahy *et al.*, 2019).

However, the utility of using solid stainless-steel microneedles is limited by the short duration (< 15 min) than the channels remain open, due to the regeneration of skin, ultimately reducing the amount of drug delivered (Bal *et al.*, 2010). Given these limitations of topical cream application, there is an
impetus to reformulate imiquimod into a dissolving microneedle system which could offer a more effective and patient friendly treatment strategy for the management of nodular BCC. The simple and straightforward one-step application using dissolving polymeric microneedle patch loaded with imiquimod relative to the two-step application process via the patch-and-poke strategy using solid microneedles and Aldara™ may be a more preferred treatment option for patients. However, there are no studies to date that have evaluated improving the delivery of imiquimod into the dermis via the use of dissolving polymeric microneedles for the management of nodular BCC.

The aim of this study is to fabricate and characterize polymeric microneedles as a drug delivery system to achieve the localised intradermal delivery of imiquimod for nodular BCC treatment. Polyvinylpyrrolidone-co-vinyl acetate (PVPVA) microneedles loaded with imiquimod were developed through a microfabrication and micromoulding technique. PVPVA is a biocompatible polymer that is widely used in the pharmaceutical industry as a dry binder in tableting, as a film-forming agent in tablet coating, as well as a film-forming agent in topical drug delivery systems (Kolter and Flick, 2000; Patel et al., 2015). Besides that, being a derivative of PVP, PVPVA is a chemically and biologically inert polymer which obviates the issues of polymer-drug compatibility along with biological toxicity (Taresco et al., 2017; Teodorescu and Bercea, 2015). A series of experiments were performed to characterise the PVPVA microneedle formulation. In addition, an in vitro permeation study, using full thickness ex vivo porcine skin was used to evaluate imiquimod delivery into and across the skin. HPLC and ToF-SIMS analysis were utilised to illustrate the permeation and dermal distribution of imiquimod into skin following the application of imiquimod loaded microneedles versus Aldara™ cream. This study can serve as a basis for future in vivo and clinical studies with imiquimod loaded PVPVA microneedles for nodular BCC treatment.
2. Materials and Methods

2.1. Materials

Imiquimod was purchased from Cayman Chemicals, USA. Aldara™ topical cream (5% w/w imiquimod), MEDA Company, Sweden was purchased from Manor pharmacy, UK. Polyvinylpyrrollidone-co-vinyl acetate (PVPVA), was kindly provided by BASF (Ludwigshafen, Germany). Polyethylene glycol, PEG 400 was purchased from Sigma Aldrich, Belgium. Sodium carboxymethyl cellulose, Mw 90,000 was purchased from Sigma Aldrich, USA. Glycerol was purchased from Sigma Aldrich, USA. Sodium acetate was purchased from Sigma-Aldrich, UK. Acetonitrile (HPLC grade) and glacial acetic acid were obtained from Fisher Scientific, UK. Teepol solution (Multipurpose detergent) was ordered from Scientific Laboratory Supplies, UK. D-Squame standard sampling discs (adhesive discs) were purchased from Cuderm corporation, USA. OCT media was obtained from VWR International Ltd. Belgium. Deionised water was obtained from an ELGA reservoir, PURELAB® Ultra, ELGA, UK. All reagents were of analytical grade, unless otherwise stated. Ex vivo porcine skin was used in imiquimod permeation studies due to the similarities in histology, thickness and permeability to human skin (Benech-Kieffer et al., 2000).

Skin samples were prepared from ears of six-month-old pigs obtained from a local abattoir prior to steam cleaning. The skins were of full skin thickness to prevent altering the biomechanical properties of the tissue that may lead to over-penetration of the microneedles into the skin (Naguib et al., 2014). The porcine skin samples were stored at -20 °C until analysis.

2.2. Design and production of microneedle master structure and microneedle PDMS moulds

To produce the custom PDMS moulds, a stainless-steel microneedle master structure was designed in SolidWorks 2018 (Dassault Systèmes), consisting of a 10 x 10 array of 300 µm x 300 µm x 1000 µm (W x L x H) pyramidal microneedles with tip-to-tip spacing of 800 µm. This master structure, and a corresponding mould housing, Figure S1, were then produced from stainless-steel using a Kern Evo CNC Micro Milling Machine at the University of Nottingham Institute for Advanced Manufacturing.
polydimethylsiloxane (PDMS) (Sylgard 184®, Dow Corning, Midland, MI) mould was then created from
the stainless-steel microneedle master structure. A mixture of elastomer and curing agent, Sylgard
184®, were prepared at a ratio of 10:1 (elastomer: curing agent). The mixture was then degassed for
45 minutes to remove any trapped air in the mixture. After degassing the PDMS mixture was poured
into the stainless-steel master mould structure and placed in an 80 °C oven for one hour to cure the
PDMS. After curing, the mould along with the cured PDMS was plunged into an ice bath to allow ease
of removal of the cured PDMS mould. The stainless-steel master structure was then cleaned with
propan-2-ol before being reused to make further PDMS moulds.

2.3. Fabrication of blank and drug loaded polyvinylpyrrolidone-co-vinyl acetate
(PVPVA) microneedles

Polymeric microneedles were prepared using a micromolding technique. The PDMS moulds produced
as described in Section 2.2 were used to fabricate the blank microneedles. The microneedle matrix
was prepared using 16.2 % w/v PVPVA (in water) by dissolving the polymer at room temperature and
pressure for one hour. Then, 2% v/v of PEG 400 was added to the polymer solution. The polymer
solution was then degassed for 30 minutes and 150 µl of the PVPVA solution was then pipetted using
a positive displacement pipette into the PDMS mould and centrifuged at 4000 RPM for 15 min at room
temperature to fill the needle cavities. Then, excess polymer was removed before leaving the needle
layer to dry overnight in a desiccator. The backing layer of the microneedle patch were prepared using
5.2 % w/w of carboxymethylcellulose, CMC (in water). The backing solution was made by dissolving
CMC under stirring at 75 °C for 2 hours. In addition, 0.66 % v/w of glycerol was added to the backing
solution as a plasticiser. Using a positive displacement pipette, 200 µl of the CMC solution was then
pipetted on top of the needle layers and centrifuged at 3500 RPM for 10 minutes. The mould was
dried at room temperature for 48 hours in a desiccator. The polymeric microneedles were then
demoulded and stored in a desiccator until further use. For imiquimod loaded microneedles the
fabrication process was repeated in a similar fashion and composition, however the drug, PEG 400 and PVPVA were dissolved in 0.05 M of hydrochloric acid under stirring for 1 hour.

2.4. Characterisation of PVPVA microneedles (SEM, tensile strength, skin insertion properties)

2.4.1. Microscopy

Polymeric microneedle images were captured using an optical microscope (Zeiss Axioplan, Germany) and an environmental scanning electron microscopy (ESEM) (FEI Quanta 650) in low vacuum mode to visualize the shape and dimensions of the microneedles. For ESEM imaging, the microneedles were mounted on a metal stub using double-sided carbon tape prior to imaging.

2.4.2. Measurement of needle fracture force

The needle fracture force of the polymeric microneedles was determined using a texture analyser (Stable Microsystems, UK) following a previously reported method (Donnelly et al., 2011). This is to investigate the effect of applying an axial force parallel to the microneedle vertical axis, similar to the force encountered by the needles during application to the skin. The polymeric microneedles were visually inspected before and after application of the compression force. For this, the force required for compression of the polymeric microneedle to a specified distance was measured. The polymeric microneedles were attached to a 10 mm cylindrical Delrin probe (part code P/ 10) using double-sided adhesive tape. The probe is connected to a 50-kg load cell and was set at the same distance from the platform for all the test measurements. The TA XT Plus Texture Analyser was set to compression, the pre-test speed was set at 2 mm/s and post-test speed at 10 mm/s. The trigger type was set to auto (force) with a trigger force of 0.009 N. The test station compresses the polymeric microneedle against a flat block of aluminium of dimensions 10.0 × 9.0 cm. Compression force versus displacement curves were plotted to calculate the fracture force. A total of five microneedle patches were used to evaluate the fracture force of the microneedles.
2.4.3. *In vitro* skin simulant insertion

As an alternative method to determine the microneedle penetration depth as a function of length, a polymeric film (Parafilm M®, a blend of a hydrocarbon wax and a polyolefin) was utilized as a skin model. This insertion study was adopted from Larrañeta *et al.* (Larrañeta *et al.*, 2014). In brief, 8 layers of Parafilm M® were stacked onto each other on a cork mat that mimics underlying muscles. The PVPVA microneedle patch was applied under thumb pressure for 10 seconds. Six replicates were generated and observed under the Zeta Profilometer (KLA-Tencor, US) for the number of micropores created.

2.4.4. Dye binding study

In order to evaluate if the microneedle patch is capable of penetrating the skin and to visualize the depth of microneedle penetration into the skin, a dye binding study using *ex vivo* porcine skin was conducted. The porcine ear skin was defrosted at room temperature for an hour prior to the experiment. Using clippers, excess hair was carefully trimmed from the skin. Regions of the skin were then selected for microneedle treatment. The skin was treated with PVPVA microneedles loaded with methylene blue dye, which is a hydrophilic dye. The microneedle was left in the skin for one hour before removing the microneedle patch. Upon removing the patch, the skin was visually inspected to see if any microneedle channels have been generated in the skin. In order to gauge the depth of microneedle penetration into the skin, skin cross-sectioning was performed. In brief, each microneedle application site was cut into 1 cm × 1 cm and fresh frozen on a metal block that was cooled with liquid nitrogen. Skin cross-sections were performed using a cryostat (Leica CM3050 S Research Cryostat, UK). The depth of microneedle penetration as visualised by methylene blue permeation was measured using an optical microscope (Zeta Profilometer, KLA-Tencor, US).

2.5. Measurement of imiquimod permeation from PVPVA microneedles

Imiquimod skin permeation was evaluated *ex vivo* using a Franz-type diffusion cell. Prior to the permeation study, skin samples were defrosted and carefully trimmed into small pieces according to
the area of the donor chamber of the Franz diffusion cell (Soham Scientific, Cambridgeshire, UK). The

ex vivo porcine skins were subjected to the following treatments: i) application of 20 mg Aldara™

cream. This is in accordance with clinical dose approved by the FDA for the treatment of BCC. ii)
imiquimod loaded PVPVA microneedles. Next, the treated porcine skins were placed on top of the
receptor compartment filled with 3 ml of degassed 100 mM acetate buffer pH 3.7. This buffer was
chosen as the receptor phase in order to maintain sink conditions throughout the permeation study.
This is due to the insolubility of imiquimod at neutral or basic pH values. Various researchers have
reported using acetate buffer pH 3.7 as the receptor phase in imiquimod permeation studies (Donnelly
et al., 2006; Sharma et al., 2019; Venturini et al., 2015). The skin was then secured between the donor
and receptor compartment of the diffusion cell using a metal clamp, with the stratum corneum side
facing the donor compartment. Upon assembling the Franz diffusion cell, the permeation experiment
was conducted over a period of 24 hours in a thermostatically controlled water bath set at 36.5 °C. 1
ml of the receptor fluid at designated time points (0.5, 1, 3, 6, 12 and 24 hours) was sampled and then
replaced with equal volume of fresh 100 mM acetate buffer pH 3.7. Upon sampling, 1000 µl of the
solution from each Franz cell after collection was then spiked with 100 µl of 100 µg/ml propranolol as
an internal standard before being filtered through 0.22 µm membrane prior to HPLC analysis.

After the 24-hour permeation experiment, the excess cream was removed from the Aldara™ cream
treated skin surface by careful application of sponges soaked with 3% v/v Teepol® solution. For the
microneedle patch treated skin, the remaining microneedle patch was removed from the skin. Upon
removing excess formulation from the skin surface, 15 sequential tape strips were collected from the
skin. The amount of imiquimod from the pooled tape strips and remaining skin after tape stripping
were extracted by the addition of 10 and 5 mL of methanol extraction mixture (Methanol 70%: Acetate
Buffer pH 3.7 100 mM 30%) respectively using a previously reported method (Paula et al., 2008).
Samples were then vortexed for 1 minute and sonicated for 30 minutes before being left overnight.
Subsequently, samples were vortexed again and sonicated for a further 30 minutes.
After sonication, 1000 µl of the extracts were collected and spiked with 100 µl of 100 µg/ml
propranolol as an internal standard. The samples were then filtered through 0.22 µm membrane prior to HPLC analysis.

2.6. High performance liquid chromatography (HPLC) analysis

HPLC analysis was carried out using an Agilent 1100 series instrument (Agilent Technologies, Germany) equipped with degasser, quaternary pump, column thermostat, autosampler and UV detector. System control and data acquisition were performed using Chemostation software. The details of the HPLC chromatographic conditions are as follow: column C18 (150 × 4.6 mm) ACE3/ACE-HPLC Hichrom Limited, UK. The mobile phase composition for analysis of extracts from skin wash, donor chamber wash, pooled tape strips and remaining skin consists of 10 mM acetate buffer: acetonitrile (79:21). Whilst, the mobile phase composition for analysis of receptor fluid consists of 10 mM acetate buffer: acetonitrile (70:30). The HPLC was operated at a flow rate of 1.0 mL/minute, UV detection at λ max=226 nm, an injection volume of 40 µL and a column temperature of 25 °C.

2.7. ToF-SIMS analysis of skin cross-sections

In order to evaluate the depth of imiquimod permeation into the skin, the permeation experiments were repeated as described above Section 2.5. After the permeation study, excess formulation was removed from skin samples treated with cream and microneedles. Then, 1 cm × 1 cm of each application site was fresh frozen with liquid nitrogen. Skin cross-sectioning was performed using a cryostat (Leica CM3050 S Research Cryostat, UK). The skin slices were then thaw mounted on a glass slides and stored at -20 °C prior to ToF-SIMS analysis. ToF-SIMS was used to analyse the cryo-sectioned porcine skin samples. ToF-SIMS analysis was performed using a ToF-SIMS IV instrument (IONTOF, GmbH) with a Bi⁺ cluster source. A primary ion energy of 25 KeV was used, the primary ion dose was preserved below 1 × 10¹² per cm² to ensure static conditions. Pulsed target current of approximately 0.3 pA, and post-acceleration energy of 10 keV were employed throughout the sample analysis. The mass resolution for the instrument was 7000 at m/z 28.
2.8. Statistical analysis

Statistical analysis was conducted using GraphPad Prism 7.02 software. Data are shown as mean ± standard error of mean. When comparing two groups an unpaired t-test analysis was used, while one-way analysis of variance (ANOVA) with Tukey’s multiple comparisons tests was used to compare multiple groups. P values < 0.05 were considered statistically significant.

3. Results and Discussion

3.1. Microneedle fabrication

In this work, polymeric PVPVA microneedles of pyramidal geometry were fabricated through four manufacturing stages; structure design via CAD, micromachining, PDMS mould production and casting as shown in the schematic of Figure S1. Micro-milling was used to fabricate the designed stainless-steel master structures with minimal surface imperfections as evidenced in Figure S1. Micro-milling was used as the technique enables simplicity in the design process while offering low manufacturing cost in generating complex geometry with high accuracy and repeatability (García-López et al., 2018).

Next, the PDMS moulds were produced by micromoulding followed by microneedle patch fabrication via casting, centrifugation and drying. The resulting polymeric microneedle patch is shown in Figure 1. Upon visual inspection as shown in Figure 1 (a), we found that the microneedle array consists of 100 uniformly distributed pyramidal microneedles. Each microneedle array had a slightly opaque and off-white appearance. The microneedle patch displays micro projections, pyramidal in structure with a length of 992.3 ± 45.3 µm (mean ± SD, n=10) and a tip diameter of 32.3 ± 3.1 µm (mean ± SD, n=4) as visualised by optical microscopy images shown in Figure 1 (b) and (c). With regards to the production method used in this study, which involved centrifugation and micromoulding, such methodology is most suited for lab-based research. However, this method of microneedle fabrication would not be ideal for the manufacture of patches at a commercial scale. However, the use of aqueous drug-polymer blend casting could be potentially be translated into the scalable roller system manufacturing
method developed by Lutton et al (Lutton et al., 2015). The use of the roller system manufacturing method would provide a potential scale-up manufacturing method at a commercial scale, enabling the transition from laboratory to industry and subsequent clinical practice.

An analysis by SEM, as shown in Figure 1 (d), revealed equally spaced, sharp pyramidal microneedles, on a clean and smooth base. This clearly indicates that the copolymer PVPVA was a suitable material for fabricating polymeric microneedles. Although, PVPVA has not been used previously for microneedle fabrication, the homopolymer PVP has been widely used. In addition, the hydrophilic nature of both of these polymers makes it easy to use them for fabricating microneedles via casting and micromoulding aqueous solutions of the polymers into the PDMS moulds. This overcomes the need for organic solvents which could damage the PDMS moulds with repeated use (Mahomed et al., 2010). Previous work has shown that PVP microneedles are capable of delivering a range of therapeutics such as sumatriptan, polymyxin B, atorvastatin, aspirin, and lisinopril (Dillon et al., 2017; Quinn et al., 2015; Ronnander et al., 2018). In addition, although PVPVA has never been used previously in microneedle fabrication, the extensive biocompatible and cytocompatible data on the polymer strongly suggest that the PVPVA microneedles are safe for this biomedical application (Moore, 1983).

Preliminary microneedle fabrication without the use of PEG 400 as a plasticiser resulted in microneedles with fractured tips as shown in Figure S2. A lack of consistency in microneedle length and tip formation may result in complications such as failure to penetrate the skin as well as inconsistent drug release from the individual microneedles within the same patch. The presence of
fractured tips is attributed to the formation of brittle polymeric microneedles post-drying. The brittleness of the preliminary PVPVA microneedles was due to the high $T_g$ of the polymer at 107 °C as shown in Figure S3. Since the microneedles were demoulded at room temperature, the polymer is well below its $T_g$ and is in glassy and brittle state making it prone to fracture (Aulton, 2011). In order to overcome this issue, PEG 400 was introduced into the needle matrix as a plasticiser. The use of PEG 400 falls under the category of an external plasticiser which has been used in several other microneedle formulations (Dillon et al., 2017; Quinn et al., 2015; Sun et al., 2013).

Due to the rigid but brittle nature of PVPVA, we discovered that using the same PVPVA and PEG 400 polymer solution as a backing layer resulted in microneedle patches that fractured during the demoulding stage as shown in Figure S4. A microneedle backing layer ought to be flexible to enable easy demoulding while allowing the microneedle patch to adapt to the skin curvature during administration (Xue et al., 2015). In order to meet these criteria, we used a different polymer blend consisting of CMC and glycerol to fabricate the backing. CMC is one of the most commonly polymer solutions used in fabricating the backing layer of microneedle patches (McGrath et al., 2014; Park et al., 2016).

3.2. Microneedle characterisation

The mechanical properties of the needles were determined using a texture analyser. The polymeric microneedle arrays were subjected to an axial compression test to measure the fracture force of the polymeric microneedles. Profiles of force versus displacement (analogous to stress-strain curves), based on average force values ($n = 5$), were generated for the PVPVA polymeric microneedle patch. This force versus displacement curve was then used to determine the average fracture force per needle. From the microneedle fracture test, the PVPVA microneedles displayed a fracture force of 0.106 ± 0.003 N/needle (mean ± SD, $n=5$). The required fracture force that microneedles need to possess in order to puncture the skin without fracturing is 0.098 N/needle (Lee et al., 2015; Yu et al., 2017). In addition, Donnelley et al. has even reported successful skin insertion with microneedles that
possess a fracture force as low as 0.03 N/needle (Donnelly et al., 2012). Therefore, it can be inferred that the fabricated microneedles displayed sufficient mechanical strength above the reported threshold needed to puncture the skin without fracturing.

It is of great importance that fracture test is coupled to insertion studies in order to evaluate the penetration capability of the fabricated microneedles. The insertion of PVPVA microneedle patch into a stack of Parafilm® layers was used as an *in vitro* skin model. This was performed by applying the patches onto the Parafilm® stacks under thumb pressure. Upon application, each Parafilm layer was separated and visualised using an optical microscope to evaluate the pore uniformity as a function of penetration depth. Figure 2 (a) shows that square shaped pores, which follows the shape of the square pyramidal PVPVA microneedle, were created on the Parafilm layers upon microneedle patch application. In addition, it can be seen that the number of microneedle channels generated decreased as a function of Parafilm layer number with the deepest layer penetrated by the microneedle patch being the fourth layer as shown in the insertion profile of Figure 2 (b). In addition, it was apparent that all replicates resulted in complete microneedle insertion in the first parafilm layer as shown in Figure 2 (b). The Parafilm® insertion test was developed by Larrañeta et al as an *in vitro* test to predict the insertion capabilities of microneedles in actual skin tissues (Larrañeta et al., 2014). Collectively, Figure 2 (a) along with the insertion profile from Figure 2 (b), suggests that fabricated PVPVA microneedle patch is capable breaching the *stratum corneum* permitting microneedle insertion into the skin.
A dye release study was then performed to evaluate the ability of the microneedle patches to puncture the skin. Methylene blue loaded microneedle patch was applied onto porcine cadaver skin, which upon application resulted in the release of the hydrophilic dye to the surrounding skin tissue upon PVPVA dissolution. This results in the formation of blue microneedle channels within the porcine skin that follows the distribution of PVPVA microneedles on the patch as shown in Figure 2 (c). The histological image of the vertical section of microneedle treated skin, shown in Figure 2 (d) provides an estimate into the depth of microneedle penetration into the skin. The application of PVPVA microneedles loaded with methylene blue breached the stratum corneum and epidermis layers as shown in Figure 2(d). The average depth of microneedle penetration was 426 ± 72 µm (mean ± SD, n=10). The penetration depth of the microneedles are of approximately the same depth as that observed with the Parafilm insertion study. With regards to clinical translation of the technology, reproducible insertion of microneedles by patients and carers is an important factor to consider. Various strategies have emerged over the years to ensure effective and reproducible insertion of microneedle patches into the skin. One of the strategies could be the use of microneedle applicators. Some examples of the microneedle applicator that could be used include MicroCor™ and Macroflux® (Singh et al., 2011). Alternatively, the use of pressure-indicating sensor film such as Pressurex-micro® Green may be an alternative, might be an option in providing feedback to patient and carers that they had pressed the microneedle with sufficient force into the skin (Vicente-pérez et al., 2016)

It was apparent that the microneedle penetration depth into ex vivo skin was shorter than the length of the microneedle. This observation agrees with earlier findings by Martanto et al. who reported partial microneedle insertion into the skin (Martanto et al., 2006). Such an observation is attributed to the inherent elastic nature of the skin which resists microneedle penetration. However, since it has been reported that the thickness of the porcine epidermal layer varies between 30-140 µm (Branski et al., 2008) it can be seen that from the vertical cross-sectional data Figure 2 (a) the microneedle application in this case has reached the skin dermis. In comparison, the human skin is reported to have an epidermal thickness of 800-1500 µm for thick skin (palm of the hands and sole of the feet) and 70-
150 µm for thin skin (face, eye lids, neck and arm) (Albanna and Holmes IV, 2016). Importantly in this instance, as BCC mainly manifests on thin skin surfaces such as the face, neck and eyelid (Carr et al., 2018), it can be postulated that application of the PVPVA microneedle patch would bypass the epidermis and reach the dermis which is the target site for the treatment of nodular BCC. Besides that, in regions where there are curvatures such as the facial cheeks, nose and eyelids the application of microneedle would still be viable. However, in this instance, the use of a very flexible backing layer such as those fabricated from polyethylene glycol diacrylate (PEGDA) would allow the microneedle patch to adapt to skin curvature whilst permeating effective skin insertion (Xue et al., 2015).

The hygroscopic nature of PVPVA could have an adverse effect on the needle architecture along with its insertion capabilities on long term storage. Hence in order for this technology to be translated into clinical practice stability studies will need to be carried out to verify the needle structure. With respect to the hygroscopicity of PVPVA, relative to PVP which is widely used in microneedle research, PVPVA is less hygroscopic than PVP. The work by Shamblin and Zografi showed that the amount of water absorbed by PVPVA was one-third of that absorbed by PVP when stored at humidity levels similar to that of ambient room conditions. Such an observation is attributed to the carbonyl group of the vinyl acetate moiety which is less basic and hence less prone to hydrogen bonding than the carbonyl group in the pyrrolidone ring (Shamblin and Zografi, 1999). Therefore, the use PVPVA which is a less hygroscopic polymer than the widely used PVP, may produce microneedles which are less susceptible to moisture than the commonly fabricated PVP microneedles. Nevertheless, one of the manufacturing and distribution challenges will be the need to manufacture, distribute and store the microneedles in a low humidity environment to reduce the exposure to moisture that may affect the architecture of the needle and ultimately the insertion of the needle into the skin. One possible suggestion to overcome this is to pack the microneedle patches in nitrogen flushed sterile packets that demonstrate protection against water ingress.
Considering the fact that microneedle dosage forms penetrate the stratum corneum rather than adhere to the surface of the skin as in a conventional transdermal patch, sterility will potentially be a key requirement by regulatory bodies. Previous work by McCrudden et al. 2014 have shown that endotoxin levels in dissolving microneedles can achieve levels below set by the Food and Drug Administration (FDA) guidelines for medical devices that are in direct contact with lymphatic tissue (20 units/device) using the appropriate sterilisation techniques (McCrudden et al., 2014). As PVPVA microneedles may be susceptible to moisture due to the hygroscopic nature of polymeric microneedle, the use of heat/steam sterilisation may damage this type of microneedle necessitating microneedle production under aseptic conditions. The use of gamma irradiation may be an alternative, however previous work has shown that this method of terminal sterilisation alters the release profile of dissolving microneedles (McCrudden et al., 2014).

3.3. Drug release study from PVPVA microneedles

3.3.1. HPLC analysis

In vitro permeation studies utilising Franz diffusion cells are widely used to study the intradermal and transdermal delivery of drugs across the skin. In this work, we investigated the delivery of imiquimod from drug loaded microneedle patches into and across the skin in comparison to the commercial imiquimod cream, Aldara™. Imiquimod was loaded into the microneedles by dissolving both the polymer (PVPVA) and the drug into a polymer blend, casting the solution into the PDMS micromoulds, centrifugation and finally drying. Imiquimod-loaded polymeric PVPVA microneedles dissolved gradually in a limited volume of the skin’s interstitial fluid to release drug into and across the skin layer. It was apparent that both drug delivery systems were capable of delivering imiquimod into the stratum corneum, remaining skin and into the receptor fluid as shown in Figure 3.

From Figure 3 (a) we observed that the mean amount of imiquimod delivered into the stratum corneum as evidenced from HPLC analysis of tape strips are 23.2 µg for microneedle treated and 8.6 µg for Aldara™ cream treated skins. The differences in amount of imiquimod delivered into the
**stratum corneum** was of statistical significance (*p*<0.05). Such enhanced permeation into the **stratum corneum** with imiquimod loaded microneedles may be attributed to the generation of microneedle channels within the skin. These channels act as focal points for imiquimod to permeate laterally and localise to the surrounding corneocytes, thus enhancing delivery to the upper layer of the skin.

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However, in the case of nodular BCC the drug delivery system must be capable of delivering the drug beyond the **stratum corneum** and deeper into the skin (Williams et al., 2017). Therefore, in order to gauge the amount of drug delivered deeper into the skin, HPLC analysis was conducted on remaining skin after tape stripping. Figure 3 (a) also shows that the mean amount of imiquimod delivered into the remaining skin from both treatment groups. It was observed that the amount drug extracted from the remaining skin treated with the microneedles was lower than the amount of drug delivered by the commercial cream, **Aldara** despite such differences were not statically significant (*p*>0.05). This would suggest that the overall amount delivered per application of both delivery system are similar.

From a dose delivered perspective, it may appear that the microneedle patch does not offer any additional benefits. However, when the two systems are viewed in terms of ease of application, the microneedle patch offers a simple one-step application to insert the microneedles into the skin under thumb pressure. This avoid the issues of dosing accuracy with the amount of cream applied and problems associated with the cream spreading to non-diseased skin which are associated with **Aldara** cream. With regards to the amount of imiquimod delivered for the management of BCC, it would be more preferable to use the **Aldara** cream in the management of superficial BCC as we would mitigate the unnecessary exposure of underlying healthy skin tissue to imiquimod. However, given the fact that at least one-third of nodular BCC coexist with superficial BCC (Goldenberg et al.,
the use of imiquimod loaded microneedles in such instances would be useful as the drug could deliver equal amounts of imiquimod to tumour located at the skin surface and underlying skin tissues.

In addition, Figure 3 (b) shows the amount of imiquimod delivered into the *stratum corneum* and remaining skin when calculated as a percentage of the applied dose. In this instance the percentage of the drug successfully delivered into the *stratum corneum* and remaining skin is significantly higher with imiquimod microneedles than that of Aldara™ cream. The drug loading in the microneedle patch was $165.6 \pm 21.4 \, \mu g$ (mean $\pm$ SD, $n=8$). Whilst, $\approx 20$ mg of Aldara cream (1000 $\mu g$ of imiquimod) was applied to the skin area of 3.8 cm$^2$, in the Franz cell, which is based on clinical dose for Aldara™ cream for the treatment of BCC (FDA, 1997). Figure 3 (b) highlights that we are able to deliver similar amounts of imiquimod into the remaining skin using a different delivery system despite a 6-fold lower drug loading with the microneedle patch.

Figure 3 (c) shows the concentration of imiquimod detected in the receptor fluid over time. It can be seen that the concentration of imiquimod in the receptor fluid overtime, was similar between Aldara™ cream and imiquimod microneedle treated skin for up to 12 hours. However, at 24 hours, the concentration of imiquimod within the receptor fluid is less for skin treated with imiquimod microneedles relative to Aldara™ cream. This highlights that over the course of 24 hours, the microneedle patch resulted in less imiquimod delivery across the skin while delivering similar quantity of the drug into the remaining skin. It is understood the amount of drug detected in receptor fluid following an *in vitro* Franz cell permeation study provides an indicator on the likelihood of systemic exposure (Benson and Watkinson, 2011). Hence, it could be postulated that the likelihood for systemic exposure to imiquimod following microneedle treatment is lower in comparison to Aldara™ application. This may limit the likelihood of influenza-like symptoms, an undesirable side effect associated with imiquimod systemic exposure (Rossi et al., 2012). Based on the current work, it is suggested that the microneedle is left in the skin for 24 hours to allow comparative application and
delivery of imiquimod into the skin as to that of Aldara™ cream. In terms of practical delivery of imiquimod using microneedle relative to other route of administrations such as oral delivery, a microneedle based intradermal delivery would be more practical as it enables more targeted delivery and avoids the likelihood of systemic side effects arising from oral delivery of imiquimod which could give rise to flu-like symptoms and result in a poor overall quality of life for the patient.

In terms of enhancing imiquimod permeation, several groups have considered alternative drug delivery systems such as the use of an emulsion gel (Stein et al., 2014), transethosomes (Ma et al., 2015) and a hydrogel/oleogel colloidal mixture (Rehman et al., 2015). However, these types of formulations are typically associated with poor sensory and cosmetic issues (e.g. tackiness and stickiness) upon application. In addition, these semisolid dosage forms could potentially stain patients’ clothes as well as spread to healthy skin regions leading to unwanted side effects (Buchmann, 2005; Devaux et al., 2012). This may ultimately limit patient compliance to the overall treatment. These limitations are not encountered with microneedle formulations as the patches are anchored in place by the micro-projections ensuring precise localised delivery at the site of application. In addition, with regards to disposal of the patch upon skin insertion, as the PVPVA microneedles are dissolving microneedles, the microneedles will dissolve in the skin leaving behind the backing layer. Therefore the microneedle patch is self-disabling post-insertion overcoming the issues of dangerous sharp waste disposal in resource-poor settings (González-vázquez et al., 2017).

3.3.2. ToF-SIMS analysis

It was apparent that HPLC analysis provided quantitative results that permit us to compare the delivery efficiency of both formulations. However, HPLC does not provide any spatial information pertaining to the dermal distribution of imiquimod. In order to complement the HPLC data, ToF-SIMS was utilised to provide insight into the dermal distribution of imiquimod.

Figure 4 shows ToF-SIMS secondary ion images from skin cross-sections analysed after a 24-hour permeation study. Due to the parallel detection capabilities of the ToF-SIMS, secondary ions
originating from both the skin tissue, polymer and drug were detected and analysed. By carefully monitoring the ion peaks from the ToF-SIMS spectra (Figure S5), we are able to visualise the localisation and distribution of these secondary ions. The fragment ion for phosphatidylcholine, \( C_5H_{15}NPO_4^+ \) was used to identify the dermis and viable epidermis. Additionally, the fragment ion for ceramide, \( C_{17}H_{32}N^+ \) is utilised to distinguish the stratum corneum from the viable epidermis and dermis (Sjövall et al., 2014). This is because, the stratum corneum displays high levels of ceramide whilst being devoid of phospholipids which makes \( C_{17}H_{32}N^+ \) a good marker for the stratum corneum (Elias, 2005; Starr et al., 2019). In a previous work, it has been found that the permeation of the imiquimod across the skin could be tracked by monitoring the molecular ion \( C_{14}H_{17}N_4^+ \) (Al-Mayahy et al., 2019). Through monitoring the fragment ion peak \( C_6H_{10}NO^+ \) we were able to detect the localisation of the PVPVA polymer within the microneedle channels as shown in Figure 4. The peak assignment for PVPVA was validated by referring to fragmentation pattern at \( m/z \) 112 with the reference spectra of pure PVPVA on silicon wafer as shown in the supplementary data (Figure S6). It is worth noting that, that the parallel detection capability of the ToF-SIMS also enabled the detection of \( Si^+ \) marker - an inorganic ion of silicon - indicated in yellow in Figures S7 and S8 used to identify the glass slide (the substrate used to mount the cross-sections) as silicon is a common fundamental constituent of glass. Figures S7 and S8 also shows the total ions image collected from the ToF-SIMS sample analysis.

Figure 4 ToF-SIMS image of skin cross sections from ex vivo porcine skin that were treated with (i) Aldara™ cream (5% w/w imiquimod) alone (ii) PVPVA microneedles loaded with imiquimod after a 24-hour permeation study. Localisation of semi-dissolved polymeric microneedles within the dermis with skin treated imiquimod loaded microneedles. \( C_{14}H_{17}N_4^+ \) indicated in green is the molecular ion for imiquimod, \( C_5H_{15}NPO_4^+ \) indicated in blue is the fragment ion for phosphatidylcholine used to identify the viable epidermis and dermis. \( C_{17}H_{32}N^+ \) indicated in red is the fragment ion for ceramide used to identify the stratum corneum. \( C_6H_{10}NO^+ \) indicated in pink is the fragment ion for PVPVA polymer. Scale bar: 500 µm

It is apparent from Figure 4 (a) that there is limited availability of imiquimod within deeper skin strata when the molecule was delivered as a topical cream, Aldara™. When the drug is applied as a topical
cream, imiquimod is localised in the stratum corneum as evidenced from overlay of imiquimod molecular ion, $\text{C}_{14}\text{H}_{17}\text{N}_4^+$ with the fragment ion for ceramide, $\text{C}_{17}\text{H}_{32}\text{N}_4^+$. However, when imiquimod is delivered as a polymeric microneedle patch, we observed delivery of imiquimod into the dermis within microchannels as shown in Figure 4 (b). It is clear from the ceramide fragment ion, $\text{C}_{17}\text{H}_{32}\text{N}_4^+$ in Figure 4 (a) that the stratum corneum looks intact when the drug is administered as a topical cream. In contrast, when the drug is delivered as a polymeric microneedle patch, we observed disruption in the ceramide fragment ion, $\text{C}_{17}\text{H}_{32}\text{N}_4^+$ distribution within top layer of the skin as shown in Figure 4 (b) that suggest that the microneedles have disrupt the stratum corneum permitting the insertion of the drug loaded polymeric microneedles into the skin. Typically nodular BCC are much harder to treat effectively as the tumour typically manifests 400 µm below the skin surface (Williams et al., 2017). The ToF-SIMS analysis of skin cross-sections from Figure 4 suggests that the microneedle patch penetrated the skin to a depth of approximately 450 µm resulting in imiquimod delivery into the dermis. With regards to penetrating the BCC tumours with microneedles, concerns may be raised on the likelihood of aggravating the tumour which may lead to unintended side effects. However, there is little to no clinical concerns with regards to penetrating BCC tumour as the tumours are routinely penetrated via punch biopsy and intraoperative incisional biopsy. Such surgical procedures do not lead to any localised or distant spread of the BCC tumour. In fact, microneedle insertions are minimally invasive, therefore the damage inflicted from penetrating the BCC tumour with microneedles is less relative to these routine surgical procedures. In addition, many BCC lesions are also frequently traumatised accidentally by patients which causes localised bleeding but again no serious consequences (Jung et al., 2012; Kamyab-Hesari et al., 2014). With regards to the dermal distribution of the various components of a microneedle system, several research groups have employed techniques such as fluorescently tagging the molecule of interest in order to visually track the delivery of compound into the skin (Saurer et al., 2010; Yu et al., 2017). This method results in modification of the physiochemical properties of the drug leading to potentially inaccurate estimation of drug permeation into the skin (Vasquez et al., 2011). However, there is no
work in the field that has demonstrated the capability to simultaneously detect the deposition of both
drug and polymer from a dissolving microneedle patch in a label free manner. ToF-SIMS analysis also
provides the capability to perform parallel detection of both endogenous and exogenous chemistry
present in the analysed samples, thus permitting simultaneous mapping the presence of polymer
within biological tissues as well as the drug. The polymer that is used in fabricating the microneedle
patch is PVPVA. Through monitoring the fragment ion peak at m/z 112 we were able to detect the co-
localisation of the polymer and imiquimod within the microneedle channels as shown in Figure 4.

By comparing this finding with the ToF-SIMS analysis of skin cross-section from samples treated
imiquimod loaded microneedles in Figure 4 (b), it was observed that imiquimod was colocalised in the
presence of PVPVA polymer within the dermis following skin application. This leaves imiquimod which
is embedded in a polymer matrix within skin. It has been previously postulated that embedded drug-
polymer matrix slowly undergoes dissolution; generating localised regions of enhanced viscosity
within the skin that slows the rate of drug release to surrounding tissues (Chu and Prausnitz, 2011;
Ribeiro et al., 2017). However, in the current work we are able to demonstrate via ToF-SIMS analysis
the existence of such semi-dissolved polymeric regions within the dermis that retains drug from
permeating across the skin and into the receptor fluid. From a clinical perspective, the reduction in
imiquimod permeation across the skin may limit the likelihood of systemic side-effects. The ability of
a microneedle patch to deliver the drug to a desired location despite having lower drug loading may
serve as a possible explanation for the dose sparing advantage conferred by microneedles. To the best
of our knowledge, this is the first report of the dose sparing advantage conferred by microneedles for
small molecule therapeutics.

Although a PVPVA polymer depot was observed, there is evidence based on this commercial polymer’s
Mw i.e 15-20 kDa that it would be eventually excreted. Indeed, based on the findings from Kagan et
al on the elimination of macromolecules from the skin, it is estimated that a majority of the polymer
will be drained into the dermal blood capillaries with some drainage into the dermal lymphatics before
reaching the systemic circulation (Kagan et al., 2007). Furthermore, as the PVPVA has a Mw less than 60 kDa, the polymer will be excreted through the kidneys once it reaches the systemic circulation (Hespe et al., 1977; Yamaoka et al., 1995).

The HPLC analysis data for Aldara™ cream treated skin shown in Figure 3 (a) appears to contradict the ToF-SIMS analysis of skin cross-section with regards to amount drug delivered into the superficial layer of the skin, i.e. the stratum corneum. In order to elucidate this discrepancy, we performed a closer analysis of skin cross-sections of Aldara™ cream treated skin as shown in Figure 5. As discussed earlier, the fragment ion for phosphatidylcholine (C₅H₁₄NO₄⁺) was used to identify the dermis and viable epidermis while the fragment ion for ceramide, C₁₇H₃₂N⁺ is now utilised to distinguish the stratum corneum from the viable epidermis and dermis. Closer analysis of the Aldara™ cream treated skin showed that the majority of the molecule of interest, imiquimod resides within the stratum corneum.

Figure 5 ToF-SIMS image of skin cross sections from porcine skin that were treated with Aldara™ cream (5% w/w imiquimod) alone. This analysis was conducted at a higher resolution closer to the skin surface to image the localisation of imiquimod near the stratum corneum and epidermis junction. C₁₄H₂₁N₄⁺ the molecular ion for imiquimod indicated in green is, C₅H₁₄NO₄⁺ the fragment ion for phosphatidylcholine used to identify the viable epidermis and dermis indicated in blue. C₁₇H₃₂N⁺ is the fragment ion for ceramide used to identify the stratum corneum indicated in red. The overlays highlights imiquimod localisation within the stratum corneum and some near the epidermis just below the stratum corneum. Scale bar: 100 µm

With regards to tape stripping as a method to quantify drug permeation into the superficial layer of the skin, various groups have observed a reduction in corneocytes removed with tape strip number. Such reduction in corneocyte extraction is attributed to the increase in corneocyte cohesion with stratum corneum depth (Chapman et al., 1991; Jacobi et al., 2006; Lademann et al., 2004). In addition, the increase in skin hydration with skin depth also reduces the ability of the tape adhesive to remove the corneocyte during the stripping process (Egawa et al., 2006). When analysing the distribution of imiquimod on the Aldara™ treated skin from Figure 4, it may appear that most imiquimod is with the top layer of the skin. However, upon closer cross-sectional analysis (Figure 5 a) majority of the...
molecule of interest, imiquimod resides within the deeper layer of the stratum corneum. These layers are not so easily removed by tape stripping and thus are extracted with the remaining skin.

4. Conclusions

In conclusion, the current work highlights the design, fabrication, evaluation and application of drug loaded polymeric microneedles as a drug delivery platform for the intradermal delivery of imiquimod for the treatment of nodular BCC. Permeation studies utilising Franz diffusion cells demonstrated that the imiquimod loaded polymeric microneedles were capable of delivering similar quantities of imiquimod to the region of tumours, despite a 6-fold lower drug loading, relative to the current clinical dose of Aldara™ cream used in BCC treatment. This ability of the polymeric microneedle to deliver the drug to the right target site despite lower drug loading may be of economic benefit while also limiting the likelihood of side effects. Using a microneedle patch, imiquimod loaded polymeric microneedles are mechanically inserted and embedded within the dermis upon application which is the target site for the treatment of nodular BCC. ToF-SIMS analysis of skin cross-sections highlighted the presence of the embedded drug-polymer matrix within the skin, which retains the drug in the dermis while reducing the permeation of the drug across the skin. This provides evidence to support the mechanistic understanding of how the embedded drug-polymer matrix following polymeric microneedles administration controls the release of drugs. In summary, this work suggests that imiquimod loaded polymeric microneedles may be of clinical utility for localised intradermal delivery of imiquimod. Such formulations may provide a less invasive intervention to patients who would prefer an alternative treatment to surgery for the treatment of nodular BCC.
Declaration of Interest: None

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References


Basal cell carcinoma: Epidemiology; pathophysiology; clinical and histological subtypes; and

Carcinoma in a Non-Sun-Exposed Area: A Rare Presentation Mimicking Recurrent Perianal


249. doi:10.1016/j.jconrel.2010.10.033


Pathol. 19. doi:10.1038/modpathol.3800512

Devaux, S., Castela, A., Archier, E., Gallini, A., Joly, P., Misery, L., Aractingi, S., Aubin, F., Bachelez, H.,
topical treatment in psoriasis: A systematic literature review. J. Eur. Acad. Dermatology


Dillon, C., Hughes, H., Reilly, N.J.O., McLoughlin, P., 2017. Formulation and characterisation of
d dissolving microneedles for the transdermal delivery of therapeutic peptides. Int. J. Pharm.

Polymeric Microneedle Arrays Prepared by a Novel Laser-Based Micromoulding Technique 41–734. doi:10.1007/s11095-010-0169-8


FDA, 1997. Food and Drug Administration approval for imiquimod - Aldara cream 5%.


Teodorescu, M., Bercea, M., 2015. Polymer-Plastics Technology and Engineering Poly (vinylpyrrolidone) – A Versatile Polymer for Biomedical and Beyond Medical Applications Poly (vinylpyrrolidone) – A Versatile Polymer for Biomedical and Beyond Medical Applications.


Figure legends

Figure 1  a) Photograph image of PVP VA microneedles post-fabrication (b) Optical microscopy image of PVP VA microneedles at 12.5x magnification (c) close up microscopy image - of a single PVP VA polymeric microneedle at 50x magnification (d) SEM images of PVP VA microneedles at 39x magnification.

Figure 2  (a) Microscopic images of first Parafilm® treated by PVP VA microneedles (b) Insertion profile of PVP VA microneedle into Parafilm® layers (c) Microchannels created in ex vivo porcine skin visualised via the release of methylene blue from PVP VA microneedles (d) Optical microscopy image of microneedle channels created when the skin is treated with PVP VA microneedles

Figure 3  Amount of drug extracted from skin (tape strips and remaining skin) after 24 hours in the permeation study. Drug concentration within receptor fluid as a function of time during permeation study from AldaraTM and imiquimod microneedles. Data are expressed as mean± SEM for n=6. Differences were calculated using one-way ANOVA, followed by Tukey’s post hoc test, and deemed significant at p<0.05. n.s = not statistically significant at p>0.05

Figure 4  ToF-SIMS image of skin cross sections from ex vivo porcine skin that were treated with (i) AldaraTM cream (5% w/w imiquimod) alone (ii) PVP VA microneedles loaded with imiquimod after a 24-hour permeation study. Localisation of semi-dissolved polymeric microneedles within the dermis with skin treated imiquimod loaded microneedles. $\text{C}_{14}\text{H}_{17}\text{N}_4^+$ inidicated in green is the molecular ion for imiquimod, $\text{C}_{6}\text{H}_{15}\text{NPO}_4^+$ indicated in blue is the fragment ion for phosphatidylcholine used to identify the viable epidermis and dermis. $\text{C}_{17}\text{H}_{32}\text{N}_4^+$ indicated in red is the fragment ion for ceramide used to identify the stratum corneum. $\text{C}_{6}\text{H}_{10}\text{NO}_4^+$ indicated in pink is the fragment ion for PVPVA polymer. Scale bar: 500 µm
Figure 5 ToF-SIMS image of skin cross sections from porcine skin that were treated with Aldara™ cream (5% w/w imiquimod) alone. This analysis was conducted at a higher resolution closer to the skin surface to image the localisation of imiquimod near the stratum corneum and epidermis junction. C$_{14}$H$_{17}$N$_4^+$ the molecular ion for imiquimod indicated in green is, C$_5$H$_{13}$NPO$_4^+$ the fragment ion for phosphatidylcholine used to identify the viable epidermis and dermis indicated in blue. C$_{17}$H$_{32}$N$^+$ is the fragment ion for ceramide used to identify the stratum corneum indicated in red. The overlays highlights imiquimod localisation within the stratum corneum and some near the epidermis just below the stratum corneum. Scale bar: 100 µm