Intradermal delivery of imiquimod using polymeric microneedles for basal cell carcinoma

Akmal H. Sabri [a], *Zachary Cater* [b], *Pratik Gurnani* [a], *Jane Ogilvie* [c], *Joel Segal* [b], *David J. Scurr* [a] *and Maria Marlow* [a*]

[a] School of Pharmacy, University of Nottingham, Nottingham NG72RD, United Kingdom.

[b] Advanced Manufacturing Technology Research Group, Faculty of Engineering, University of Nottingham, Nottingham, NG8 1BB

[c] Walgreens Boots Alliance, Thane Road, Nottingham, NG90 1BS.

*Corresponding author

Dr Maria Marlow: Maria.Marlow@nottingham.ac.uk

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. I*n 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 groups (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. Polyvinylpyrollidone-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. Polyvinylpyrollidone-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 p*orcine 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. A

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 ^{o}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 ^{o}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

191 fabrication process was repeated in a similar fashion and composition, however the drug, PEG 400
192 and PVPVA were dissolved in 0.05 M of hydrochloric acid under stirring for 1 hour.

193 2.4. Characterisation of PVPVA microneedles (SEM, tensile strength, skin insertion
194 properties)

195 2.4.1. Microscopy

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

200 2.4.2. Measurement of needle fracture force

201 The needle fracture force of the polymeric microneedles was determined using a texture analyser
202 (Stable Microsystems, UK) following a previously reported method (Donnelly et al., 2011). This is to
203 investigate the effect of applying an axial force parallel to the microneedle vertical axis, similar to the
204 force encountered by the needles during application to the skin. The polymeric microneedles were
205 visually inspected before and after application of the compression force. For this, the force required
206 for compression of the polymeric microneedle to a specified distance was measured. The polymeric
207 microneedles were attached to a 10 mm cylindrical Delrin probe (part code P/ 10) using double-sided
208 adhesive tape. The probe is connected to a 50-kg load cell and was set at the same distance from the
209 platform for all the test measurements. The TA XT Plus Texture Analyser was set to compression, the
210 pre-test speed was set at 2 mm/s and post-test speed at 10 mm/s. The trigger type was set to auto
211 (force) with a trigger force of 0.009 N. The test station compresses the polymeric microneedle against
212 a flat block of aluminium of dimensions 10.0 × 9.0 cm. Compression force versus displacement curves
213 were plotted to calculate the fracture force. A total of five microneedle patches were used to evaluate
214 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 utilised 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 visualise 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 ^{o}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^{TM} 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 ^{o}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_3^+ cluster source. A primary ion energy of 25 KeV was used, the primary ion dose was preserved below 1×10^{12} per cm^2 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.

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

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 ^oC 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 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.

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

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 that 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.

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 Aldara™ 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

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™ although 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.,

2010), 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 ± 21.4 µg (mean ± SD, *n*=8). Whilst, ≈20 mg of Aldara cream (1000 µg 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 a 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. $C_{14}H_{17}N_4^+$inidicated 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

565 cream, imiquimod is localised in the *stratum corneum* as evidenced from overlay of imiquimod
566 molecular ion, $C_{14}H_{17}N_4^+$ with the fragment ion for ceramide, $C_{17}H_{32}N^+$. However, when imiquimod is
567 delivered as a polymeric microneedle patch, we observed delivery of imiquimod into the dermis within
568 microchannels as shown in Figure 4 (b). It is clear from the ceramide fragment ion, $C_{17}H_{32}N^+$ in
569 Figure 4 (a) that the *stratum corneum* looks intact when the drug is administered as a topical cream.
570 In contrast, when the drug is delivered as a polymeric microneedle patch, we observed disruption in
571 the ceramide fragment ion, $C_{17}H_{32}N^+$ distribution within top layer of the skin as shown in Figure 4 (b)
572 that suggest that the microneedles have disrupt the *stratum corneum* permitting the insertion of the
573 drug loaded polymeric microneedles into the skin. Typically nodular BCC are much harder to treat
574 effectively as the tumour typically manifests 400 µm below the skin surface (Williams et al., 2017).
575 The ToF-SIMS analysis of skin cross-sections from Figure 4 suggests that the microneedle patch
576 penetrated the skin to a depth of approximately 450 µm resulting in imiquimod delivery into the
577 dermis. With regards to penetrating the BCC tumours with microneedles, concerns may be raised on
578 the likelihood of aggravating the tumour which may lead to unintended side effects. However, there
579 is little to no clinical concerns with regards to penetrating BCC tumour as the tumours are routinely
580 penetrated via punch biopsy and intraoperative incisional biopsy. Such surgical procedures do not lead
581 to any localised or distant spread of the BCC tumour. In fact, microneedle insertions are minimally
582 invasive, therefore the damage inflicted from penetrating the BCC tumour with microneedles is less
583 relative to these routine surgical procedures. In addition, many BCC lesions are also frequently
584 traumatised accidentally by patients which causes localised bleeding but again no serious
585 consequences (Jung et al., 2012; Kamyab-Hesari et al., 2014).

586 With regards to the dermal distribution of the various components of a microneedle system, several
587 research groups have employed techniques such as fluorescently tagging the molecule of interest in
588 order to visually track the delivery of compound into the skin (Saurer et al., 2010; Yu et al., 2017). This
589 method results in modification of the physiochemical properties of the drug leading to potentially
590 inaccurate estimation of drug permeation into the skin (Vasquez et al., 2011). However, there is no

591 work in the field that has demonstrated the capability to simultaneously detect the deposition of both
592 drug and polymer from a dissolving microneedle patch in a label free manner. ToF-SIMS analysis also
593 provides the capability to perform parallel detection of both endogenous and exogenous chemistry
594 present in the analysed samples, thus permitting simultaneous mapping the presence of polymer
595 within biological tissues as well as the drug. The polymer that is used in fabricating the microneedle
596 patch is PVPVA. Through monitoring the fragment ion peak at *m/z* 112 we were able to detect the co-
597 localisation of the polymer and imiquimod within the microneedle channels as shown in Figure 4.

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

612 Although a PVPVA polymer depot was observed, there is evidence based on this commercial polymer's
613 Mw i.e 15-20 kDa that it would be eventually excreted. Indeed, based on the findings from Kagan *et*
614 *al* on the elimination of macromolecules from the skin, it is estimated that a majority of the polymer
615 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 ($C_5H_{15}NPO_4^+$) was used to identify the dermis and viable epidermis while the fragment ion for ceramide, $C_{17}H_{32}N^+$ 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. $C_{14}H_{17}N_4^+$ the molecular ion for imiquimod indicated in green is, $C_5H_{15}NPO_4^+$ 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

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

642 molecule of interest, imiquimod resides within the deeper layer of the *stratum corneum*. These layers
643 are not so easily removed by tape stripping and thus are extracted with the remaining skin.

644 4. Conclusions

645 In conclusion, the current work highlights the design, fabrication, evaluation and application of drug
646 loaded polymeric microneedles as a drug delivery platform for the intradermal delivery of imiquimod
647 for the treatment of nodular BCC. Permeation studies utilising Franz diffusion cells demonstrated that
648 the imiquimod loaded polymeric microneedles were capable of delivering similar quantities of
649 imiquimod to the region of tumours, despite a 6-fold lower drug loading, relative to the current clinical
650 dose of Aldara™ cream used in BCC treatment. This ability of the polymeric microneedle to deliver the
651 drug to the right target site despite lower drug loading may be of economic benefit while also limiting
652 the likelihood of side effects. Using a microneedle patch, imiquimod loaded polymeric microneedles
653 are mechanically inserted and embedded within the dermis upon application which is the target site
654 for the treatment of nodular BCC. ToF-SIMS analysis of skin cross-sections highlighted the presence of
655 the embedded drug-polymer matrix within the skin, which retains the drug in the dermis while
656 reducing the permeation of the drug across the skin. This provides evidence to support the
657 mechanistic understanding of how the embedded drug-polymer matrix following polymeric
658 microneedles administration controls the release of drugs. In summary, this work suggests that
659 imiquimod loaded polymeric microneedles may be of clinical utility for localised intradermal delivery
660 of imiquimod. Such formulations may provide a less invasive intervention to patients who would prefer
661 an alternative treatment to surgery for the treatment of nodular BCC.

662

663

664

665

Declaration of Interest: None

Acknowledgement

This work was supported by the following funding: The University of Nottingham Centre for Doctoral Training in Advanced Therapeutics and Nanomedicine, Walgreens Boots Alliance and the EPSRC [grant number: EP/L01646X/1] via a PhD sponsorship for Akmal Sabri. Optical microscopy and skin cryo-sectioning was carried out in the University of Nottingham, School of Life Sciences Imaging Unit (SLIM): https://www.nottingham.ac.uk/life-sciences/facilities/slim/index.aspx . This research is also funded by the Department of Health and Social Care using UK Aid funding and is managed by the Engineering and Physical Sciences Research Council (EPSRC, grant number: EP/R013764/1). The views expressed in this publication are those of the author(s) and not necessarily those of the Department of Health and Social Care.

References

Al-Mayahy, M.H., Sabri, A.H., Rutland, C.S., Holmes, A., McKenna, J., Marlow, M., Scurr, D.J., 2019. Insight into imiquimod skin permeation and increased delivery using microneedle pre-treatment. Eur. J. Pharm. Biopharm. 139, 33–43. doi:10.1016/j.ejpb.2019.02.006

Albanna, M., Holmes IV, J., 2016. Skin Tissue Engineering and Regenerative Medicine, First Edit. ed. Academic Press, London.

Aulton, M.E., 2011. Aulton's Pharmaceutics The Design And Manufacture of Medicines, Third. ed. Churchill Livingstone, China.

Bal, S., Kruithof, A.C., Liebl, H., Tomerius, M., Bouwstra, J., Lademann, J., Meinke, M., 2010. In vivo visualization of microneedle conduits in human skin using laser scanning microscopy. Laser Phys. Lett. 7, 242–246. doi:10.1002/lapl.200910134

Benech-Kieffer, F., Wegrich, P., Schwarzenbach, R., Klecak, G., Weber, T., Leclaire, J., Schaefer, H., 2000. Percutaneous Absorption of Sunscreens in vitro: Interspecies Comparison, Skin Models and Reproducibility Aspects. Skin Pharmacol. Physiol. 13, 324–335. doi:10.1159/000029940

Benson, H.A.E., Watkinson, A.C. (Eds.), 2011. Topical and Transdermal Drug Delivery. John Wiley & Sons, Inc., Hoboken, NJ, USA. doi:10.1002/9781118140505

Branski, L.K., Mittermayr, R., Herndon, D.N., Norbury, W.B., Masters, O.E., Hofmann, M., Traber, D.L., Redl, H., Jeschke, M.G., 2008. A porcine model of full-thickness burn, excision and skin autografting. Burns 34, 1119–1127. doi:10.1016/j.burns.2008.03.013

Buchmann, S., 2005. Main Cosmetic Vehicles, in: Barel, A., Paye, M., Maibach, H. (Eds.), Handbook of Cosmetic Science and Technology. Taylor & Francis, New York, United States of America, pp. 99–124.

Cameron, M.C., Lee, E., Hibler, B.P., Barker, C.A., Mori, S., Cordova, M., Nehal, K.S., Rossi, A.M., 2019.

711 Basal cell carcinoma: Epidemiology; pathophysiology; clinical and histological subtypes; and
712 disease associations. J. Am. Acad. Dermatol. 80, 303–317. doi:10.1016/j.jaad.2018.03.060

713 Carr, A. V., Feller, E., Zakka, F.R., Griffith, R.C., Schechter, S., 2018. A Case Report of Basal Cell
714 Carcinoma in a Non-Sun-Exposed Area: A Rare Presentation Mimicking Recurrent Perianal
715 Abscess. Case Rep. Surg. 2018, 1–5. doi:10.1155/2018/9021289

716 Chapman, S.J., Walsh, A., Jackson, S.M., Friedmann, P.S., 1991. Lipids, proteins and corneocyte
717 adhesion. Arch. Dermatol. Res. 283, 167–73. doi:10.1007/BF00372057

718 Chu, L.Y., Prausnitz, M.R., 2011. Separable arrowhead microneedles. J. Control. Release 149, 242–
719 249. doi:10.1016/j.jconrel.2010.10.033

720 Colver, G.B., 2002. Skin Cancer A Practical Guide to Management, First. ed. Taylor & Francis, London.

721 Crowson, A.N., 2006. Basal cell carcinoma : biology , morphology and clinical implications. Mod.
722 Pathol. 19. doi:10.1038/modpathol.3800512

723 Devaux, S., Castela, A., Archier, E., Gallini, A., Joly, P., Misery, L., Aractingi, S., Aubin, F., Bachelez, H.,
724 Cribier, B., Jullien, D., Le Maître, M., Richard, M.A., Ortonne, J.P., Paul, C., 2012. Adherence to
725 topical treatment in psoriasis: A systematic literature review. J. Eur. Acad. Dermatology
726 Venereol. 26, 61–67. doi:10.1111/j.1468-3083.2012.04525.x

727 Diffey, B.L., Langtry, J.A.A., 2005. Skin cancer incidence and the ageing population. Br. J. Dermatol.
728 153, 679–680. doi:10.1111/j.1365-2133.2005.06799.x

729 Dillon, C., Hughes, H., Reilly, N.J.O., Mcloughlin, P., 2017. Formulation and characterisation of
730 dissolving microneedles for the transdermal delivery of therapeutic peptides. Int. J. Pharm.
731 526, 125–136. doi:10.1016/j.ijpharm.2017.04.066

732 Donnelly, R.F., Majithiya, R., Raghu, T., Singh, R., Morrow, D.I.J., Garland, M.J., Demir, Y.K., Ryan, E.,
733 Gillen, D., Scott, C.J., Woolfson, A.D., 2011. Design , Optimization and Characterisation of

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

736 Donnelly, R.F., McCarron, P.A., Zawislak, A.A., David Woolfson, A., 2006. Design and physicochemical
737 characterisation of a bioadhesive patch for dose-controlled topical delivery of imiquimod. Int. J.
738 Pharm. 307, 318–325. doi:10.1016/j.ijpharm.2005.10.023

739 Donnelly, R.F., Singh, T.R.R., Garland, M.J., Migalska, K., Majithiya, R., McCrudden, C.M., Kole, P.L.,
740 Mahmood, T.M.T., McCarthy, H.O., Woolfson, A.D., 2012. Hydrogel-forming microneedle arrays
741 for enhanced transdermal drug delivery. Adv. Funct. Mater. 22, 4879–4890.
742 doi:10.1002/adfm.201200864

743 Egawa, M., Arimoto, H., Hirao, T., Takahashi, M., Ozaki, Y., 2006. Regional difference of water
744 content in human skin studied by diffuse-reflectance near-infrared spectroscopy: Consideration
745 of measurement depth. Appl. Spectrosc. 60, 24–28. doi:10.1366/000370206775382866

746 Elias, P.M., 2005. Stratum Corneum Defensive Functions : An Integrated View. J. Invest. Dermatol.
747 125, 183–200. doi:10.1111/j.0022-202X.2005.23668.x

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

749 García-López, E., Siller, H.R., Rodríguez, C.A., 2018. Study of the fabrication of AISI 316L microneedle
750 arrays. Procedia Manuf. 26, 117–124. doi:10.1016/j.promfg.2018.07.014

751 Goldenberg, G., Golitz, L.., Fitzpatrick, J., 2010. Histopathology of Skin Cancer, in: Stockfleth, E.,
752 Rosen, T., Schumaak, S. (Eds.), Managing Skin Cancer. Springer-Verlag Berlin Heidelberg,
753 London, pp. 17–35. doi:10.1007/978-3-540-79347-2

754 González-vázquez, P., Larrañeta, E., Mccrudden, M.T.C., Jarrahian, C., Rein-weston, A., Quintanar-
755 solares, M., Zehrung, D., Mccarthy, H., Courtenay, A.J., Donnelly, R.F., 2017. Transdermal
756 delivery of gentamicin using dissolving microneedle arrays for potential treatment of neonatal
757 sepsis. J. Control. Release 0–1. doi:10.1016/j.jconrel.2017.07.032

758 Hespe, W., Meier, A.M., Blankwater, Y.J., 1977. Excretion and distribution studies in rats with two
759 forms of 14carbon-labelled polyvinylpyrrolidone with a relatively low mean molecular weight
760 after intravenous administration - PubMed [WWW Document]. Arzneimittelforschung. URL
761 https://pubmed.ncbi.nlm.nih.gov/578432/ (accessed 7.25.20).

762 Jacobi, U., Tassopoulos, T., Surber, C., Lademann, J., 2006. Cutaneous distribution and localization of
763 dyes affected by vehicles all with different lipophilicity. Arch. Dermatol. Res. 297, 303–310.
764 doi:10.1007/s00403-005-0621-5

765 Jansen, M.H.E., Mosterd, K., Arits, A.H.M.M., Roozeboom, M.H., Sommer, A., Essers, B.A.B., Pelt,
766 H.P.A. Van, Quaedvlieg, P.J.F., Steijlen, P.M., Nelemans, P.J., Kelleners-smeets, N.W.J., 2017.
767 Five-Year Results of a Randomized Controlled Trial Comparing Effectiveness of Photodynamic
768 Therapy , Topical Imiquimod , and Topical 5-Fluorouracil in Patients with Superficial Basal Cell
769 Carcinoma. J. Invest. Dermatol. 138, 527–533. doi:10.1016/j.jid.2017.09.033

770 Jung, J.E., Rah, D.K., Kim, Y.O., 2012. Effects of preoperative biopsies on recurrence in head and neck
771 skin cancer. Arch. Plast. Surg. 39, 518–521. doi:10.5999/aps.2012.39.5.518

772 Kagan, L., Gershkovich, P., Mendelman, A., Amsili, S., Ezov, N., Hoffman, A., 2007. The role of the
773 lymphatic system in subcutaneous absorption of macromolecules in the rat model. Eur. J.
774 Pharm. Biopharm. 67, 759–765. doi:10.1016/j.ejpb.2007.04.002

775 Kamyab-Hesari, K., Seirafi, H., Naraghi, Z.S., Shahshahani, M.M., Rahbar, Z., Damavandi, M.R.,
776 Naraghi, M.M., Rezvani, M., Aghazadeh, N., 2014. Diagnostic accuracy of punch biopsy in
777 subtyping basal cell carcinoma. J. Eur. Acad. Dermatology Venereol. 28, 250–253.
778 doi:10.1111/j.1468-3083.2012.04695.x

779 Kolter, K., Flick, D., 2000. Structure and dry binding activity of different polymers, including Kollidon®
780 VA 64. Drug Dev. Ind. Pharm. 26, 1159–1165. doi:10.1081/DDC-100100987

781 Kuijpers, D.I.M., Thissen, M.R.T.M., Neumann, M.H.A., 2002. Basal cell carcinoma: treatment options

782 and prognosis, a scientific approach to a common malignancy. Am. J. Clin. Dermatol. 3, 247–
783 259.

784 Lacarrubba, F., Nasca, M.R., Micali, G., 2008. Advances in the use of topical imiquimod to treat
785 dermatologic disorders. Ther. Clin. Risk Manag. 4, 87–97. doi:10.2147/tcrm.s1109

786 Lademann, J., Rudolph, A., Jacobi, U., Weigmann, H.-J., Schaefer, H., Sterry, W., Meinke, M., 2004.
787 Influence of nonhomogeneous distribution of topically applied UV filters on sun protection
788 factors. J. Biomed. Opt. 9, 1358. doi:10.1117/1.1805557

789 Larrañeta, E., Moore, J., Vicente-Pérez, E.M., González-Vázquez, P., Lutton, R., Woolfson, A.D.,
790 Donnelly, R.F., 2014. A proposed model membrane and test method for microneedle insertion
791 studies. Int. J. Pharm. 472, 65–73. doi:10.1016/j.ijpharm.2014.05.042

792 Lee, J.Y., Park, S.H., Seo, I.H., Lee, K.J., Ryu, W.H., 2015. Rapid and repeatable fabrication of high A/R
793 silk fibroin microneedles using thermally-drawn micromolds. Eur. J. Pharm. Biopharm. 94, 11–
794 19. doi:10.1016/j.ejpb.2015.04.024

795 Lien, M.H., Sondak, V.K., 2011. Nonsurgical treatment options for Basal cell carcinoma. J. Skin Cancer
796 2011, 571734. doi:10.1155/2011/571734

797 Lutton, R.E.M., Larrañeta, E., Kearney, M., Boyd, P., Woolfson, A.D., Donnelly, R.F., 2015. A novel
798 scalable manufacturing process for the production of hydrogel-forming microneedle arrays. Int.
799 J. Pharm. 494, 417–429. doi:10.1016/j.ijpharm.2015.08.049

800 Ma, M., Wang, J., Guo, F., Lei, M., Tan, F., Li, N., 2015. Development of nanovesicular systems for
801 dermal imiquimod delivery: physicochemical characterization and in vitro/in vivo evaluation. J.
802 Mater. Sci. Mater. Med. 26. doi:10.1007/s10856-015-5524-1

803 Mackiewicz-Wysocka, M., Bowszyc-Dmochowska, M., Strzelecka-Węklar, D., Dańczak-Pazdrowska,
804 A., Adamski, Z., 2013. Reviews Basal cell carcinoma – diagnosis. Współczesna Onkol. 4, 337–
805 342. doi:10.5114/wo.2013.35684

806 Mahomed, A., Hukins, D.W.L., Kukureka, S.N., 2010. Swelling of medical grade silicones in liquids and
807 calculation of their cross-link densities. Med. Eng. Phys. 32, 298–303.
808 doi:10.1016/j.medengphy.2009.12.004

809 Martanto, W., Moore, J.S., Couse, T., Prausnitz, M.R., 2006. Mechanism of fluid infusion during
810 microneedle insertion and retraction. J. Control. Release 112, 357–361.
811 doi:10.1016/j.jconrel.2006.02.017

812 Mccrudden, M.T.C., Alkilani, A.Z., Courtenay, A.J., Mccrudden, C.M., Mccloskey, B., Walker, C.,
813 Alshraiedeh, N., Lutton, R.E.M., Gilmore, B.F., Woolfson, A.D., Donnelly, R.F., 2014.
814 Considerations in the sterile manufacture of polymeric microneedle arrays. Drug Deliv. Transl.
815 Res. 5, 3–14. doi:10.1007/s13346-014-0211-1

816 McGrath, M.G., Vucen, S., Vrdoljak, A., Kelly, A., O'Mahony, C., Crean, A.M., Moore, A., 2014.
817 Production of dissolvable microneedles using an atomised spray process: Effect of microneedle
818 composition on skin penetration. Eur. J. Pharm. Biopharm. 86, 200–211.
819 doi:10.1016/j.ejpb.2013.04.023

820 Mehta, K.S., Mahajan, V.K., Chauhan, P.S., Sharma, A.L., Sharma, V., Abhinav, C., Khatri, G., Prabha,
821 N., Sharma, S., Negi, M., 2012. Metastatic Basal Cell Carcinoma: A Biological Continuum of
822 Basal Cell Carcinoma? Case Rep. Dermatol. Med. 2012, 1–4. doi:10.1155/2012/157187

823 Moore, A., 1983. 7 Final Report on the Safety Assessment of Polyvinylpyrrolidone/Vinyl Acetate
824 Copolymer. Int. J. Toxicol. 2, 141–159. doi:10.3109/10915818309140719

825 Naguib, Y.W., Kumar, A., Cui, Z., 2014. The effect of microneedles on the skin permeability and
826 antitumor activity of topical 5-fluorouracil. Acta Pharm. Sin. B 4, 94–99.
827 doi:10.1016/j.apsb.2013.12.013

828 Nijssen, A., Bakker Schut, T.C., Heule, F., Caspers, P.J., Hayes, D.P., Neumann, M.H.A., Puppels, G.J.,
829 2002. Discriminating basal cell carcinoma from its surrounding tissue by raman spectroscopy. J.

Invest. Dermatol. 119, 64–69. doi:10.1046/j.1523-1747.2002.01807.x

Park, Y., Ha, S.K., Choi, I., Kim, K.S., Park, J., Choi, N., Kim, B., 2016. Fabrication of Degradable Carboxymethyl Cellulose (CMC) Microneedle with Laser Writing and Replica Molding Process for Enhancement of Transdermal Drug Delivery 118, 110–118. doi:10.1007/s12257-015-0634-7

Patel, D., Kumar, P., Thakkar, H.P., 2015. Journal of Drug Delivery Science and Technology Lopinavir metered-dose transdermal spray through microporated skin : Permeation enhancement to achieve therapeutic needs. J. Drug Deliv. Sci. Technol. 29, 173–180. doi:10.1016/j.jddst.2015.07.004

Paula, D. De, Martins, C.A., Bentley, M.V.L.B., 2008. Development and validation of HPLC method for imiquimod determination in skin penetration studies. Biomed. Chromatogr. 22, 1416–1423. doi:10.1002/bmc.1075

Prausnitz, M.R., 2004. Microneedles for transdermal drug delivery. Adv. Drug Deliv. Rev. 56, 581–587. doi:10.1016/J.ADDR.2003.10.023

Quinn, H.L., Bonham, L., Hughes, C.M., Donnelly, R.F., 2015. Design of a Dissolving Microneedle Platform for Transdermal Delivery of a Fixed-Dose Combination of Cardiovascular Drugs. J. Pharm. Sci. 104, 3490–3500. doi:10.1002/jps.24563

Rehman, K., Aluwi, M.F.F.M., Rullah, K., Wai, L.K., Mohd Amin, M.C.I., Zulfakar, M.H., 2015. Probing the effects of fish oil on the delivery and inflammation-inducing potential of imiquimod. Int. J. Pharm. 490, 131–141. doi:10.1016/j.ijpharm.2015.05.045

Ribeiro, L.N.M., Alcântara, A.C.S., Rodrigues, G.H., Franz-montan, M., Nista, S.V.G., Castro, S.R., Couto, V.M., Guilherme, V.A., Paula, E. De, 2017. Advances in Hybrid Polymer-Based Materials for Sustained Drug Release 2017. doi:10.1155/2017/1231464

Ronnander, P., Simon, L., Spilgies, H., Koch, A., Scherr, S., 2018. Dissolving polyvinylpyrrolidone-based microneedle systems for in-vitro delivery of sumatriptan succinate. Eur. J. Pharm. Sci.

114, 84–92. doi:10.1016/j.ejps.2017.11.031

Rossi, A., Cantisani, C., Melis, L., Iorio, A., Scali, E., Calvieri, S., 2012. Minoxidil Use in Dermatology, Side Effects and Recent Patents. Recent Pat. Inflamm. Allergy Drug Discov. 6, 130–136. doi:10.2174/187221312800166859

Sabri, A., Ogilvie, J., Abdulhamid, K., Shpadaruk, V., McKenna, J., Segal, J., Scurr, D.J., Marlow, M., 2019. Expanding the applications of microneedles in dermatology. Eur. J. Pharm. Biopharm. 140, 121–140. doi:10.1016/j.ejpb.2019.05.001

Sabri, A., Ogilvie, J., McKenna, J., Segal, J., Scurr, D., Marlow, M., 2020. Intradermal delivery of an immunomodulator for basal cell carcinoma; expanding the mechanistic insight in solid microneedle enhanced delivery of hydrophobic molecules. Mol. Pharm. doi:10.1021/acs.molpharmaceut.0c00347

Sauder, D.N., 2000. Immunomodulatory and pharmacologic properties of imiquimod. J. Am. Acad. Dermatol. 43, S6–S11. doi:10.1067/mjd.2000.107808

Saurer, E.M., Flessner, R.M., Sullivan, S.P., Prausnitz, M.R., Lynn, D.M., 2010. Layer-by-layer assembly of DNA- and protein-containing films on microneedles for drug delivery to the skin. Biomacromolecules 11, 3136–3143. doi:10.1021/bm1009443

Shamblin, S.L., Zografi, G., 1999. The effects of absorbed water on the properties of amorphous mixtures containing sucrose. Pharm. Res. doi:10.1023/A:1018960405504

Sharma, M., Sharma, G., Singh, B., Katare, O.P., 2019. Systematically Optimized Imiquimod-Loaded Novel Hybrid Vesicles by Employing Design of Experiment (DoE) Approach with Improved Biocompatibility, Stability, and Dermatokinetic Profile. AAPS PharmSciTech 20. doi:10.1208/s12249-019-1331-1

Singh, T.R.R., Dunne, N.J., Cunningham, E., Donnelly, R.F., 2011. Review of patents on microneedle applicators. Recent Pat. Drug Deliv. Formul. 5, 11–23. doi:10.2174/187221111794109484

878 Sjövall, P., Greve, T.M., Clausen, S.K., Moller, K., Eirefelt, S., Johansson, B., Nielsen, K.T., 2014.
879 Imaging of distribution of topically applied drug molecules in mouse skin by combination of
880 time-of-flight secondary ion mass spectrometry and scanning electron microscopy. Anal. Chem.
881 86, 3443–3452. doi:10.1021/ac403924w

882 Starr, N.J., Abdul, K., Wibawa, J., Marlow, I., Bell, M., Pérez-garcía, L., Barrett, D.A., Scurr, D.J., 2019.
883 Enhanced vitamin C skin permeation from supramolecular hydrogels , illustrated using in situ
884 ToF-SIMS 3D chemical profiling. Int. J. Pharm. 563, 21–29. doi:10.1016/j.ijpharm.2019.03.028

885 Stein, P., Gogoll, K., Tenzer, S., Schild, H., Stevanovic, S., Langguth, P., Radsak, M.P., 2014. Efficacy of
886 Imiquimod-Based Transcutaneous Immunization Using a Nano-Dispersed Emulsion Gel
887 Formulation. PLoS One 9, e102664. doi:10.1371/journal.pone.0102664

888 Sun, W., Araci, Z., Inayathullah, M., Manickam, S., Zhang, X., Bruce, M.A., Marinkovich, M.P., Lane,
889 A.T., Milla, C., Rajadas, J., Butte, M.J., 2013. Polyvinylpyrrolidone microneedles enable delivery
890 of intact proteins for diagnostic and therapeutic applications. Acta Biomater. 9, 7767–7774.
891 doi:10.1016/j.actbio.2013.04.045

892 Suppa, M., Micantonio, T., Di Stefani, A., Soyer, H.P., Chimenti, S., Fargnoli, M.C., Peris, K., 2015.
893 Dermoscopic variability of basal cell carcinoma according to clinical type and anatomic location.
894 J. Eur. Acad. Dermatology Venereol. 29, 1732–1741. doi:10.1111/jdv.12980

895 Taresco, V., Louzao, I., Scurr, D., Booth, J., Treacher, K., McCabe, J., Turpin, E., Laughton, C.A.,
896 Alexander, C., Burley, J.C., Garnett, M.C., 2017. Rapid Nanogram Scale Screening Method of
897 Microarrays to Evaluate Drug-Polymer Blends Using High-Throughput Printing Technology. Mol.
898 Pharm. 14, 2079–2087. doi:10.1021/acs.molpharmaceut.7b00182

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

902 Polym. Plast. Technol. Eng. 54, 923–943. doi:10.1080/03602559.2014.979506

903 Tinelli, M., Ozolins, M., Bath-hextall, F., Williams, H.C., 2012. What determines patient preferences
904 for treating low risk basal cell carcinoma when comparing surgery vs imiquimod ? A discrete
905 choice experiment survey from the SINS trial. BMC Dernatology 12, 1–11. doi:10.1186/1471-
906 5945-12-19

907 Vasquez, K.O., Casavant, C., Peterson, J.D., 2011. Quantitative Whole Body Biodistribution of
908 Fluorescent- Labeled Agents by Non-Invasive Tomographic Imaging. PLoS One 6.
909 doi:10.1371/journal.pone.0020594

910 Venturini, C.G., Bruinsmann, F.A., Contri, R. V., Fonseca, F.N., Frank, L.A., D'Amore, C.M., Raffin, R.P.,
911 Buffon, A., Pohlmann, A.R., Guterres, S.S., 2015. Co-encapsulation of imiquimod and copaiba oil
912 in novel nanostructured systems: Promising formulations against skin carcinoma. Eur. J. Pharm.
913 Sci. 79, 36–43. doi:10.1016/j.ejps.2015.08.016

914 Vicente-pérez, E.M., Quinn, H.L., Mcalister, E., Neill, S.O., Hanna, L., Barry, J.G., Ryan, F., Mcalister,
915 E., Neill, S.O., 2016. The Use of a Pressure-Indicating Sensor Film to Provide Feedback upon
916 Hydrogel-Forming Microneedle Array Self-Application In Vivo. Pharm. Res. 33, 3072–3080.
917 doi:10.1007/s11095-016-2032-z

918 Williams, H.C., Bath-Hextall, F., Ozolins, M., Armstrong, S.J., Colver, G.B., Perkins, W., Miller, P.S.J.,
919 2017. Surgery Versus 5% Imiquimod for Nodular and Superficial Basal Cell Carcinoma: 5-Year
920 Results of the SINS Randomized Controlled Trial. J. Invest. Dermatol. 137, 614–619.
921 doi:10.1016/j.jid.2016.10.019

922 Wu, S., Han, J., Li, W.Q., Li, T., Qureshi, A.A., 2013. Basal-cell carcinoma incidence and associated risk
923 factors in U.S. women and men. Am. J. Epidemiol. 178, 890–897. doi:10.1093/aje/kwt073

924 Wu, X., Elkin, E.E., Marghoob, A.A., 2015. Burden of basal cell carcinoma in USA. Futur. Oncol. 11,
925 2967–74. doi:molm10.2217/fon.15.180

926 Xue, P., Zhang, X., Chuah, Y.J., Wu, Y., Kang, Y., 2015. Flexible PEGDA-based microneedle patches
927 with detachable PVP-CD arrowheads for transdermal drug delivery. RSC Adv. 5, 75204–75209.
928 doi:10.1039/c5ra09329e

929 Yamaoka, T., Tabata, T., Ikada, Y., 1995. Comparison of Body Distribution of Poly (viny1 alcohol)
930 with Other Water-soluble Polymers after Intravenous Administration 479–486.

931 Yang, Y., Sunoqrot, S., Stowell, C., Ji, J., Lee, C.-W., Kim, J.W., Khan, S.A., Hong, S., 2012. Effect of
932 Size, Surface Charge, and Hydrophobicity of Poly(amidoamine) Dendrimers on Their Skin
933 Penetration. Biomacromol 13, 2154−2162. doi:10.1021/bm300545b

934 Yu, W., Jiang, G., Zhang, Y., Liu, D., Xu, B., Zhou, J., 2017. Polymer microneedles fabricated from
935 alginate and hyaluronate for transdermal delivery of insulin. Mater. Sci. Eng. C 80, 187–196.
936 doi:10.1016/j.msec.2017.05.143

937

938

939

940

941

942

943

944

945

946

947

948 **Figure legends**

949 **Figure 1** a) Photograph image of PVPVA microneedles post-fabrication (b) Optical microscopy image
950 of PVPVA microneedles at 12.5x magnification (c) close up microscopy image - of a single PVPVA
951 polymeric microneedle at 50x magnification (d) SEM images of PVPVA microneedles at 39x
952 magnification.

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

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

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

970

971

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, $C_5H_{15}NPO_4^+$ 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