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Design and physicochemical characterisation of a bioadhesive patch for dose-controlled topical delivery of imiquimod

Ryan F. Donnelly [a,*], Paul A. McCarron [a], Agnieszka A. Zawislak [b], A. David Woolfson [a]

[a] *School of Pharmacy, Queens University Belfast, Medical Biology Centre, 97 Lisburn Road, Belfast BT9 7BL, UK*
[b] *Department of Obstetrics and Gynaecology, The Royal Group of Hospitals, Grosvenor Road, Belfast BT12 6BA, UK*

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Abstract

Clinical use of the imidazoquinoline immunomodulator imiquimod for the topical treatment of dysplastic and neoplastic lesions has increased markedly in recent years. However, despite guidance from the manufacturer of the proprietary imiquimod cream, there seems to be little consensus between clinicians as to the topically applied dose. Given that patients often apply the cream themselves at home, further dosing variability is expected and, consequently, accurate comparison of the results of different published studies is difficult. This paper describes, for the first time, the formulation and physicochemical characterisation of a bioadhesive patch for dose-controlled topical delivery of imiquimod as well as a new HPLC method for sensitive fluorescence determination of imiquimod released from such systems. Patches containing imiquimod loadings of 4.75, 9.50 and 12.50 mg cm^{-2} all released significantly more drug across a model membrane than the proprietary cream over a period of 6 h. Inclusion of imiquimod in patches did not adversely affect their physicochemical properties. Of major importance, patches contained defined drug loadings per unit area; therefore, their use could reduce inter-clinician variability. This would make critical comparison of clinical studies and determination of an appropriate imiquimod dose for successful treatment much simpler. Since bioadhesive formulations are capable of adhering to body tissues in moist environments, the use of a bioadhesive patch system may allow extension of the clinical uses of imiquimod to the treatment of neoplastic conditions of the oral cavity and cervix, as well as the vulva.
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Keywords: Imiquimod; Bioadhesive; Neoplastic; Drug delivery

1. Introduction

Imiquimod is a member of the imidazoquinoline amine family, as shown in Fig. 1. It is an immunomodulating agent that was approved in 1997 by the U.S. Food and Drugs Administration for treating external genital and perianal warts (Sauder, 2003; Schon and Schon, 2004). Since then, unlicensed administration has grown to include use in the management of benign diseases, such as molluscum contagiosum (Bayerl et al., 2003) and xeroderma pigmentosum (Nagore et al., 2003), as well as neoplastic conditions, such as basal (Berman et al., 2003) and squamous cell carcinomas (Florez et al., 2004), actinic keratosis (Bianchi et al., 2003) and Bowen's disease (Alette, 2003).

Imiquimod is formulated as a 5% (w/w) cream (Aldara®, 3M) intended for topical application. It is recommended that approximately 250 mg of this cream is applied to each 20 cm^2 of the lesional area for optimum results (Aldara® patient information leaflet, 2001). However, Berman et al. (2004) have shown recently that 250 mg of cream can be made to cover an area of 386 cm^2 of skin. Clearly, there is scope for considerable variation in the mass of applied cream per unit area of skin, driven presumably by the clinical preference and experience of those using the formulation. Frequently, the cream is supplied on an outpatient basis, with the patient instructed to apply to the affected area when at home. The variability in cream thickness, and hence applied dose, is likely to be even greater. The manufacturer recommends that the cream should be left on the lesion for 6–10 h (Aldara® patient information leaflet, 2001). If the lesion is on an exposed area of skin, such as on the face or lower arm, then problems are unlikely. However, if the lesion is situated in the lower female gynaecological tract, then cream retention for such

* Corresponding author. Tel.: +44 28 90 972 251; fax: +44 28 90 247 794.
E-mail address: r.donnelly@qub.ac.uk (R.F. Donnelly).

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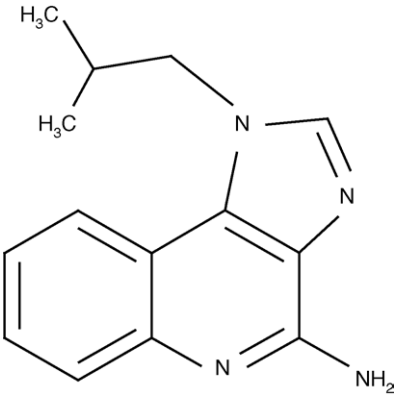

Fig. 1. Chemical structure of imiquimod.

long periods may prove difficult. Shear forces are high in this region, particularly in mobile patients and the cream may simply be sloughed away.

It is conceivable that variability in imiquimod dose may lead to observed inconsistencies in the clinical response. Moreover, this makes interpretation of the clinical outcomes of published studies more difficult. For example, Table 1 shows a summary of recent studies employing imiquimod for the topical treatment of selected neoplastic conditions. As can be observed, the applied dose is often not stated; it is debatable, therefore, if variations in clearance rate can be correlated to lesion sensitivity, poor control over imiquimod dose or a combination of both. With this in mind, the aim of this work is to design an imiquimod-loaded bioadhesive patch and evaluate its physicochemical characteristics in an attempt to achieve predictable and reproducible imiquimod delivery. As the patch is of fixed dimension, variations in its thickness and the applied footprint do not occur. It will take the form of a topical dosage system, containing a defined imiquimod loading available for release within a defined surface area. Such a system would ideally be self-adhesive, backed with a protective material and capable of delivering a comparable drug dose to the proprietary cream. This paper describes, for the first time, the design and physicochemical characterisation of a bioadhesive patch for dose-controlled topical delivery of imiquimod. A new HPLC method, employing fluorescence detection, for sensitive determination of imiquimod released from topical formulations is also detailed.

2. Materials and methods

2.1. Chemicals

Imiquimod was provided by 3M, Leicestershire, UK. Aldara® cream (5%, w/w, imiquimod) was purchased from AAH Ltd., Belfast, UK. Gantrez® AN-139, a co-polymer of methy vinyl ether and maleic anhydride (PMVE/MA), was provided by ISP Co. Ltd., Guildford, UK. Plastisol® medical grade poly(vinyl chloride) emulsion, containing diethylphthalate as plasticiser, was provided by BASF Coatings Ltd., Clwyd, UK. Tripropyleneglycol methyl ether (Dowanol™ TPM) was purchased from Sigma–Aldrich, Dorset, UK. All other chemicals used were of analytical reagent quality. Poly(ester) film, one-side siliconised, release liner (FL2000™ PET 75 μ 1S) was purchased from Rexam Release B.V., Apeldoorn, The Netherlands. Moisture-impermeable, heat-sealable poly(ester) foils were purchased from Transparent Film Products Ltd., Newtownards, N. Ireland.

2.2. Determination of drug loadings for bioadhesive patches

Determination of an approximate loading of imiquimod to be included in bioadhesive patches was by consultation with the clinician involved in the study. It was decided to load each square centimetre of the patch with an equivalent dose of imiquimod to that contained in the amount of proprietary cream typically applied per square centimetre to treated lesions.

A cream (Unguentum® Merck), similar in consistency and composition to Aldara® cream, was applied in the thickness used

Table 1
Summary of some recent studies employing imiquimod for the topical treatment of selected neoplastic conditions

Neoplastic lesion	Number of patients/lesions	Complete clearance rate (%)[a]	Frequency of application per week	Duration of treatment (weeks)	Side effects	Reference
AK	6	100	5	8	LR	Bianchi et al. (2003)
	286	57	1–3	16	LR	Szeimies et al. (2004)
	436	45	1–2	16	LR	Lebwohl et al. (2004)
BCC						
Superficial	NR	82	5	6	LR	Geisse et al. (2004)
Nodular	15	100	1–3	15	NR	Huber et al. (2004)
Nodular	19	53	1–3	12	NR	Peris et al. (2005)
Superficial	70	93	1–3	12	NR	Peris et al. (2005)
BD	16	93	7	16	LR	Mackenzie-Wood et al. (2001)
	5	80	3–14	8–24	LR	Mandekou-Lefaki et al. (2005)
VIN	13	62	NR	14	LR	Jayne and Kaufman (2002)
	15	27	1–3	6–34	NR	van Seters et al. (2002)

AK, actinic keratosis; BCC, basal cell carcinoma; BD, Bowen's disease; VIN, vulval intraepithelial neoplasia; NR, not reported; LR, local reactions, which included pruritis, burning sensations, erythema, erosion and scabbing in the studies presented.

[a] Complete clearance rate at follow-up examination, which varied between 3 and 5.5 months after completion of treatment in the studies presented.

clinically, to each of 25 cm^2, ruled out on the back of a gloved hand, by a clinician experienced in the use of topical imiquimod (AZ). Each square centimetre was individually cleared of cream using a microspatula and each aliquot of cream weighed. As Aldara® contains 5% (w/w) imiquimod, the mean drug dose per square centimetre was determined by calculation. This estimation of imiquimod loading was then used as a starting point in the patch design process.

2.3. Formulation of bioadhesive patches

Aqueous polymer blends, containing 20% (w/w) of the copolymer PMVE/MA and 10% (w/w) of the plasticiser tripropylene glycol methyl ether, were prepared, as described previously (McCarron et al., 2003, 2004). The required weight of PMVE/MA was added to ice-cooled water and stirred vigorously. The mixture was then heated and maintained between 95 and 100 °C until a clear solution was formed. Upon cooling, the required amount of TPM was added and the casting blend adjusted to final weight with water. Due to the increasing insolubility of imiquimod as pH is increased, the blend pH was not adjusted and, therefore, was around pH 2.

We have previously prepared bioadhesive films by slowly pouring 30 g of the aqueous blend into a mould of internal dimensions 100 mm × 100 mm (McCarron et al., 2004). To avoid wastage of imiquimod, a suitable glass mould (50 mm × 30 mm) was constructed. An amount (4.5 g) of aqueous blend was used to produce a film of area 15 cm^2, with a thickness consistent with that of those described previously. The appropriate amount of imiquimod was dissolved directly into the aqueous blend immediately prior to casting. The mould, lined with release liner, siliconised side-up, attached with high vacuum grease, was placed on a levelled surface to allow the blend to spread evenly across the area of the mould. The cast blend was dried under a constant air flow at 25 °C.

Films were removed from the mould by simply peeling the release liner, with attached film, off the base of the mould. The vacuum grease was then wiped off the non-siliconised side of the release liner. Bi-laminar bioadhesive patches were prepared by attaching the exposed side of the films containing imiquimod, to equivalent areas of PVC backing films with the aid of gentle pressure. The PVC films were prepared separately by heating (160 °C, 15 min) Plastisol® emulsion, which had been smeared onto a glass plate at a thickness of approximately 150 μm. For protection, the release liner was allowed to remain with its siliconised side attached to what had now become the release surface of the formed patch. Patches were then placed in moisture-impermeable poly(ester) foils, which were immediately heat-sealed.

2.4. Bioadhesion measurements

The bioadhesion properties of all films were evaluated quantitatively using a TA-XT2 Texture Analyser (Stable Microsystems, Haslemere, UK) in tensile mode. Full thickness, shaved, neonate porcine skin was attached with cyanoacrylate adhesive to a lower platform. Film segments (1 cm^2) were attached to the probe of the Texture Analyser using double-sided adhesive tape. Adhesion was initiated by adding a defined amount of water (10 μl) over an exposed skin sample (1 cm^2) and immediately lowering the probe with attached film. Upon contact, a force of 5 N for 30 s was applied before the probe was moved upwards at a speed of 0.1 mm s^{-1}. Adhesion was recorded as the force required to detach the sample from the surface of the excised skin. The distance to separation of a test film from the skin substrate, that is, the normal displacement from the skin surface that the probe had travelled at the instant the film and substrate lost contact with each other, was also recorded to provide some measure of the cohesion within the film sample. Results were reported as the mean (±S.D.) of five replicates.

2.5. Determination of tensile properties

The tensile strength and percentage elongation at break of films were determined using the Texture Analyser. Film thicknesses were determined prior to testing using a digital micrometer (Digital Micrometer, Mitutoyo Corporation, Japan). Film strips of 5-mm width were grasped using an upper and lower flat-faced metal grip laminated with a smooth rubber grip. The distance between the grips was set at 20 mm and this distance, therefore, represented the length of film under stress. A cross-head speed of 6 mm s^{-1} was used for all measurements. The resultant force–time profiles were analysed using propriety software (Dimension 3.7E). Only results from films that were observed to break in the middle region of the test strip during testing were used. The percentage elongation at break, E_b, of tested films was determined using Eq. (1) (Radebaugh, 1992), where E is the extension to break of the film and L_0 is its original length. The break strength, B, of tested films was determined using Eq. (2) (Radebaugh, 1992), where F is the break force of the film and A_R is its cross-sectional area. Results were reported as the mean (±S.D.) of five replicates.

$$E_b = \frac{E}{L_0}100 \quad (1)$$

$$B = \frac{F}{A_R} \quad (2)$$

2.6. Swelling studies

Segments of bioadhesive films of area 4 cm^2, backed on one side with an equal area of release liner, were weighed using a five-figure electronic balance (Denver Instruments, Denver, CO, USA) and individually placed in 50 ml of a 0.9% (w/w) saline solution. Such segments were removed from the solution every 2.5 min, shaken to remove excess fluid and reweighed. Each experiment was performed for 45 min. At this time, any residual film on the release liner was removed, the liner dried by blotting with filter paper and weighed. This allowed calculation of the initial film weight. Results were reported as the mean (±S.D.) of five replicates.

2.7. Drug release studies

The release of imiquimod from patch formulations was investigated using a modified Franz cell apparatus. The orifice diameter in both donor and receptor compartments was 15 mm. Receptor compartment volumes, approximately 10 ml, were exactly determined by triplicate measurements of the weights of water they could accommodate. Account was taken for the volumes occupied by magnetic stirring bars. Compartment temperatures were kept constant at 37 °C by recirculating water from a thermostatically controlled bath. The receiver phase was acetate buffer BP pH 3.7. This buffer was used to allow maintenance of sink conditions, due to the insolubility of imiquimod at neutral or basic pH values. The buffer was degassed prior to use by vacuum filtration through a HPLC filter. Continuous stirring was provided by Teflon-coated stirring bars, rotating at 600 rpm. Stainless steel filter support grids were used to support Cuprophan® membranes. The membranes and support grids were sandwiched between the donor and receptor compartments. High vacuum grease and spring clips were used to hold the entire assembly together. The donor compartments were covered with laboratory film (Parafilm®).

Release from imiquimod-loaded patches was investigated by first cutting circular discs from 3 cm × 5 cm patches using a sharp circular cork borer of inside diameter 1.5 cm. The bioadhesive surfaces of these discs were attached to the Cuprophan® membranes in the donor compartments using 10 μl of deionised water. Using a long needle, samples (0.10 ml) were removed from the receptor compartment at defined time intervals (5, 10, 15, 30, 60, 120, 180, 240, 300 and 360 min). This volume was immediately replaced using blank, pre-warmed buffer. Aliquots (10 μl) of samples removed were diluted to 10 ml with buffer and analysed by HPLC, as described in Section 2.8. Results were reported as the mean (±S.D.) of five replicates. The release of imiquimod from the proprietary Aldara® cream was also investigated in this way. The cream was applied, in the thickness used clinically, as calculated in this study, to the membranes in the donor compartments. Again, imiquimod was determined using HPLC and results reported as the mean (±S.D.) of five replicates.

2.8. Determination of imiquimod

There was some initial uncertainty concerning the quantities of imiquimod capable of being released from patches and the proprietary cream. Therefore, HPLC with fluorescence detection was used as the method of quantification, due to the noted superior sensitivity when compared to HPLC employing ultraviolet absorption detection (Skoog et al., 1998). The mobile phase was acetonitrile/acetate buffer (50/50, v/v) and the flow rate (LKB Bromma 2150 liquid chromatography pump, LKB Company, Upsala, Sweden) was 1.5 ml min^{-1}. Sample aliquots (15 μl) were injected (Waters WISP 712 autoinjector, Waters Associates, Harrow, UK) onto a C_{18} column (Spherisorb®; 250 mm × 4.6 mm, C_{18} ODS_2 with 5 μm packing, Waters Associates, Harrow), equipped with a C_{18} guard column (Spherisorb® S5; 10 mm × 4.6 mm, C_{18} ODS_2 with 5 μm packing). Detection (Shimadzu RF-535, Dyson Instruments Ltd., Tyne & Wear, UK) was by fluorescence, with excitation at 260 nm and emission at 340 nm.

2.9. Statistical analysis

Data were analysed, where appropriate, using a one-way analysis of variance (ANOVA). Post hoc comparisons were made using Fisher's PLSD test. In all cases, $p < 0.05$ denoted significance.

3. Results

Due to its only relatively recent use, reports concerning the determination of imiquimod by HPLC are scarce. Ravichandran et al. (2003) report determination by HPLC using UV detection at 239 nm. The limit of detection was <5.0 μg ml^{-1}. Soria et al. (2000) describe a similar procedure capable of a linear calibration range of 1.0–50 ng ml^{-1}. Harrison et al. (2004) report determination of imiquimod in serum using HPLC with mass spectrometric determination. Due to initial concerns about the quantities of imiquimod capable of being released from the tested formulations, a new HPLC method with fluorescence

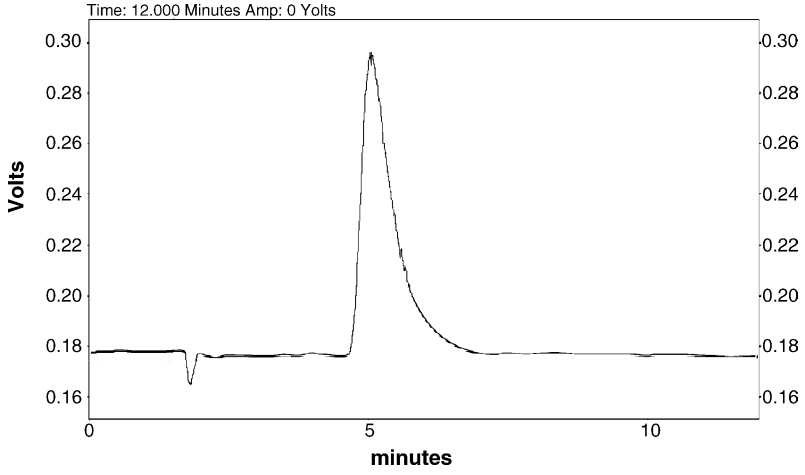

Fig. 2. Typical chromatogram obtained after an injection of a solution containing 500 ng imiquimod ml^{-1}.

Table 2
Outcome of experiment to determine an approximate imiquimod loading for a bioadhesive patch (mean ± S.D., $n = 25$)

Mean weight of cream applied (mg cm^{-2}) (±S.D.)	190 ± 50
Imiquimod delivered (approximate amount) (mg cm^{-2})	9.50

detection was used, considering the known greater sensitivity of fluorescence over UV detection. The retention time was approximately 5 min and the calibration plot was linear ($R^2 = 0.9902$) in the range 0–1000 ng ml^{-1}). The limit of detection was 0.16 ng ml^{-1}. A typical chromatogram is shown in Fig. 2.

Table 2 shows the mean (±S.D.) weights of cream applied per square centimetre. Table 2 also shows the calculated mean imiquimod dose available per square centimetre. An imiquimod loading of 9.50 mg cm^{-2} was, therefore, used as a starting point for patch design. Imiquimod loadings of 4.75, 9.50 and 12.50 mg cm^{-2} were all investigated with respect to the influence of different imiquimod contents on patch properties.

As may be seen from Table 3, increasing imiquimod loading had no significant effect on bioadhesive properties of cast films. For example, films containing 12.50 mg imiquimod cm^{-2} required forces of approximately 1.76 N cm^{-2} for removal from neonate porcine skin ($p = 0.994$) and distances to separation of skin and film of 4.69 mm ($p = 0.9795$) were typically observed, neither of which were significantly different from those of blank films containing no drug. Similarly, increasing imiquimod loading did not significantly alter the tensile properties of bioadhesive films. For example, films containing 9.50 mg imiquimod cm^{-2} had tensile strengths of 2.70×10^6 N m^{-2} ($p = 0.7397$) and percentage elongations at break of approximately 347.93% ($p = 0.8460$), neither of which were significantly different from those of blank films containing no drug.

As may be seen from Fig. 3 and Table 4, increasing imiquimod loading did not significantly increase the maximum swollen mass of bioadhesive films. For example, films containing 12.50 mg imiquimod cm^{-2} had a maximum swollen mass of approximately 121.25%, achieved after 5 min immersion, which

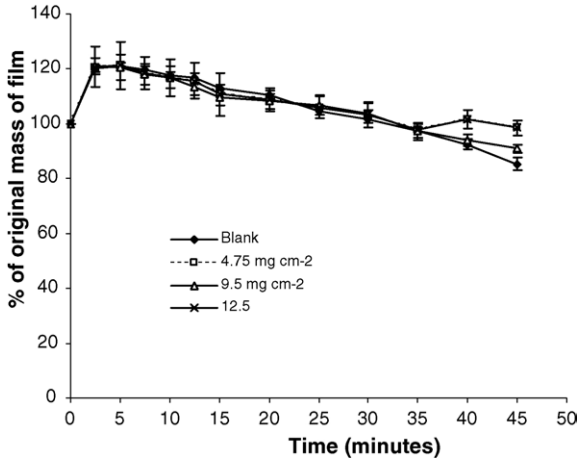

Fig. 3. Influence of imiquimod content on swelling and dissolution behaviour of bioadhesive films cast from aqueous blends containing 20% (w/w) PMVE/MA and 10% (w/w) TPM (mean ± S.D., $n = 5$).

was not significantly different ($p = 0.0916$) from those of blank films containing no drug. The imiquimod films acquired a white colouration on immersion in PBS, an effect most pronounced in films containing 9.50 and 12.50 mg cm^{-2} imiquimod, and not seen in blank films. Films containing 4.75 mg imiquimod cm^{-2} had remaining masses of approximately 85.20% after 45 min immersion, which were not significantly different from those of blank films. However, films containing 9.50 and 12.75 mg imiquimod cm^{-2} had remaining masses of 90.75% ($p = 0.0161$) and 98.50% ($p = 0.0004$), respectively, after the same time of immersion, which were significantly greater than those of blank films immersed for the same time.

As may be seen from Fig. 4, even the patch containing 4.75 mg cm^{-2} imiquimod released significantly ($p = 0.0073$) more drug (2.52 mg) over 6 h than the proprietary Aldara® cream (2.00 mg), even though the cream contained approximately twice as much drug. The patch containing 12.50 mg imiquimod cm^{-2} released significantly ($p = 0.0066$) more drug (4.34 mg) over 6 h that that containing 4.75 mg cm^{-2}. However, the patch

Table 3
Influence of imiquimod loading on bioadhesive and tensile properties of films cast from aqueous blends containing 20% (w/w) PMVE/MA and 10% (w/w) TPM (mean ± S.D., $n = 5$)

Imiquimod loading (mg cm^{-2})	Adhesion (N)	Distance to separation (mm)	Tensile strength (N m^{-2} × 10^6)	Elongation at break (%)
0.00	1.77 ± 0.32	4.72 ± 2.78	2.65 ± 0.39	353.74 ± 23.79
4.75	1.77 ± 0.28	4.73 ± 0.80	2.58 ± 0.72	347.92 ± 53.31
9.50	1.83 ± 0.20	4.74 ± 0.78	2.70 ± 0.40	347.93 ± 60.19
12.50	1.76 ± 0.07	4.69 ± 0.20	2.77 ± 0.66	357.53 ± 65.53

Table 4
Influence of imiquimod content on swelling and dissolution behaviour of bioadhesive films cast from aqueous blends containing 20% (w/w) PMVE/MA and 10% (w/w) TPM (mean ± S.D., $n = 5$)

Imiquimod loading (mg cm^{-2})	Maximum mass (% of original mass)	Time to achieve maximum mass (min)	Mass at 45 min (% of original mass)
0.00	127.68 ± 1.66	5.00	83.06 ± 6.06
4.75	121.16 ± 1.13	5.00	85.20 ± 2.33
9.50	121.00 ± 2.94	2.50	90.75 ± 1.50
12.50	121.25 ± 8.500	5.00	98.50 ± 2.65

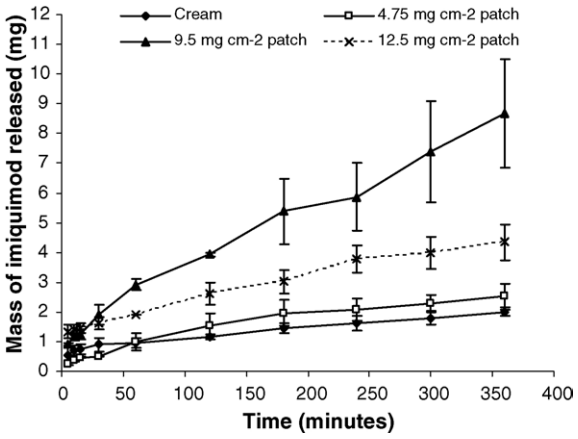

Fig. 4. Release of imiquimod from bioadhesive patches and the proprietary Aldara® cream.

Table 5
Total imiquimod and percentage of total drug, released after 6 h by the cream and patch formulations (mean ± S.D., $n = 5$)

Formulation	Mass of imiquimod released after 6 h (mg)	Percentage of total imiquimod released after 6 h
Aldara® cream	2.00 ± 0.15	11.93 ± 0.87
4.75 mg cm^{-2} patch	2.53 ± 0.44	30.08 ± 5.23
9.50 mg cm^{-2} patch	8.69 ± 1.83	51.78 ± 10.87
12.50 mg cm^{-2} patch	4.34 ± 0.60	19.66 ± 2.72

containing 9.50 mg cm^{-2} released significantly ($p = 0.0001$) more drug (8.69 mg) over 6 h than the patch with the higher loading (Table 5). All of the patches were observed to draw buffer from the receiver compartment across the membranes during the drug release experiment, leading to gel formation. The gels derived from the patches containing the two higher imiquimod loadings were observed to be white in colour, with this effect particularly pronounced when the original patch loading was 12.5 mg cm^{-2}.

4. Discussion

The exact mechanism of action of imiquimod has yet to be elucidated, but it clearly affects both major divisions of the immune system: the innate and the activated or adaptive immune system (Sauder, 2003). This activation occurs via the Toll-like receptors (TLR), particularly TLR-7 (Dummer et al., 2003). It has been thought that imiquimod exerts it anti-tumoural activity primarily through induction of a profound cellular tumour-directed immune response. However, recent experimental and clinical data have demonstrated that the mode of action of the compound extends beyond its known function as an immune response modifier, in that imiquimod also confers direct pro-apoptotic activity against malignant and benign tumours of different origin (Schon et al., 2003, 2004; Sidbury et al., 2003; Sullivan et al., 2003). This mode of action appears to be independent of membrane-bound death receptors, but involves caspase activation (Schon and Schon, 2004).

Conventional treatments for skin neoplasias include surgical excision and radiotherapy, which are highly successful, with clearance rates as high as 95% on 5-year follow-up reported. However, these treatments are unsuitable for large or multiple lesions and can lead to poor cosmesis (Pearse, 2002). Cryotherapy and curettage are only suitable for very superficial tumours and are associated with high rates of recurrence (Szeimies et al., 2002). Initially, topical imiquimod was largely employed in the treatment of human papilloma virus (HPV)-induced skin lesions, such as warts and condyloma acuminate (Hengge and Cusini, 2003). Recently, however, imiquimod has been increasingly used in the topical treatment of superficial skin neoplasias, despite this being an unlicensed indication. In most cases, successful clinical outcomes have been produced, with high complete clearance rates and no evidence of scarring. However, local skin excoriation and moderate to severe pain and pruritis at the site of application have been frequently reported (Salasche and Shumack, 2003).

Table 1 summarises the results of several clinical studies using imiquimod for the topical treatment of selected skin neoplasias. While good complete clearance rates are noted in each case, none of the detailed studies report the dose of cream applied topically. Consequently, critical comparison of the outcomes of studies that are otherwise similar is problematic. From Table 2, it can be seen that the amount of cream applied per square centimetre (190 mg), used to determine patch loadings, is more than 10 times greater than that recommended by the manufacturers (12.5 mg cm^{-2}). However, Berman et al. (2004) applied approximately 20 times less cream (0.64 mg cm^{-2}) than that recommended. This clearly illustrates the inter-clinician variability observed in the topical use of imiquimod clinically.

Our group has previously described the design of bioadhesive patches for topical delivery of defined doses of drugs to the vulva for photodynamic therapy (PDT) of vulval intraepithelial neoplasia (McCarron et al., 2003) and extramammary Paget's disease (Zawislak et al., 2004) and to the cervix for cytotoxic treatment (CT) of cervical intraepithelial neoplasia (Sidhu et al., 1997). Since imiquimod may be of use in the treatment of such conditions, either alone or as an adjuvant to PDT or CT, the present paper extends our work to this immunomodulator.

Maintenance of an imiquimod cream in the oral cavity, or on the cervix or vulva may prove difficult, if not impossible, due to the moist environments of the former areas and the high shear forces at the latter. In addition to our work with the vulva and cervix, the group has also produced a patch for use in the oral cavity (Mahdi et al., 1996). Moreover, our group has successfully produced a bioadhesive patch containing tetracaine for local skin anesthesia (McCafferty et al., 2000). Inclusion of such a compound in an imiquimod-loaded patch may alleviate the pain and pruritis associated with the topical use of the immunomodulator.

The human body is known to produce 3–10 g m^{-2} h^{-1} of sweat from the average of 200–250 sweat ducts found per square centimetre of normal skin (Guyton and Hall, 1996). The number of sweat glands found on the labia majora of the vulva and, hence, the rate of sweat production is even higher (Shaw et al., 1997). In addition, the vulva also possesses many sebaceous and apocrine glands (Moore and Hacker, 1998). The amount

of moisture present in the oral cavity is, obviously, even higher due to the continuous production of saliva. We have previously conducted systematic studies on the in vitro and in vivo performance of bioadhesive films cast from aqueous blends containing different amounts of PMVE/MA and TPM. It was found that films cast from blends containing PMVE/MA contents lower than 20% (w/w) demonstrated excessive aqueous solubilities and became very gel-like on topical application to the vulva, exuding from beneath the backing layer (McCarron et al., 2005). Films cast from blends containing polymer:plasticiser ratios lower than 2:1 were found to be inflexible and, therefore, unlikely to conform well to contoured areas of the body, such as the vulva or oral cavity (McCarron et al., 2004). Therefore, the aqueous blend used for formulation of imiquimod patches in the present study contained 20% (w/w) PMVE/MA and 10% (w/w) TPM. As may be observed from Fig. 3 and Table 4, increasing imiquimod loading had no significant effect on the maximum swollen mass of bioadhesive films after immersion in PBS. However, drug-loaded films were observed to turn white on immersion, with this effect most striking in the films containing the higher drug loadings. This phenomenon is likely to be due to the insolubility of the imiquimod base in neutral and basic environments. The white colour observed is, therefore, probably due to imiquimod that has come out of solution in the film. This may explain the fact that the films containing the higher drug loadings showed significantly less dissolution than the blank film after 45 min immersion. As may be observed from Table 3, increasing imiquimod loadings did not significantly alter the physical or bioadhesive properties of cast films.

While limited reports on HPLC determination of imiquimod exist, publications concerning the release of the drug from topically applied dosage forms are completely absent from the literature. Cuprophan® is a dialysis membrane, with a molecular weight cut-off of 10,500 Da (Cuprophan® product information sheet, 1994). In simple terms, this means that it consists of a lipophilic polymeric membrane, interspersed with small pores filled by the aqueous receiver phase. It may, to some extent, approximate the disordered *stratum corneum* overlying neoplastic skin lesions. The mass of imiquimod released from bioadhesive patches was, in each case, significantly greater than that from the proprietary Aldara® cream. This was despite the fact that the quantity of the 5% (w/w) cream (336.4 mg) applied to membranes in the donor compartments contained twice as much imiquimod as in 1.77 cm^2 (the area of the orifice in the donor compartment) of the 4.75 mg cm^{-2} patch and the same amount as in 1.77 cm^2 of the 9.50 mg cm^{-2} patch. This is probably due to the hydrophobic imiquimod having little propensity for release out of a w/o cream into an aqueous environment. The patch containing 9.50 mg cm^{-2} released significantly more drug than all of the other formulations, including the patch containing 12.50 mg cm^{-2}. This may be due to the fact that the precipitation of imiquimod was most pronounced in the gel derived from the 12.5 mg cm^{-2} patch, thereby preventing its release. This precipitation is likely to be due to the formed gels having their pH increased by the ingress of buffer. Due to the very small volumes of these gels, the imiquimod present exceeded its maximum solubility and came out of solution. This effect will obviously be most pronounced in the formulation with the highest drug loading and could be prevented by using the hydrochloride salt of imiquimod. The use of this salt would, however, prevent accurate comparison with the proprietary cream, which contains the free base.

In summary, a bioadhesive patch has been produced which, under the conditions investigated, is capable of releasing more drug than the proprietary cream. Increasing imiquimod loadings did not significantly alter the physical or bioadhesive properties of the patch. Patches containing the two highest drug loadings maintained most of their original mass after 45 min immersion in PBS pH 7.4, meaning that they are unlikely to lose their structure following absorption of perspiration, saliva or other bodily fluids and, hence, should remain in place. Since patches contain a defined drug loading per unit area, this system could reduce the inter-clinician variability currently observed in terms of amount of drug topically applied. This would then make critical comparison of clinical studies and the determination of an appropriate imiquimod dose for successful treatment much simpler. Since bioadhesive formulations are capable of adhering to body tissues in moist environments (Woolfson et al., 1995), the use of a bioadhesive patch system may allow extension of the clinical uses of imiquimod to the treatment of neoplastic conditions of the oral cavity and cervix, as well as the vulva.

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