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Evaluation of a water-soluble bioadhesive patch for photodynamic therapy of vulval lesions

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Abstract

An innovative bioadhesive patch intended primarily as a vulval drug delivery system and, specifically, as a means to deliver photosensitisers, or their prodrugs, for photodynamic purposes is described. The patch was formulated with a copolymer of methyl vinyl ether and maleic anhydride (PMVE/MA) as a bioadhesive matrix and poly(vinyl chloride) as a drug-impervious backing layer. Adhesive strength to neonate porcine skin, as a model substrate, was evaluated using peel and tensile testing measurements. Acceptabilities of non-drug loaded patches were appraised using human volunteers and visual-analogue scoring devices. An optimal formulation, with water uptake and peel strengths appropriate for vulval drug delivery, was cast from a 20% (w/w) PMVE/MA solution and adhered with a strength of approximately 1.7 N cm^{-2}. Patient evaluation demonstrated comfort and firm attachment for up to 4 h in mobile patients. Aminolevulinic acid, a commonly used photosensitiser, was formulated into the candidate formulation and applied to vulval intraepithelial neoplastic lesions. Fluorescence under ultraviolet illumination revealed protoporphyrin synthesis. The patch achieves the extended application times obligatory in topical photodynamic therapy of vulval lesions, thereby contributing to potential methods for the eradication of neoplastic lesions in the lower female reproductive tract.

Keywords: Vulval; Patient acceptability; Photodynamic therapy; Bioadhesive; Neoplastic; Aminolevulinic acid

1. Introduction

Photodynamic therapy of superficial neoplastic lesions combines localised delivery of a photosensitiser followed by illumination with light of an appropriate wavelength. Although light delivery is relatively uncomplicated, topical and prolonged drug delivery to the vulva is more difficult to achieve, due mostly to the inability of the dosage form to withstand the physical attributes of this site. Attempts have involved incorporation of therapeutic agents in emulsion-based creams (Carson et al., 1976; Sakakura et al., 1993) or, less

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commonly, solutions (Frenga et al., 1997; Hillemanns et al., 1999a). In many instances, creams and solutions are covered with an occlusive foil or dressing immediately after application, to aid retention at the vulval site and enhance drug absorption (Hillemanns et al., 2000a; Kurwa et al., 2000). Pragmatically, occlusive dressings are poor at staying in place, particularly around the female lower reproductive tract area, where shear forces are high in the ambulant patient. They interfere frequently with movement, micturition and bowel function, thereby causing additional distress for the patient. Moreover, determination of an exact and proper dose of drug for successful treatment is difficult, given that there is no control over cream thickness under occlusion (Price, 1999). Finally, given the moist nature around the labial area, the performance of solvent-based adhesives is expected to diminish after a short period of time, leading to detachment of the occlusive dressing. Notwithstanding these problems, treatment of vulval pathologies using topical application has been reported widely and includes administration of diverse agents, such as povidone–iodine for bacterial eradication (Sakakura et al., 1993) and flutrimazole for treatment of fungal infection (Del Palacio et al., 2000). Fluorouracil, podophillotoxin, α-interferon and β-interferon have been used to treat genital warts (Frenga et al., 1997). Vulval intraepithelial neoplasia III (VIN III), or carcinoma in situ of the vulva, has been treated using application of dinitrochlorobenzene, imiquomod and 5-fluorouracil (Foster and Woodruff, 1981; Davis et al., 2000; Krupp and Bohm, 1978).

One approach that may overcome the difficulties associated with vulval drug delivery is the formulation of bioadhesive delivery systems based on water-swellable or water-soluble polymers. This strategy is analogous to the formulation of polymeric powders used for drug delivery to the nasal mucosa (Nagai and Konishi, 1987) and compacts developed for use in the oral cavity (Ponchel et al., 1987). Perhaps the most useful dosage form configuration for administration to the vulva is the patch, consisting of a bioadhesive layer and a non-adhesive backing layer, as described for topical drug delivery to the skin (McCafferty et al., 2000).

The aim of this study was to produce a bioadhesive patch to act as a generic platform for drug delivery to the vulva as part of the overall management of vulval abnormalities, especially those of neoplastic and dysplastic characters. Such a system would be flexible enough to conform to the contours of the vulva and facilitate normal ambulation when in place, but be of sufficient robustness to allow handling and manipulation. The patch would possess strong bioadhesive properties and remain stable during storage. While the bioadhesive layer would be water-based, the backing layer would be water-impermeable and, therefore, resist the action of moisture and vaginal exudates when in place. The patch would be capable of withstanding prolonged periods of shear, in the region of 6 h or so, while in place and would be capable of being removed intact. It should not cause pain or irritation during use.

It is envisaged that the patch would be a unit dosage form, thus allowing for determination of an exact and appropriate dose. This is relevant for administration of cytotoxic agents, such as fluorouracil, where the mass of drug available per unit surface area of skin can be defined, something that is not achievable using a semi-solid formulation. Additionally, the patch is designed to accommodate photodynamic therapy (PDT) and, in particular, the administration of a commonly used photosensitising prodrug, such as 5-aminolevulinic acid (ALA). Lichen sclerosus and vulval intraepithelial neoplasia III have been treated recently using PDT, based on topically applied ALA (Hillemanns et al., 1999b, 2000b; Kurwa et al., 2000). ALA is an example of a molecule that penetrates the stratum corneum poorly, due to its zwitterionic nature. Consequently, prolonged application times, usually in the region of 4 h, are needed to achieve therapeutic concentrations within targeted lesions. It is clear that enhancing drug permeation using occlusive dressings will be problematic and the patch described in this work is designed to circumvent many of these difficulties.

2. Materials and methods

2.1. Materials

Gantrez® AN-139, a copolymer of methyl vinyl ether and maleic anhydride (PMVE/MA), was obtained from International Specialty Products (ISP) Co. Ltd. Guildford, UK. Tripropyleneglycol methyl ether (Dowanol®, TPM) was obtained from Sigma Aldrich, Dorset, UK. Plastisol®, a medical grade poly(vinyl chloride) emulsion containing diethylphthalate as plasticiser, was obtained from BASF Coatings Ltd., Clwyd,

UK. All other chemicals and reagents used during the formulation and evaluation work were of a suitable analytical reagent quality.

2.2. Preparation of bioadhesive films

Aqueous polymer solutions were prepared using the required weight of PMVE/MA, added to ice-cooled water (reagent grade 1) and stirred vigorously. The mixture was heated and maintained between 95 and 100 °C until a clear solution was formed. Once cool, the required amount of TPM was added, the pH adjusted to 4.5 using 10 M NaOH and the casting blend adjusted to final weight with water.

Bioadhesive films were prepared by casting the aqueous solution (30 g) into a glass rectangular mould (internal dimensions of 30 mm × 50 mm) positioned on a levelled surface. This was placed into a moving air stream at 25 °C and allowed to dry for 24 h, at which point residual water was determined at 11% (w/w), as found using Karl Fischer titration. The mechanical properties of the bioadhesive matrices were evaluated using the film formulation without any non-adhesive backing film.

2.3. Preparation of non-adhesive backing films

Poly(vinyl chloride) (PVC) films were prepared using a film-coating procedure. Plastisol® PVC emulsion (5 g) was placed into a channel on a flat plate, bordered with two parallel runners, each 200 μm in height. A suitable edge, such as a glass stirring rod, was run along the runner guides, creating an emulsion smear down the plate with a precisely controlled thickness. In this way, PVC films, 200 μm thick, were produced. These films were cured by heating at 160 °C for 15 min.

2.4. Bi-laminar patch assembly and storage

Bi-laminar bioadhesive patches were prepared by attaching PMVE/MA films, plasticised with TPM, to equivalent areas of cured PVC backing film with the aid of gentle pressure. Adhesion between the two layers was sufficiently tenacious to ensure that separation during patch use and handling did not occur. The exposed bioadhesive surface was protected by means of attachment to the siliconised side of an equivalent area of a poly(ester)-based release liner (FL2000 PET 75 μ 1S, Rexam Release B.V., The Netherlands). Patches were heat-sealed in moisture-impermeable poly(ester) foils (Transparent Film Products Ltd., Northern Ireland) and stored at 4 °C before use. The fully assembled bi-laminar patch was used for bioadhesive performance and clinical evaluation purposes.

2.5. Determination of bioadhesive tensile and bioadhesive peel strengths

The bioadhesive properties of PMVE/MA films plasticised with TPM were evaluated quantitatively using a texture analyser in tensile mode (TA-XT2 Texture Analyser, Stable Microsystems, Haslemere, UK). Full thickness, shaved, neonate porcine skin harvested from the animal flank was used as a model substrate for vulval skin. A large prepared section, approximately 90 mm × 90 mm, was attached with cyanoacrylate adhesive to a lower platform. Patch segments (1 cm^2) were attached to the upper probe 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 the 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 maximal force required to detach the sample from the surface of the excised skin. After three determinations, the lower platform was relocated in such a fashion as to expose a fresh portion of skin. In addition, the fresh skin was adjacent to that just used, so as to minimise variability between samples. Results were reported as the mean of five replicates.

The peel strength of bi-laminar patch formulations was investigated using neonate porcine skin secured to a sliding platform using cyanoacrylate adhesive. The initial 1 cm section of an elongated patch, measuring 1 cm × 7 cm, was secured in the grips of a clamp, such that the remaining 6 cm of length pointed vertically downwards. The clamp was brought to within 1 cm of the porcine skin and water (10 μl) placed on each 1 cm^2 to which the patch would be adhered. An area of the film measuring 1 cm × 5 cm was attached to the wetted skin using a force of 10 N cm^{-2} applied for 30 s. The clamp then moved upwards at a speed of 6 mm s^{-1}. Simultaneously, the sliding section was free to move horizontally, such that the angle formed at the interface

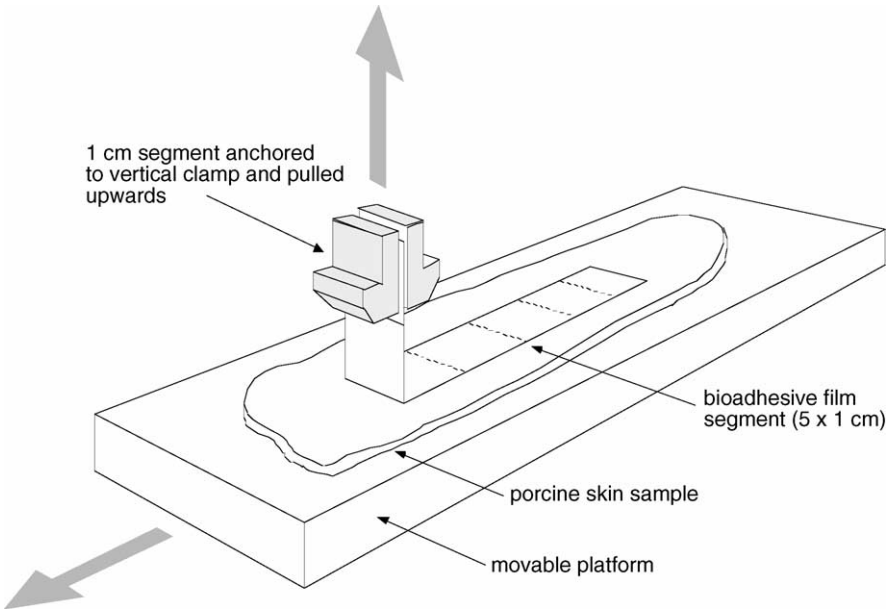

Fig. 1. Simplified representation of the test set-up used to determine peel strength of bioadhesive films. The tissue is adhered to a low friction, stainless steel sliding platform and a bioadhesive strip is applied, with one end gripped firmly in a vertically moving clamp.

between the film and skin was maintained at approximately 90°. This arrangement is shown diagrammatically in Fig. 1.

Data was analysed using proprietary software (Dimension 3.7E) and the peel strength was recorded as the maximal force per unit length of the separating interface. This linear interface is formed at the point where tissue and bioadhesive matrix are pulled apart and represents the transverse width of the formulation (1 cm). Results were reported as the mean (±S.D.) of five replicates.

2.6. Determination of tensile properties and swelling indices

The tensile strength and percentage elongation at break of all bioadhesive films were determined using the Texture Analyser as described above. Only results from films that were observed to break in the middle region of the test strip during testing were used. Results were reported as the mean (±S.D.) of five replicates.

The water uptake rate of bioadhesive film segments was evaluated using sections (2 cm × 2 cm), attached to release liner primarily for support. These were weighed and placed in 50 ml of a 0.9% (w/w) saline solution. Segments were removed after each 2.5 min interval, shaken to remove excess fluid and reweighed. Each experiment was performed over 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 bioadhesive matrix weight. Results were reported as the mean (±S.D.) of five replicates.

2.7. Clinical evaluation

Bi-laminar patches, which were shown to have suitable characteristics after in vitro tests, were evaluated clinically using an observational study. Local ethical committee approval and fully informed patient consent was obtained in all cases. Unlabelled patches were applied to the shaved, pre-wetted, vulvae of in-patient volunteers. Feedback was obtained by means of a patient and clinician questionnaire. Clinician responses were graded according to the extent of agreement with a particular statement, such that a response of “strongly agree” (SA) was given a value of 4, and a response of “strongly disagree” (SD) was given a value of 0. Patient responses were simply recorded as “yes” or “no”. Particular attention was paid to clinician responses con-

cerning ease of handling, application, removal and irritation. Each type of patch was applied to the vulvae of at least three patients.

Results from both pharmaceutical and clinical evaluations were assessed and a formulation chosen for the manufacture of ALA-loaded patches. Sufficient ALA was added to the polymer blend so that after casting and drying, a patch was produced containing 38 mg ALA cm^{-2}. The patch was adhered to the vulvae of five trail patients, who were participating in a larger clinical evaluation of patch delivery of ALA for photodynamic therapy of vulval intraepithelial neoplasia (grade III). The patch was allowed to remain in place for 4 h, during which time the patient was free to leave the clinic. Upon return, the presence of protoporphyrin IX (PpIX), the cellular product of ALA administration, was assessed photographically using fluorescence induced by ultraviolet illumination from a Wood's lamp.

2.8. Statistical analysis

Data was 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

Bioadhesive films were cast from aqueous blends containing ratios of PMVE/MA (5.0–20% (w/w)) and TPM (0.5–10% (w/w)). Owing to the high glass transition temperature of PMVE/MA (Chung et al., 1990), unplasticised films without any TPM were not prepared as such films are brittle and shatter easily into pointed shards. Conversely, films prepared from aqueous blends in which the ratio of plasticiser to copolymer exceeded unity did not form properly, resulting instead in a tacky semi-solid mass. Those solutions that did form appropriate films were found to have a pH of approximately 2. In addition to the potential for cutaneous irritancy, films produced from such blends have been shown to exhibit significantly lower adhesive properties compared to those at pH 4.5 (Woolfson et al., 1995a). Therefore, all blends had their pH adjusted to 4.5 using 10 M NaOH, prior to casting.

Film thickness, as shown in Table 1, was observed to increase significantly with increasing copolymer content in the casting blend. It was shown that this increase became less significant upon moving from higher concentrations, such as from 15% to 20% (w/w) ($p = 0.0899$). As expected, films cast from blends rich in polymer produced thicker films with a thickness of approximately 0.60 mm. The content of TPM in casting blends had no significant influence on film thickness. For films cast from blends containing 10% (w/w) PMVE/MA, no significant increase in thickness was observed as the TPM content in the original blend was increased from 3.5% to 8% (w/w) ($p = 0.345$) and from 8% to 10% (w/w) ($p = 0.174$). However, the plasticising effect of TPM is clear from inspection of percentage elongation at break. For example, films cast from blends containing 15% (w/w) PMVE/MA show significant increases in this response as the TPM content

Table 1
Influences of copolymer and plasticiser contents of aqueous blends on properties of cast bioadhesive films (mean ± S.D.)

Ratio (PMVE/MA:TPM) (%, w/w)	Film thickness (mm)	Elongation at break (%)	Tensile strength ($\times 10^6$ N m^{-2})
5.0:0.5	0.21 ± 0.01	TB	TB
5.0:1.5	0.22 ± 0.01	2.8 ± 2.6	2.75 ± 2.70
5.0:3.0	0.26 ± 0.01	889.0 ± 92.8	4.18 ± 1.76
10.0:3.5	0.33 ± 0.01	0.0	2.05 ± 2.65
10.0:8.0	0.36 ± 0.02	513.0 ± 111.0	1.52 ± 0.77
10.0:10.0	0.39 ± 0.02	946.0 ± 67.4	2.58 ± 0.72
15.0:3.5	0.53 ± 0.01	0.0	0.24 ± 0.22
15.0:8.0	0.57 ± 0.04	468.0 ± 87.7	3.00 ± 0.99
15.0:10.0	0.61 ± 0.04	637.0 ± 110.0	1.45 ± 0.56
20.0:3.5	0.55 ± 0.02	0.0	2.70 ± 4.04
20.0:8.0	0.64 ± 0.01	348.0 ± 53.3	2.23 ± 0.39
20.0:10.0	0.59 ± 0.02	354.0 ± 23.8	2.65 ± 0.39

TB: too brittle to test.

was increased from 3.5% to 8% (w/w) ($p < 0.0001$) and from 8% to 10% (w/w) ($p = 0.0066$). One notable exception was for films cast from blends containing 20% (w/w) PMVE/MA. While a significant increase in percentage elongation at break was observed as the TPM content in the original blend was increased from 3.5% to 8% (w/w) ($p < 0.0001$), no significant increase was observed when the TPM content was increased from 8% to 10% (w/w) ($p = 0.7896$). No discernable trend was observed with respect to the influence of the plasticiser to copolymer ratio on the tensile strength of the films.

Fig. 2 shows a typical trace of the force resulting across the area of the adhesive bond during tensile stressing. The force rises rapidly until fracture occurs and returns immediately to a baseline value. As can be seen in Fig. 3, there was no statistically significant difference in the force of removal of bioadhesive films cast from blends containing 5% or 10% (w/w) PMVE/MA ($p = 0.0971$). Films cast from blends containing 3.5% or 8% (w/w) TPM exhibited significant decreases in their forces of removal as the PMVE/MA content in the original blend was increased. Fig. 3 allows a comparison to be made also in films where the PMVE/MA content was kept constant, but the degree of plasticisation was altered. Increasing TPM contents in the original blends did not significantly alter the mean forces of removal of the final films. Films cast from blends containing 10% (w/w) PMVE/MA with increasing TPM contents in the original blend from 3.5% to 8% (w/w) ($p = 0.1359$) and from 8% to 10% (w/w) ($p = 0.0731$) did not display significantly different forces of removal. Indeed, it takes approximately 2 N of force to remove a 1 cm^2 patch formulation from porcine skin.

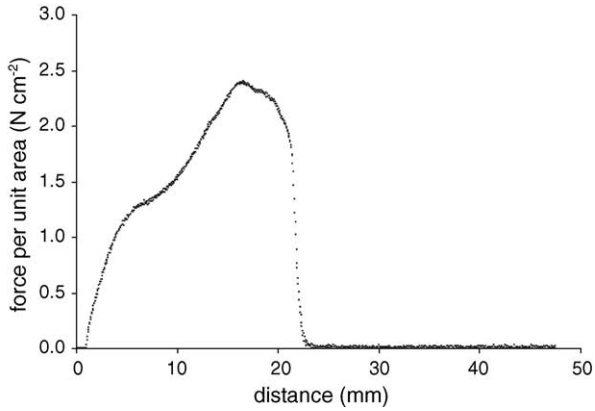

Fig. 2. A distance-resolved trace showing the typical variation in force acting on the bioadhesive-tissue interface during the detachment process. The maximal force, taken as the peak value, is recorded as the bioadhesive force of adherence.

Films cast from aqueous blends of PMVE/MA and TPM were capable of being restuck after initial positioning. This is an important consideration during clinical use and may arise if the patch has been mis-

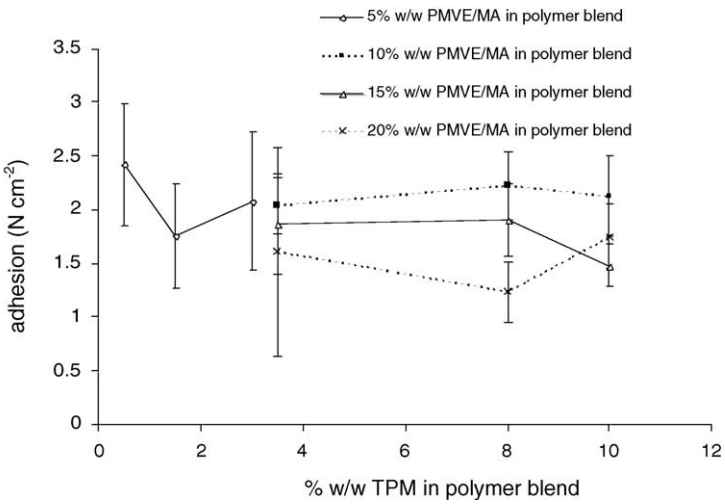

Fig. 3. Influence of varying plasticiser and copolymer contents in aqueous blends on the subsequent bioadhesive tensile strength of bi-laminar films to neonate porcine skin (mean ± S.D., $n = 5$).

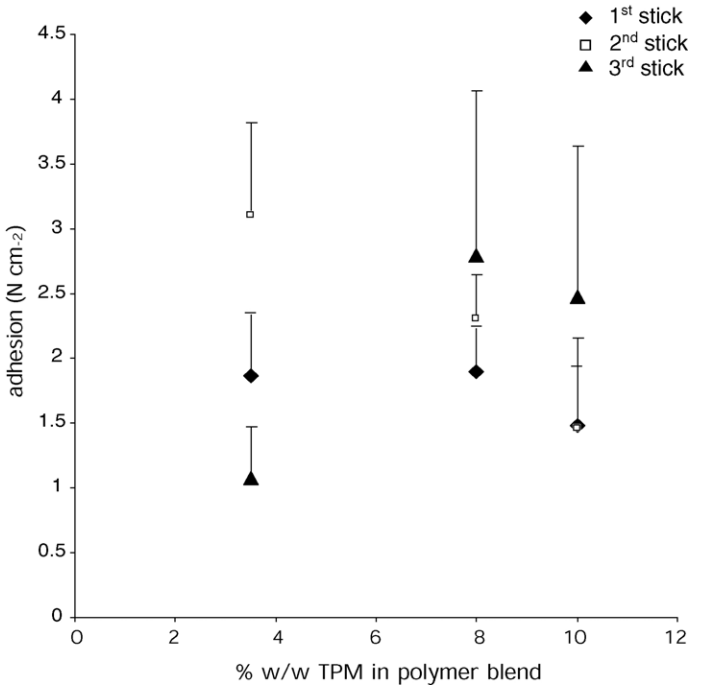

Fig. 4. Restick capabilities, as determined after sequential application, of differently plasticised films cast from polymer blends containing 15% (w/w) PMVE/MA to neonate porcine skin. In vitro forces of bioadhesion are measured in tensile mode (mean ± S.D., $n = 5$).

aligned during the first application. As can be seen from Fig. 4, which shows the adhesion of films cast from blends containing 15% (w/w) PMVE/MA, adhesion was generally increased by repositioning. The force of removal of films cast from blends containing 15% (w/w) PMVE/MA and 3.5% (w/w) TPM, for example, increased significantly from the first initial stick to the second stick ($p = 0.0036$), but showed an unusual reduction in going from the second stick to the third stick ($p < 0.0001$). Films cast from all other blends demonstrated similar properties.

The in vitro peel strength of a topical bioadhesive film is an important parameter to measure as this will mimic the procedure used to remove the patch from skin. As shown in Table 2, peel strength was not significantly affected by the plasticiser or copolymer content in the casting blend and was typically in the range 0.96–1.64 N cm^{-1}. For example, films cast from blends containing 10% (w/w) PMVE/MA and 8% (w/w) TPM had peel strengths that were not significantly different from those of films cast from blends containing 15% (w/w) PMVE/MA and 8% (w/w) TPM ($p = 0.06$), or from those of films cast from blends containing 10% (w/w) PMVE/MA and 3.5% (w/w) TPM ($p = 0.07$).

As can be seen from Fig. 5, the films gain mass initially as they hydrate and initiate adhesion, but loose

Table 2
Copolymer and plasticiser contents of aqueous blends and peel strengths of resulting cast bioadhesive films from neonate porcine skin (mean ± S.D.)

Ratio (PMVE/MA:TPM) (%, w/w)	Peel strength (N cm^{-1})
5.0:0.5	TB
5.0:1.5	1.64 ± 0.57
5.0:3.0	1.58 ± 0.51
10.0:3.5	1.21 ± 0.39
10.0:8.0	1.17 ± 0.28
10.0:10.0	1.24 ± 0.36
15.0:3.5	TB
15.0:8.0	1.21 ± 0.18
15.0:10.0	0.98 ± 0.29
20.0:3.5	TB
20.0:8.0	0.96 ± 0.26
20.0:10.0	0.98 ± 0.17

TB: too brittle to test.

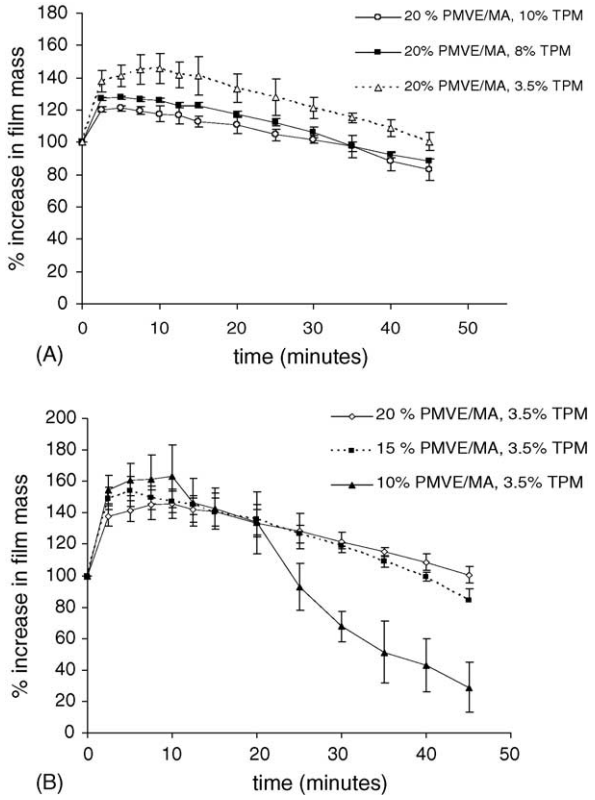

Fig. 5. (A) Influence of TPM content on swelling and dissolution behaviour of bioadhesive films cast from aqueous blends containing 20% w/w PMVE/MA (mean ± S.D., $n = 5$). (B) Influence of PMVE/MA content on swelling and dissolution behaviour of bioadhesive films cast from aqueous blends containing 3.5% (w/w) TPM (mean ± S.D., $n = 5$).

mass if sufficient moisture is present, which brings about film dissolution. Both TPM and PMVE/MA contents in the original aqueous blends significantly affected the swelling and dissolution behaviour of cast bioadhesive films. As PMVE/MA content in the aqueous blend was increased, the maximum swelling weight of cast films in 0.9% (w/w) saline significantly decreased, for a given TPM concentration in the original blend, as shown in Table 3. For example, the mean maximum swollen weight of films cast from blends containing 10% (w/w) PMVE/MA and 3.5% (w/w) TPM was 163.28% of the original film weight, while that of films cast from blends containing 20% (w/w) PMVE/MA and 3.5% (w/w) TPM was significantly less ($p = 0.0462$) at 145.82% of the original film weight. In addition, the extent of dissolution of the films also decreased with increasing PMVE/MA content for a given TPM concentration in the original blend. For example, the mean percentage of film remaining after a period of 45 min immersion in 0.9% (w/w) saline for films cast from blends containing 10% (w/w) TPM and 8% (w/w) TPM was only 26.76% of the original film weight, while that for films cast from blends containing 20% (w/w) PMVE/MA and 8% (w/w) TPM was significantly greater ($p < 0.0001$) at 88.31% of the original film weight. As the TPM content in the original blend was increased, for a given content of PMVE/MA, the mean maximum swelling weight decreased significantly, but the extent of film dissolution after 45 min immersion increased significantly. For example, films cast from blends containing 10% (w/w) PMVE/MA and 3.5% (w/w) TPM had significantly greater mean maximum swollen weights than those cast from blends containing 10% (w/w) PMVE/MA and 10% (w/w) TPM ($p = 0.0049$). In addition, these films

Table 3
Influence of PMVE/MA and TPM contents on swelling and dissolution behaviour of bioadhesive films cast from aqueous blends (mean ± S.D.)

Ratio (PMVE/MA:TPM) (%, w/w)	Maximum weight (% of original weight)	Time to achieve maximum weight (min)	Weight at 45 min (% of original weight)
10.0:3.5	163.28 ± 5.02	10.0	28.95 ± 5.85
10.0:8.0	142.74 ± 8.90	5.0	26.76 ± 4.89
10.0:10.0	131.86 ± 6.96	2.5	16.46 ± 6.02
15.0:3.5	153.58 ± 9.54	7.5	84.09 ± 7.54
15.0:8.0	142.02 ± 8.35	5.0	78.18 ± 2.16
15.0:10.0	127.25 ± 5.59	2.5	71.54 ± 3.36
20.0:3.5	145.82 ± 9.17	10.0	100.53 ± 5.39
20.0:8.0	127.68 ± 1.66	5.0	88.31 ± 1.29
20.0:10.0	121.16 ± 1.13	5.0	83.06 ± 6.36

cast from blends containing 10% (w/w) PMVE/MA and 3.5% (w/w) TPM had significantly greater weights after 45 min immersion than those cast from blends containing 10% (w/w) PMVE/MA and 10% (w/w) TPM ($p = 0.04$). Films cast from blends containing 5% (w/w) PMVE/MA dissolved too quickly to produce reliable results.

Films cast from blends containing 5% (w/w) PMVE/MA, as shown in Table 1, had thicknesses of around 0.2 mm. They were difficult to handle and not suitable for clinical evaluation. Table 4 shows the formulations brought forward into clinical appraisal, together with associated mean scores awarded to each of six characteristics of the patch as derived from the questionnaire. To reiterate, a score of 4 corresponded to a response of "strongly agree", while a score of 0 corresponded to a response of "strongly disagree".

Patches based on films cast from blends containing 3.5% (w/w) TPM were found to be inflexible and too brittle for further use. Patches based on films cast from blends containing 10% and 15% (w/w) PMVE/MA became distorted during use with the bioadhesive film layer reverted to a gel-like material which oozed from beneath the backing layer. As a result, the patients' clothes became adhered to skin. To exacerbate matters, these formulations were strongly adhesive in vivo and difficult to remove.

Patches based on films cast from blends containing 20% (w/w) PMVE/MA and, either 8% or 10% (w/w) TPM performed well. They were flexible, easy to apply and conformed well to the contours at the site of application. They were strongly adhesive, but were capable of being removed in one piece. No pain or irritation at the site of application was reported by patients while patches were in place. Pain on removal was, however, widely reported, even for formulations that could be removed intact.

ALA-loaded patches, prepared using 20% (w/w) PMVE/MA and 10% (w/w) TPM, were shown to induce fluorescence after application for 4 h, as shown in Fig. 5. PpIX is shown to fluoresce under UV illumination, indicating that cutaneous drug absorption has occurred, which is a requisite for successful photodynamic therapy of lesions of intraepithelial nature.

Table 4
Influence of PMVE/MA and TPM contents in original blends on the in vivo performance of bi-laminar bioadhesive patches composed of a cast bioadhesive layer and a non-adhesive PVC packing layer

Ratio (PMVE/MA:TPM) (%, w/w)	A	B	C	D	E	F
10.0:3.5	1	4	1	2	0	3/3
10.0:8.0	4	4	4	3	0	3/3
10.0:10.0	4	1	4	4	0	3/3
15.0:3.5	TB	TB	TB	TB	TB	TB
15.0:8.0	4	4	4	4	0	4/4
15.0:10.0	4	3	4	4	0	3/3
20.0:3.5	TB	TB	TB	TB	TB	TB
20.0:8.0	4	4	4	4	3	4/4
20.0:10.0	4	4	4	4	3	5/5

A, flexibility; B, ease of handling; C, conformability; D, initial adherence; E, easily removed; F, remained attached/fraction of applied patches. TB: too brittle to test in vivo.

4. Discussion

Photodynamic therapy is a treatment method with several merits. This is especially so for therapies based on administration of ALA, which produce selective cellular destruction without significant scarring. The success of the therapy relies on adequate penetration of the drug through intact skin, but given the poor penetration characteristics of ALA, prolonged administration under occlusive dressings is needed to achieve this prerequisite (Kurwa et al., 2000). This has been met with some success on lesions occupying regions of planar skin, such as basal cell lesions on exposed limbed skin, where such systems remain anchored reasonably well (Morton et al., 1998). It is clear from the vulval form that this approach is problematic and such dressings will be difficult to keep in place. It can be concluded that the success of treatments, such as PDT, to the vulva will be improved by using a dosage form designed specifically for application to this area, such as a bioadhesive patch. Prototype patch designs have been described for similar uses, such as transdermal (McCafferty et al., 2000) and cervical applications (Woolfson et al., 1995b). They are suited ideally as a generic system for the administration of cytotoxic drugs in the treatment of cervical intraepithelial neoplasia (Sidhu et al., 1997). To date, there have been no reports of the use of a bioadhesive patch for drug delivery to the vulva. This present study now describes the design of a novel bioadhesive patch possessing suitable characteristics for a vulval drug delivery system.

The patch was based on a bioadhesive layer cast from an aqueous blend of PMVE/MA and TPM and a non-adhesive PVC backing layer prepared from Plastisol® emulsion. PMVE/MA is an ideal polymer to use for vulval application as it is water-soluble and has a documented safety profile (Gantrez® AN-139 Production Bulletin, ISP Europe Ltd., 1996). It has been shown to possess strong bioadhesive properties in vitro (Woolfson, 1996) and determination of this adhesive strength is an important factor in the design of patches of this nature. In this study, porcine skin was used as a model for vulval skin. The former has been shown to be a good model for human skin with regard to hair sparseness, presence of subcutaneous fat, epidermal proliferation and both the orientation and distribution of blood vessels (Fourtenier and Berreb, 1989). Adhesion studies were performed using the bi-laminar system as this represents the envisaged patch as intended for clinical use. For the patch to operate successfully the bioadhesive matrix must not only retain some degree of structural integrity and act as a drug reservoir, it ought to hold the non-adhesive backing layer in place also. Adhesion studies revealed that the polymer–tissue interface was the point of fracture; in all cases, the bioadhesive film remained anchored to the PVC film. Furthermore, adhesion to porcine skin showed that thicker films, produced by casting more concentrated polymer blends, showed significantly weaker bioadhesion in vitro than thinner films, as shown in Fig. 3. Indeed, as can be seen in Table 1, films cast from blends containing 5% (w/w) PMVE/MA had thicknesses around 0.20 mm. Films cast from blends containing 20% (w/w) PMVE/MA had thicknesses around 0.55 mm. This effect may arise due to the ability of thicker films to remove water from the adhesive joint giving a suboptimal concentration required for effective adhesion. At the interface, polymer molecules are solvent-poor and unable to form effective interpenetrations with the biological substrate (Ahuja et al., 1997). Thinner films have a lesser capacity for water sequestering and give a more hydrated interface. Regardless of this effect, adhesion was found to be in the region of 2.0 N cm^{-2}, which is sufficiently tenacious to secure the complete patch assembly.

Bioadhesive films cast from aqueous blends containing PMVE/MA require a plasticiser due to the high glass transition temperature of the unplasticised copolymer (Chung et al., 1990). Polyhydric alcohols, such as glycerol, are generally employed as plasticisers of these films (Woolfson et al., 1995c). However, these formulations, have been shown by McCarron et al. (2004) to lose their adhesion and become increasingly brittle over time. Consequently, TPM, which does not bring about this alteration of film properties, was used as the plasticiser in this study. As can be seen from Table 1, increasing the TPM content in the original blend significantly increased the percentage elongation at break of formed films. This represents a measure of the conformability of the bioadhesive film, which is a required property if the patch is to be shaped and moulded over potential lesions. As the PVC layer is considerably more flexible than the bioadhesive matrix, the majority of the tensile properties leading to ease of conformability arise from the latter. Higher TPM contents allowed extremely flexible films to be produced. Films cast from blends containing 10% (w/w) PMVE/MA and 8% (w/w) TPM had a mean percentage elongation at break of 513%, while those cast from blends containing 10% (w/w) PMVE/MA and 10% (w/w) TPM had a mean percentage elongation at break of 946%.

The ability to relocate a misaligned patch was an important consideration. As shown in Fig. 4, all films were capable of being successfully restuck on more than one occasion. Restick strength was found to increase, presumably due to improved hydration after successive exposures to moisture on the target surface. This would allow a greater interpenetration of bioadhesive polymeric chains into the surface features on the skin substrate.

Films cast from blends containing 5% (w/w) PMVE/MA were very thin and, consequently difficult to handle. They were not evaluated clinically, nor were films cast from blends containing 3.5% (w/w) TPM and 15% (w/w) and 20% (w/w) PMVE/MA, respectively, which were brittle. Patches based on films cast from aqueous blends containing 10%, 15% and 20% (w/w) PMVE/MA exhibited strong adhesion in vivo. This was estimated using an in vitro peel test, which closely mimics the mechanism used to remove the film in clinical use. As shown in Table 2, in vitro peel strengths were typically in the order of 1.0 N cm^{-1} and were not significantly affected by copolymer or plasticiser contents in the original casting blends. This data suggests that the patch is held firmly in location, as judged by tensile adhesion measurements, but could be removed

from the skin or tissue using moderate amounts of force applied in a peeling mode.

Patches based on films cast from blends containing 10% and 15% (w/w) PMVE/MA performed poorly in vivo. They were unable to maintain their solid nature, becoming increasingly more fluid as the application time increased, eventually resembling gels that exuded from beneath the periphery of the PVC backing layer. This effect was predicted based on water uptake studies. Films cast from blends containing 10% (w/w) PMVE/MA were highly soluble; their mass decreasing rapidly in 0.9% (w/w) saline, such that only around 27% of films cast from blends containing 8% (w/w) TPM remained after 45 min immersion. Increasing the TPM content to 10% (w/w) in the original blend significantly increased the solubility of these films, such that only around 17% of the film remained after 45 min. A similar effect was observed in the enhanced dissolution of PVA films as the proportion of water-soluble plasticisers, such as glycerol, was increased (Lim and Wan, 1994). Increasing the PMVE/MA content in the original blends to 15% reduced the solubility of the films significantly, presumably because a higher percentage of the weight of the final cast film was due to the copolymer. Again, increasing the TPM content significantly reduced the maximum swollen weight and the final weight of these films. This data is important as sweating from skin under occlusion is expected, together with exposure to vaginal exudates and secretions from sebaceous and apocrine glands. Films cast from blends containing 10% and 15% (w/w) PMVE/MA are unable to withstand this hydration, due to their dissolution characteristics. Overhydration occurred in vivo with films cast from blends containing 10% and 15% (w/w) PMVE/MA. These films were difficult to remove from the vulva. In fact, none could be removed in one coherent piece and adherent remnants were observed upon removal of the backing layer.

Bi-laminar patches based on films cast from blends containing 20% (w/w) PMVE/MA and either 8% or 10% (w/w) TPM performed well in vivo. They maintained their shape throughout the 4 h application times used in this study, with the patch still securely anchored on the lower vulval area, as shown in Fig. 6(A). As a result, they were capable of being removed in one piece and none of the bioadhesive formulation oozed from beneath the PVC backing layer. The maximum swollen weight of films cast from blends containing 20% (w/w) PMVE/MA and 8% (w/w) TPM was approximately 127% of their original mass on immersion in 0.9% (w/w) saline. Films cast from such a blend also maintained most of their original weight (88%) even after 45 min immersion. Films cast from blends containing 20% (w/w) PMVE/MA and 10% (w/w) TPM performed similarly, the increased TPM content only slightly enhancing dissolution. Table 4 shows a summary of clinical evaluations based on qualitative measures and shows clearly that the patches cast from 20% (w/w) PMVE/MA gels performed well. Indeed, it is clear that the 20.0:10.00 formulation possesses an overall assessment that meets most of the design criteria and further drug administration studies were performed using this formulation.

The in vivo ALA delivery resulting from the 20.0:10.00 formulation was evaluated by detecting the fluorescence of protoporphyrin (PpIX) in vulval skin after administration of ALA. A dose of 38 mg cm^{-2} was chosen based on the clinical use of a 20% (w/w) ALA proprietary cream formulation, as detailed by McCarron et al. (2003). As can be seen in Fig. 6(B) and subsequent images, the red fluorescence observed under ultraviolet illumination is clear, demonstrating that neoplastic cells have been exposed to a sufficient concentration of ALA to elicit PpIX production. This came about in ambulant patients who was not immobilised, as is often required using the conventional occlusion dressing approach. Once removed, the patch induced PpIX formation and subsequent fluorescence in the five patients observed in this study. The vulval area is now in a photosensitised state and ready for light application. Further studies are now underway to evaluate the clinical effectiveness of the patch following photodynamic therapy.

In conclusion, bi-laminar patches, of optimal design for vulval application, were produced, comprising a bioadhesive layer cast from an aqueous blend containing 20% (w/w) PMVE/MA, 10% (w/w) TPM and a non-adhesive backing layer composed of medical grade PVC. The bioadhesive and non-adhesive layers both demonstrated excellent flexibility in vitro and the patches conformed well to the contours of the vulva in vivo. These patches adhered strongly to the vulva, but could be removed easily. They remained in position, maintained their structures during application times of 4 h in vivo and did not cause pain or irritation. As such, they represent a generic delivery system

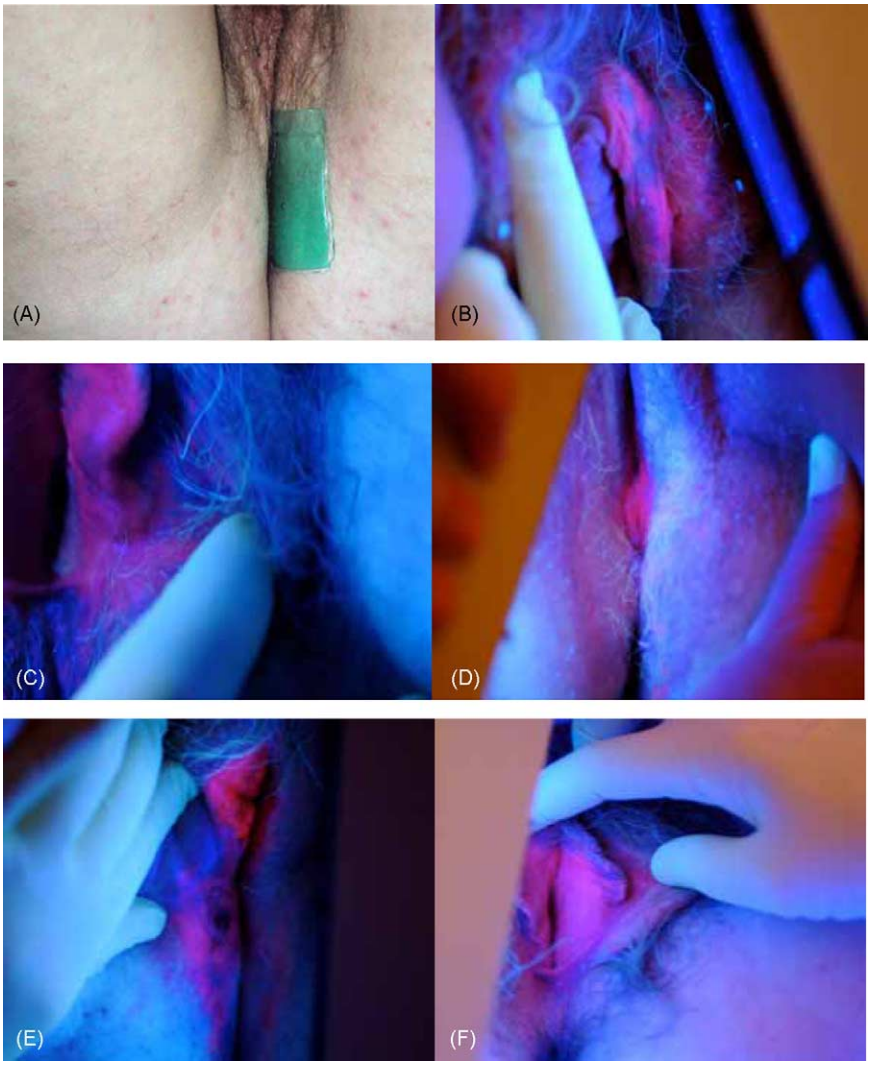

Fig. 6. (A) Photograph of bi-laminar bioadhesive patch in place at the female lower reproductive tract. The patch is composed of a bioadhesive film layer and a PVC backing layer. Note the green color of the backing layer and the presence of a removal tag, shown at the point of the arrow. (B)–(F) Fluorescence of intracellular protoporphyrin IX, as shown by the areas of red coloration, induced in vulval intraepithelial neoplasia after patch-based administration of ALA and illumination under ultraviolet light (Wood's lamp).

that may be adapted to administer a range of drugs to the vulva, in particular, cytotoxic drugs and photosensitising agents. The latter group is of particular relevance given the need to achieve prolonged application times. This study showed that administration of ALA was achieved over a 4 h period, as demonstrated by PpIX production. Further clinical studies are now underway to evaluate the patch as a treatment modality for vulval intraepithelial neoplasia, vulvodynia, lichen sclerosus et atrophicus and other neoplastic conditions of the vulva. Similarly, drug release studies and resultant tissue penetration data shall be reported shortly.

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