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Research paper

# A novel *in-vitro* skin model to assess secondary transfer of applied testosterone following skin to skin contact

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ABSTRACT

Transdermal testosterone gels are popular and widely used for testosterone replacement therapy. However, unintended exposure of other individuals to testosterone through secondary transfer of the product caused by skin-to-skin contact is a recognised risk with their use. Case reports have described children developing clinical symptoms following inadvertent transfer, despite controlled *in vivo* studies generally suggesting that secondary transfer is minimal once the gel has dried. At present, there is limited consensus regarding the guidance patients should follow to minimise transfer risk, and regulatory agencies have highlighted the need for an *in vitro* model capable of investigating transfer mechanisms and mitigation strategies. In this study, an *in vitro* model was developed to evaluate the influence of the time interval between gel application and skin-to-skin contact on testosterone transfer and absorption, as well as the impact of sweat on these processes. The model was based on the Franz diffusion cell system and simulated controlled skin-to-skin contact between 'primary' skin, representing the treated individual, and 'secondary' skin, representing the exposed individual. Experiments were conducted using both porcine and human skin following application of Testogel to the primary skin surface. As expected, the lowest transfer of testosterone to secondary skin occurred when contact was made after the testosterone film had fully dried, with a 7-fold reduction in transferred testosterone compared to when contact was made immediately after dosing. This resulted in a 14-fold reduction in testosterone absorption into the secondary skin. However, although the greatest amount of testosterone remained on the primary skin under fully dried conditions, skin-to-skin contact at this stage resulted in a 2-fold reduction in testosterone absorption into the primary skin itself. This effect has not previously been reported. Raman microscopy demonstrated that this reduction was associated with disruption of intimate contact between the dried testosterone film and the primary skin following skin-to-skin contact, suggesting that such contact may also compromise therapeutic efficacy. In the presence of sweat, secondary transfer and absorption were increased approximately 5-fold and 3-fold, respectively, compared with contact in the absence of sweat, demonstrating for the first time the important role of perspiration in enhancing transfer risk. Overall, the developed model provided new mechanistic insight into factors governing secondary transfer and absorption from topical hormonal products and represents a promising approach to support formulation development and reduce the need for *in vivo* clinical studies.

## 1. Introduction

Transdermal testosterone T) and oestradiol OE) gels are widely used to treat symptomatic hypogonadism in ageing men and women [1–4]. Topical gels offer several advantages over alternative delivery systems for T and OE, such as bypassing the first-pass metabolism associated with oral preparations [5], improved patient acceptability compared to intramuscular injections [6,7] and reduce the inconvenience and irritation associated with patch-based transdermal delivery systems [8,9]. Currently, T gels are the most prescribed T formulation in England [10].

T and OE gels are applied to low-hair areas such as the abdomen, shoulders, upper arms, and thighs (Electronic Medicines [11,12]. They are volatile and undergo dynamic formulation changes, leaving dried residual films on the application site shortly after use [13]. If another individual comes into physical contact with this site, even after the formulation has dried to the residual film, transfer of the drug may occur, posing a health risk to the exposed individual [14,15]. A group of controlled *in-vivo* studies investigating T gel transfer have demonstrated

*Abbreviations:* T, Testosterone; OE, Oestradiol; CB, Cotton bud; SD, Standard deviation; IPM, Isopropyl myristate; API, Active pharmaceutical ingredients.

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that direct skin contact can result in the transfer of T, albeit at low levels. These studies also show that covering the application site and washing of the skin can lower the transfer of T to the exposed individual, [16–19];). OE gels have also demonstrated transference [20]. Current T gel guidance advises patients to wash their hands and cover the application site. However, there is a lack of consensus across products regarding the appropriate time frame for safe contact with a partner or the recommended duration for leaving the product on before washing the site [21–24].

Despite the available guidance, case studies of transference occurring in real world situations have been extensively reported, in particular through incidents involving children displaying precocious pubertal symptoms following inadvertent transfer of T and OE gel from their parents [25–27]. Although less common, spousal transfer has also been documented [28]. Both the UK's Medicines and Healthcare products Regulatory Agency MHRA) and US's Food and Drug Administration FDA) have issued warnings to clinicians and manufacturers about the risk of secondary transfer from T and OE gels [29,30]. These reported case studies may reflect poor adherence to treatment guidance and/or differing conditions in real world situations such as the presence of sweat affecting transference.

To date, no *in-vitro* research has investigated the secondary transfer of T and OE gels [15] with the FDA expressing interest in conducting such research [31]. Implementation of an *in-vitro* model would serve as a cost-effective alternative to *in-vivo* studies and enable the effect of different conditions that may impact the transferability of these hormonal gels, including the presence of sweat, to be more easily assessed. Moreover an *in vitro* model could be employed in formulation development, to improve formulations to be less transferrable, mitigating the impact of contact between individuals on transference or poor adherence to treatment guidance.

This work introduces a novel *in-vitro* model to investigate secondary transfer scenarios of T gel from patient-modelled primary) skin to exposed individual-modelled secondary) skin. It assesses the transferability to, and exposure of, secondary skin, as well as the impact of contact on primary skin absorption under varying time intervals after dosing. Additionally, it investigates the hypothesised influence of sweat on transference [32,33], a factor that has not been previously explored in *in-vivo* studies.

## 2. Materials and methods

### 2.1. Materials

Testosterone "T"; Eu. Ph. Grade) was obtained from Sigma-Aldrich Gilllingham, UK) and Testogel® 40.5 mg T per 2.5 g) manufactured by Besins Healthcare London, UK) was obtained from ASDA pharmacy Hatfield, UK). High-performance liquid chromatography HPLC) grade methanol, absolute ethanol 99%+), and "Oxoid" PBS tablets were sourced from Fisher Scientific Loughborough, UK). Isopropyl myristate IPM) 96%), sodium chloride, urea 98+%), lactic acid 90%), ammonium hydroxide solution were obtained from Sigma-Aldrich Dorset, UK). Carbopol 980 was sourced from IMCD Sutton, UK). MerckMillipore >18.0 MΩ.cm ultrapure type 1 water was obtained from an in-lab dispenser Dorset, UK).

### 2.2. Methods

#### 2.2.1. High Performance Liquid Chromatography HPLC)

Quantitative analysis of T was performed using an Agilent 1260 infinity system coupled with an Agilent 1260 multi wavelength UV/Vis detector set to 244 nm from Agilent Technologies Stockport, UK). Analysis was performed on a Kinetex$^{TM}$ $C_{18}$ reverse phase liquid chromatography column 250 x 4.6 mm 5 μm particle size with a sample injection volume of 20 ul at a flow rate of 1 mL min$^{-1}$. A gradient method was employed using the following ratios of methanol to water: 5:5 from 0 to 2 minutes, 9:1 from 2 minutes to 9 minutes, 5:5 from 9 minutes to 12 minutes. Under these conditions the elution time of T was at 9.1 minutes. The HPLC method was fit for purpose with respect to ICH guidelines [34] with a linearity of $R^2 > 0.999$ across a range of 0.025 – 50 ug/ml. The limit of detection and limit of quantification were calculated as 0.32 μg/ml and 0.96 μg/ml respectively. Precision was less than 2% RSD and accuracy ranged from 98.75% to 100.77 %.

#### 2.2.2. Porcine skin preparation

Skin from 12-week-old female pigs was obtained from a reputable supplier and on the same day of culling, the back, flank and abdomen was clipped and cut with a scalpel. Skin was dermatomed with a D80 dermatome from Humeca Borne, NL), to a thickness of 500 μm, wrapped in foil and placed in zip lock bags to be stored at -20°C. On the day of experimentation, dermatomed sheets were thawed and cut into 2 cm$^2$ sized squares for mounting onto Franz cells.

#### 2.2.3. Human skin preparation

Human abdominal skin from a single, caucasian, female donor without pre-existing health complications, dermatomed to 500 μm was obtained from BioIVT Glasgow, UK). The skin was stored at -20°C and thawed before use. Ethical approval for the use of human skin was provided through NHS Research Ethics Committee Approval 19/WS/0164).

#### 2.2.4. In-vitro secondary transfer model of dosed T gel.

Skin was mounted SC facing side up on individual calibrated Franz cells purchased from Soham Scientific Cambridge, UK) with a 3 mL receptor chamber volume and an average surface area of 1 cm$^2$. Across 12 Franz cells, 6 were designated as primary skin, receiving the T gel dose and serving as the patient skin model. The other 6 Franz cells were designated as secondary skin, contacting the dosed primary skin in differing scenarios and representing the exposed individual model.

Franz cells were prepared by filling the receptor chamber with EtOH: PBS at a ratio of 3:7, equilibrated to a skin surface temperature of 32°C, confirmed using a thermal laser gun. This was achieved by placing the cells in a water bath set to 37°C and continuously stirring at 350 rpm with a 2 mm micro stirrer in the receptor chamber. Regardless of contact condition, all primary skin Franz cells were dosed with 10 μL of formulation from a freshly opened Testogel® sachet and spread using a positive displacement pipette. Spreading was standardised by performing 5 clockwise and 5 anticlockwise motions over a 10 second period.

To initiate contact representing the secondary transfer scenario), pairs of primary and secondary skin were swiftly unmounted from their respective Franz cells and placed on glass slides. The secondary skin was lifted from the slide, placed on a gloved thumb, and contact was made with the primary skin for 10 clockwise and 10 anticlockwise motions over 20 seconds, ensuring consistent pressure equivalent to that of a D500 D-Squame Pressure Instrument from CuDerm Corporation Dallas, USA) across the 1 cm$^2$ diffusive area of the secondary skin. Both sets of skin were then swiftly re-mounted onto their respective Franz cells

*2.2.4.1. Assessing the effect of time between dosing and skin contact on secondary transfer..* Contact initiated between porcine skin samples occurred in four separate conditions at different timepoints after dosing primary skin, to investigate whether the amount of T transferred was associated with the dryness of the residual T gel film. For these conditions, contact by secondary skin after dosing primary skin with T gel occurred either: immediately after dosing, 1 minute after dosing, 5 minutes after dosing or 10 minutes after dosing. Experiments with human skin were performed similarly to those that used pig skin, with select conditions of no contact following dosing therapeutic dose) and contact with secondary skin occurring 10 minutes after dosing.

*2.2.4.2. Assessing topical secondary transfer in the presence of sweat..* A

physiologically relevant 'exercise-induced' amount of sweat, equating to 36 μL $cm^{-2}$ [35], was used to simulate sweat on the surface of primary skin. Artificial sweat was prepared on the day according to the nickel release standard BS EN 1811:2011, with a final composition of deionised water, sodium chloride 0.5% w/v), urea 0.1% w/v), and lactic acid 0.1% w/v), with the pH adjusted to 6.5 using ammonium hydroxide $NH_4OH$).

In the first sweat condition, "30 minutes of dose pre-sweat " DPS30 min), primary skin mounted in a Franz cell, received a T gel dose and was left to dry for 30 minutes. This was followed by sweat administration via a pipette. The primary skin was then left for another 10 minutes to allow the sweat to be exposed to the residual film and evaporate before contact was initiated. The second condition, "10 minutes of sweat pre-dose" SPD10 min), involved administering sweat to skin mounted in a Franz cell for 10 minutes before applying the Testogel® dose. Once dosed, the primary skin was left for 10 minutes before contact was initiated with secondary skin. Again, experiments with human skin were performed similarly to those that used pig skin, with select conditions of no contact following dosing therapeutic dose) and contact occurring 10 minutes after dosing. A schematic illustration of the model is shown in Fig. 1.

#### 2.2.5. *Skin distribution of testosterone.*

Samples 250 μL) of receiver fluid were taken after dosing primary skin) or exposure to primary skin secondary skin) at pre-determined intervals for 24 hours and analysed by HPLC-UV. Control conditions, involving primary skin receiving no contact with secondary skin to represent the usual therapeutic profile, followed the same dosing and sampling regimen. After the 24-hour *in-vitro* permeation experiments, the skin was unmounted and unabsorbed T was recovered using three wipes of a cotton bud CB)—the first soaked in methanol and the last two dry. Two scotch tape strips sourced from 3M Company Bracknell, UK) were then taken from the stratum corneum SC) on the skin surface, before the epidermal and dermal layers were separated by heating the skin samples on glass slides in a pre-heated oven set to 60°C for 1 minute, followed by peeling the epidermis from the dermis [36]. All separated skin matrix sections were placed in individual glass vials, to which 1 mL of methanol was added. The vials were sonicated for 15 minutes and then placed on a roller overnight at room temperature 20°C). Prior to HPLC analysis, the skin distribution samples were filtered using 0.22 μm PTFE polytetrafluoroethylene) syringe filters. Using these procedures, total drug recovery for each condition was calculated as the sum of T recovered from all matrices across each paired primary and secondary skin replicate. According to OECD guidelines for *in vitro* studies involving volatile formulations, recovery should be 100 ± 20%, which was met in this study [37,38].

#### 2.2.6. *Raman microscopy of testosterone gel films on the skin surface*

Raman microscopy of T gel films was conducted using a Renishaw inVia Raman microscope Renishaw Plc, UK), calibrated with a reference silicon block. To obtain the reference spectra for each excipient of Testogel, samples were placed on foil-covered glass slides and imaged using a x50 magnification lens with an excitation wavelength of 785 nm, with three accumulations over an acquisition time of 10 seconds.

For imaging the T gel film on skin following contact, primary porcine) skin was set up as described in section 2.2.4 and imaged immediately after dosing and after 10 minutes when contact was initiated with secondary skin. Digital images and Raman spectra to confirm the presence or absence of T on the film or skin were taken using the x50 magnification lens with the same settings used for the reference spectra, with three accumulations.

#### 2.2.7. *Data treatment and statistical analysis*

Statistical analysis of experimental data was performed using GraphPad Prism software version 10.3.0 461) for MacOS Boston, USA), and all experimental data was expressed as mean n = 6) ± standard deviation SD) unless stated otherwise. The Shapiro-Wilk test was used to determine the normality of all data sets. Parametric data involving multiple comparisons followed a one-way ANOVA with Tukey's post hoc analysis. For unequal standard deviations between data groups, a Brown-Forsythe and Welch ANOVA was conducted with Dunnett T3 for post hoc analysis. For pairwise comparisons, an unpaired T-test was used as all data was parametric. Alternatively, for non-parametric data involving multiple comparisons, a Kruskal-Wallis test with Dunn's post hoc test was used.

## 3. Results

### 3.1. *The effect of secondary transfer contact on drug absorption into exposed secondary) skin assessed by the in-vitro model.*

Figs. 2A and 2B show the amount of T transferred to secondary porcine and human skin respectively following contact with primary dosed skin and, for comparison, the T present on primary skin following dosing without secondary contact. T is separated into unabsorbed and absorbed fractions at 24 hours for porcine P; Fig. 2A) and human H; Fig. 2B) skin. Porcine skin was contacted immediately after T gel application IC) or following delays of 1 C1), 5 C5), or 10 minutes C10), whereas human skin was evaluated following contact at 10 minutes C10) only. The corresponding permeation profiles over 24 h are shown in Fig. 2C P) and Fig. 2D H).

Across the porcine skin data, a clear trend was observed whereby the

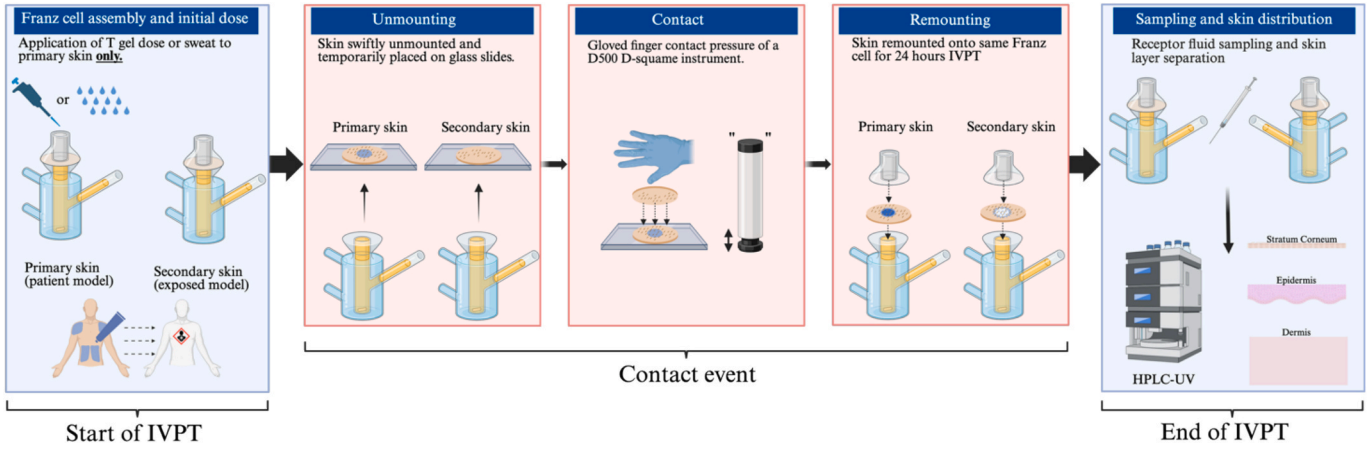



**Fig. 1.** Overview of methodology for the *in-vitro* secondary transfer model. Primary skin is firstly dosed with T gel alone/and/or sweat depending on condition). After defined intervals of time, this skin is dismounted and contact is initiated between the primary and secondary skin. The skin is then remounted on to the Franz cells with skin permeation recorded across the 24 h and skin matrices separation and analysis at the end of the experiment.

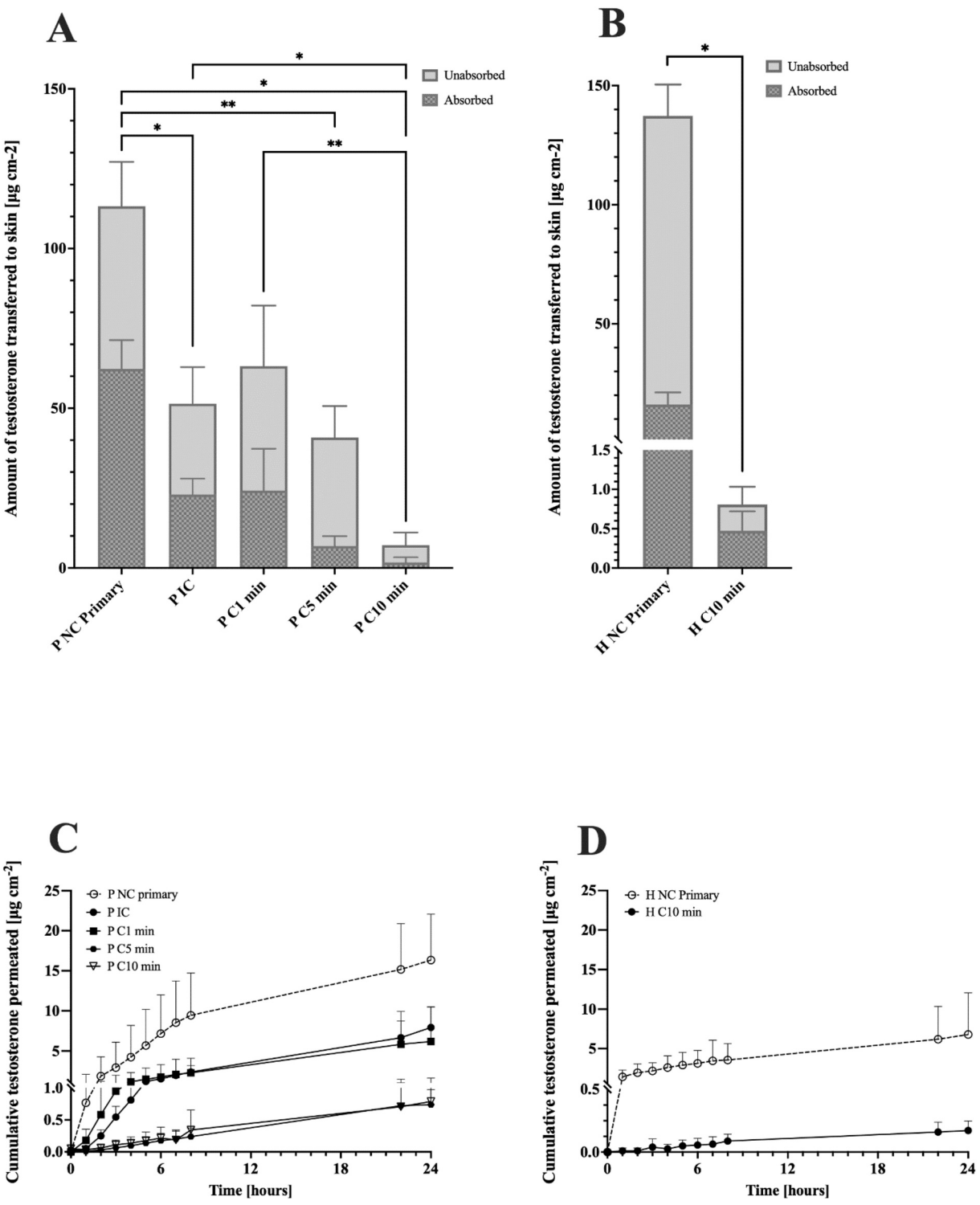



**Fig. 2.** A) Total T transferred to porcine P) secondary skin after 24 h following contact with dosed primary skin immediately after gel application IC) or after 1 C1), 5 C5) or 10 C10) min. Transferred T is presented as unabsorbed surface-associated) and absorbed within skin) fractions. B) Total T transferred to human H) secondary skin after a 10 min contact delay C10), presented as in A). C) Cumulative permeation of T into porcine secondary skin over 24 h following the contact conditions shown in A). D) Cumulative permeation of T into human secondary skin over 24 h following the contact condition shown in B). Statistical comparisons were performed on total transferred T unabsorbed + absorbed) relative to the corresponding no-contact NC) control. Bars and permeation profiles represent mean ± SD $p \leq 0.05$, $p \leq 0.01$; $n = 6$).

amount of T transferred to secondary skin decreased with increasing time between dosing and contact Fig. 2A). Secondary skin contacting primary skin 10 min after dosing resulted in significantly lower T transfer 7.1 µg $cm^{-2}$), representing a 7- and 9-fold reduction compared with the 51.4 and 63.2 µg $cm^{-2}$ transferred when contact occurred immediately or 1 min after dosing, respectively. For human skin, transfer at 10 min post-dose was lower still, with only 0.9 µg $cm^{-2}$ of T being transferred to secondary skin Table 1).

Absorption of T into secondary skin decreased with longer time intervals between dosing and contact as shown in Table 1. Contact

**Table 1**
Amounts of T recovered from secondary skin and compared with primary no-contact skin therapeutic dose). Data are presented as mean ± SD n = 6) or %RSD. Statistical comparisons are indicated by * $p \leq 0.05$), ** $p \leq 0.01$) or **** $p \leq 0.0001$). Matching numbered superscripts identify the groups included within each statistical significant comparison, with asterisks indicating the corresponding level of statistical significance. Percent of transferred T that was absorbed represents the proportion of transferred T that permeated into and across the secondary skin relative to the total transferred amount. Percent delivery of therapeutic dose represents the absorbed amount of T in the secondary skin expressed as a percentage of the absorbed amount associated with the therapeutic dose.

| Contact condition | Transferred T [μg $cm^{-2}$] | Unabsorbed T [μg $cm^{-2}$] | Absorbed T [μg $cm^{-2}$] | Percent of transferred T that was absorbed. [%] | Percent delivery of therapeutic dose. [%] | Stratum corneum surface [μg $cm^{-2}$] | Epidermis [μg $cm^{-2}$] | Dermis [μg $cm^{-2}$] | Receptor fluid [μg $cm^{-2}$] |
|---|---|---|---|---|---|---|---|---|---|
| P IC | 51.4 ± 14.1) | 28.5 ± 11.4) [1] | 23.0 ± 5.0) [1, 2] | 46.1 ± 9.3) [2] | 36.9 ± 8.0) | 5.4 ± 1.9) | 2.1 ± 1.3) | 7.5 ± 1.8) | 7.2 ± 2.6) |
| P C1 min | 63.2 ± 13.9) | 39.0 ± 19.0) [1] | 24.2 ± 13.1) [1, 2] | 39.5 ± 23.5) | 38.9 ± 21.0) | 4.5 ± 2.0) | 2.1 ± 2.2) | 11.5 ± 7.9) | 6.2 ± 4.3) |
| P C5 min | 40.8 ± 10.7) | 34.0 ± 9.9) [1] | 6.9 ± 3.1) [1] | 17.1 ± 8.0) [2**] | 11.0 ± 5.0) | 3.1 ± 1.4) | 0.8 ± 0.6) | 2.2 ± 1.8) | 0.7 ± 0.2) |
| P C10 min | 7.1 ± 5.3) | 5.4 ± 4.0) [1*] | 1.7 ± 1.6) [1, 2**] | 25.1 ± 12.4) [1] | 2.7 ± 2.6) | 0.4 ± 1.6) | 0.1 ± 0.1) | 0.5 ± 0.5) | 0.7 ± 0.8) |
| *P NC Primary* | *N/A* | *50.9 ± 13.9) [1]* | *62.3 ± 9.1) [1*]* | *55.5 ± 9.5) [1**, 2]* | *100.0 ± 14.5)* | *27.8 ± 8.4)* | *8.0 ± 7.7)* | *10.2 ± 3.6)* | *16.3 ± 5.7)* |
| H C10 min | 0.9 ± 0.5) | 0.4 ± 0.5) [3] | 0.5 ± 0.3) [3] | 57.9 ± 15.4) [3****] | 2.9 ± 1.6) | 0.1 ± 0.0) | 0.1 ± 0.1) | 0.2 ± 0.1) | 0.2 ± 0.1) |
| *H NC Primary* | *N/A* | *121.2 ± 16.1) [3****]* | *16.1 ± 5.1) [3****]* | *11.8 ± 4.1) [3]* | *100.0 ± 31.7)* | *7.7 ± 2.6)* | *0.4 ± 0.4)* | *0.9 ± 0.6)* | *7.1 ± 4.8)* |

occurring 10 minutes after dosing porcine skin resulted in the lowest amount of T absorption by secondary skin equating to 2.7% of absorption of the therapeutic dose on primary skin $p \leq 0.0001$). This reduction in absorption was reflected in the corresponding permeation profiles, where lower cumulative permeation over 24 h was observed with increasing intervals between dosing and contact Fig. 2C and D). In contrast, when contact with secondary skin was made immediately after dosing, 36.9% of the therapeutic dose $p \leq 0.05$) was absorbed into the secondary porcine skin. Absorption of T into human skin was lower than that observed with porcine skin, however when contact occurred after 10 minutes, a similar percentage of the therapeutic dose was absorbed into secondary human skin and porcine skin 2.9% and 2.7% respectively). Across the porcine skin conditions, a general observation was that with longer intervals i.e. 5 min and 10 min) between dosing and contact resulted in a reduction in the proportion of transferred T that was absorbed. Specifically, contact at 5 min and 10 min resulted in 17.1% and 25.1% absorption of transferred T respectively, compared with 46.1% following immediate contact $p \leq 0.01$).

### 3.2. The effect of secondary transfer contact on drug absorption into dosed primary) skin assessed by the in-vitro model.

The data presented in Fig. 3 and Table 2 are shown in the same format as Fig. 1 and Table 1, but instead they present the amount of T remaining on primary skin following contact with secondary skin, again distinguishing between absorbed and unabsorbed T over a 24-hour permeation timeline. As before this is compared to primary skin receiving no contact therapeutic dose) across both porcine and human skin experiments.

The amount of T remaining on porcine primary skin following secondary transfer contact increased with longer time intervals between dosing and contact Fig. 3A). Contact occurring 10 min after dosing resulted in a significantly higher amount of T remaining on primary skin 124.8 μg $cm^{-2}$), representing 1.7-, 1.7- and 1.3-fold increases compared with the 75.6, 72.4 and 95.5 μg $cm^{-2}$ remaining when contact occurred immediately, 1 min and 5 min after dosing, respectively. For human skin, contact at 10 min post-dose resulted in a modest but significant 1.2-fold reduction in total T remaining on primary skin, from 137.3 μg $cm^{-2}$ in the absence of contact to 111.7 μg $cm^{-2}$ Fig. 3B).

Despite a greater amount of T remaining on the primary skin at longer contact intervals, absorption into porcine primary skin decreased. The lowest absorption occurred following contact at 5 and 10 min post-dose, with both conditions reducing absorption to approximately 21 μg $cm^{-2}$ $p \leq 0.05$), representing an approximately 2-fold reduction compared with 45.2 μg $cm^{-2}$ following immediate contact Table 2). This trend was also reflected in the corresponding permeation profiles Fig. 3C). When compared to the therapeutic dose, these conditions corresponded to 34.5% and 35.1% absorption $p \leq 0.05$) of the therapeutic dose, respectively. In contrast, contact at 1 min post-dose resulted in significantly greater absorption equating to 53.7% of the therapeutic dose $p \leq 0.05$) despite an overall lower amount of T remaining on the primary skin compared to contact at 5 and 10 min. For human primary skin, secondary contact at 10 min post-dose resulted in a reduction in T absorption to 63.8% of the therapeutic dose $p \leq 0.05$), consistent with the trend observed in porcine skin, although the change in absorption was of a lower magnitude.

### 3.3. Raman microscopy of T gel films on the skin surface

Raman spectral analysis of the T gel excipients and porcine skin identified two peaks in a spectral window of 1575 $cm^{-1}$ to 1700 $cm^{-1}$ Fig. 4) that were unique to T present in dried T gel spectra. These were labelled in Fig. 5 as peak 1619 $cm^{-1}$ and peak 1655 $cm^{-1}$. The peak at 1619 $cm^{-1}$ is associated with C=C) alkene stretching from the unsaturated bonds at position C4 and C5 on T, whilst peak 1655 $cm^{-1}$ is associated with carbonyl C=O) stretching at position C3 [39,40].

Once applied to porcine skin, the T gel dried within 10 minutes, forming a relatively continuous film containing T on the skin surface Fig. 5A and C). After contact was initiated, light microscopy suggested disruption to this film occurred, with areas where the film had been previously deposited losing intimate contact with the skin Fig. 5B and D). Raman spectra of areas previously covered with the dried T gel film, and of areas where the film seemed to have lost contact with the skin, confirm this interpretation through the loss of Raman peaks associated with T 1619 and 1655 $cm^{-1}$) at these disrupted regions. Fig. 4E shows the obtained spectra, indicating the removal of T from disrupted areas of the film following skin-to-skin contact.

### 3.4. The effect of sweat on drug absorption into exposed secondary) skin following secondary transfer contact assessed by the in-vitro model

Both sweat-modified conditions resulted in a marked increase in T transfer to secondary skin compared with contact occurring 10 min after dosing in the absence of sweat Fig. 6). Sweat applied 10 min before T gel application followed by secondary skin contact 10 min after dosing is referred to as the sweat before dose condition SBD10 min), whereas T gel applied 30 min before sweat application followed by secondary skin contact 10 min later is referred to as the dose before sweat condition DBS30 min). Under the SBD10 min condition, 34.4 μg $cm^{-2}$ of T was transferred to secondary skin, approximately a 5-fold increase compared

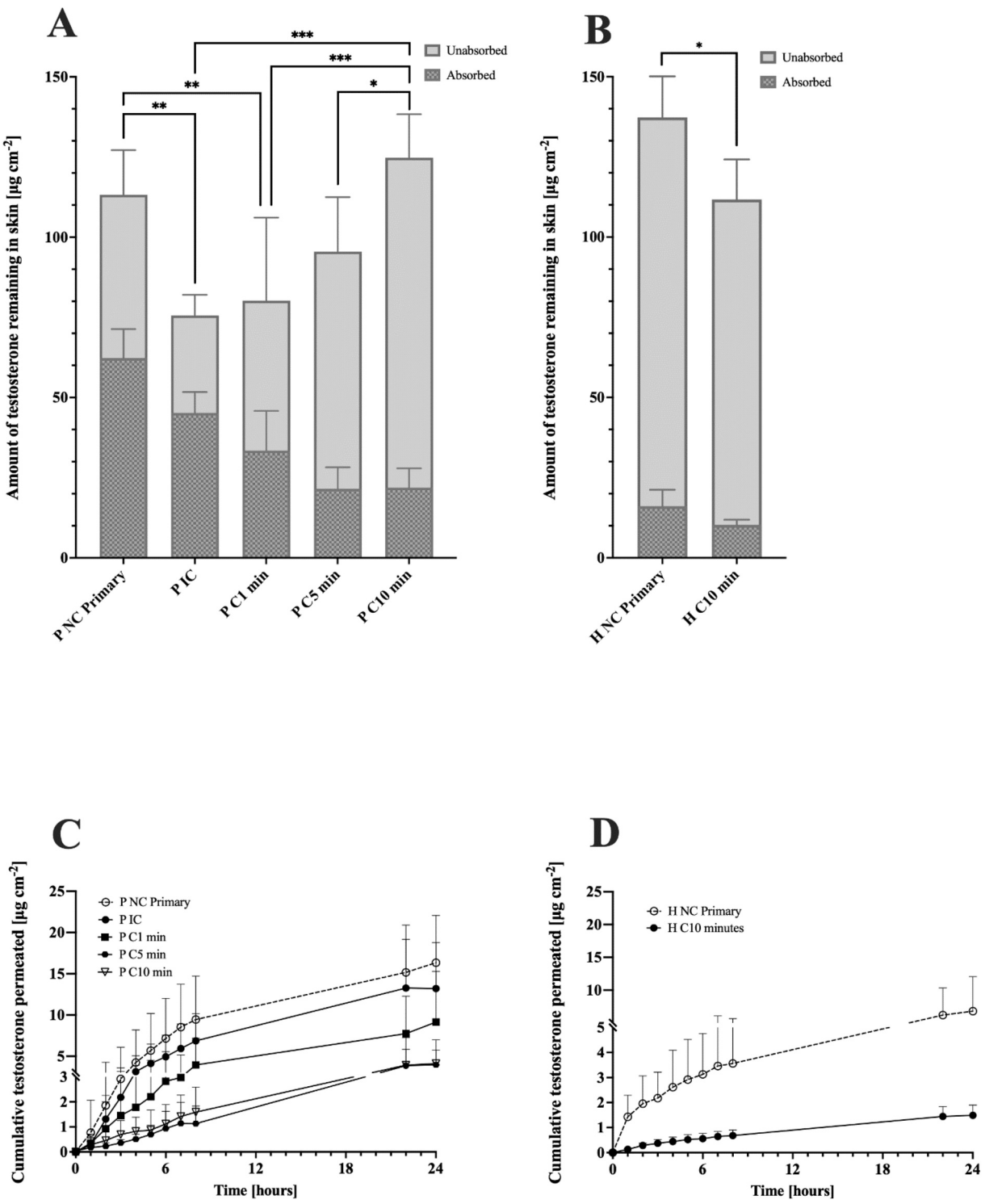



**Fig. 3.** A) Total T remaining on porcine P) primary skin after 24 h following contact with secondary skin immediately after gel application IC) or after 1 C1), 5 C5) or 10 C10) min. T remaining on the primary skin is presented as unabsorbed surface-associated) and absorbed within skin) fractions. B) Total T remaining on human H) primary skin after a 10 min contact delay C10), presented as in A). C) Cumulative permeation of T across porcine primary skin over 24 h following the contact conditions shown in A). D) Cumulative permeation of T across human primary skin over 24 h following the contact condition shown in B). Statistical comparisons were performed on total T remaining on primary skin unabsorbed + absorbed) relative to the corresponding no-contact NC) control. Bars and permeation profiles represent mean ± SD $p \leq 0.05$, $p \leq 0.01$; n = 6).

with 7.1 µg $cm^{-2}$ following contact at 10 min in the absence of sweat $p \leq 0.05$). Similarly, the DBS30 min condition resulted in 27.1 µg $cm^{-2}$ of T transferred to secondary skin, representing a 3.8-fold increase compared with contact at 10 min in the absence of sweat $p \leq 0.05$) Table 3). Both SBD10 min and DBS30 min also resulted in greater absorption of T into secondary skin, with 19.6 µg $cm^{-2}$ and 10.5 µg $cm^{-2}$ absorbed, respectively, representing 12- and 6-fold increases compared with 1.7 µg $cm^{-2}$ following contact at 10 min in the absence of sweat $p < 0.05$ and $p < 0.0001$, respectively) Table 3). These differences were reflected in the corresponding permeation profiles, where higher cumulative

**Table 2**
Amounts of T recovered from primary skin and compared with primary no-contact skin therapeutic dose). Data are presented as mean ± SD n = 6) or %RSD. Statistical comparisons are indicated by * $p \leq 0.05$), ** $p \leq 0.01$) or *** $p \leq 0.001$). Matching numbered superscripts identify the groups included within each statistical significant comparison, with asterisks indicating the corresponding level of statistical significance. Combined recovery represents the total T recovered from the corresponding primary and secondary skin within each condition. Percent of remaining T that was absorbed represents the proportion of remaining T that permeated into and across the primary skin relative to the total remaining amount. Percent delivery of therapeutic dose represents the absorbed amount of T in primary skin expressed as a percentage of the absorbed amount associated with the therapeutic dose.

| Contact condition | Remaining T [μg $cm^{-2}$] | Unabsorbed T [μg $cm^{-2}$] | Absorbed T [μg $cm^{-2}$] | Percent of remaining T that was absorbed. [%] | Percent delivery of therapeutic dose. [%] | Stratum corneum surface [μg $cm^{-2}$] | Epidermis [μg $cm^{-2}$] | Dermis [μg $cm^{-2}$] | Receptor fluid [μg $cm^{-2}$] | Combined recovery [80–120 % OECD] |
|---|---|---|---|---|---|---|---|---|---|---|
| P IC | 75.6 ± 11.6) | 30.4 ± 6.4) [1, 2**] | 45.2 ± 6.5) [2*] | 60.0 ± 4.3) [2**] | 72.5 ± 10.5) | 13.0 ± 2.7) | 4.8 ± 2.1) | 14.6 ± 4.1) | 20.8 ± 12.5) | 95.3 ± 15.0) |
| P C1 min | 72.4 ± 23.1) | 46.8 ± 25.9) [1] | 33.4 ± 12.4) [1] | 53.1 ± 29.7) [3*] | 53.7 ± 19.9) | 9.2 ± 3.5) | 1.7 ± 0.9) | 13.8 ± 6.9) | 9.1 ± 6.2) | 95.3 ± 19.5) |
| P C5 min | 95.5 ± 13.2) | 74.0 ± 17.0) [2**] | 21.5 ± 6.7) [1, 2] | 23.2 ± 8.7) [1, 2, 3] | 34.5 ± 10.8) | 10.5 ± 3.4) | 2.1 ± 0.7) | 5.1 ± 2.8) | 3.9 ± 3.1) | 102.3 ± 6.5) |
| P C10 min | 124.8 ± 18.1) [2*] | 102.9 ± 13.6) [1**] | 21.9 ± 6.0) [1, 2] | 17.4 ± 3.2) [1, 2, 3] | 35.1 ± 9.7) | 13.4 ± 5.1) | 1.5 ± 0.7) | 2.9 ± 1.0) | 4.1 ± 1.6) | 99.0 ± 16.8) |
| *P NC* | *113.2 ± 13.4)* [1**] | *50.9 ± 13.9)* [1] | *62.3 ± 9.1)* [1*] | *55.5 ± 9.5)* [1**] | *100.0 ± 14.5)* | *27.8 ± 8.4)* | *8.0 ± 7.7)* | *10.2 ± 3.6)* | *16.3 ± 5.7)* | *85.0 ± 10.1)* |
| *Primary* | | | | | | | | | | |
| H C10 min | 111.7 ± 13.3) [3] | 101.4 ± 12.5) [3] | 10.3 ± 1.6) [3] | 9.2 ± 1.3) | 63.8 ± 9.9) | 5.9 ± 1.7) | 1.0 ± 0.7) | 1.9 ± 1.2) | 1.5 ± 0.4) | 87.7 ± 7.8) |
| *H NC* | *137.3 ± 13.3)* [3*] | *121.1 ± 13.8)* [3*] | *16.1 ± 5.1)* [3*] | *11.8 ± 4.1)* | *100.0 ± 3.7)* | *7.7 ± 2.6)* | *0.4 ± 0.4)* | *0.9 ± 0.6)* | *7.1 ± 4.8)* | *103.1 ± 10.0)* |
| *Primary* | | | | | | | | | | |

permeation was observed under both sweat-modified conditions Fig. 6B), consistent with the increased transferred and absorbed amounts of T. Furthermore, the proportion of the transferred T that was absorbed was significantly greater for SBD10 min, corresponding to 56.3% compared with 25.1% following contact at 10 min in the absence of sweat $p \leq 0.01$). When expressed relative to the therapeutic dose of the no-contact primary skin condition, absorption corresponded to 31.5% and 16.8% for the SBD10 min and DBS30 min conditions, respectively.

### *3.5. The effect of sweat on drug absorption into dosed primary) skin following secondary transfer contact assessed by the in-vitro model*

Primary skin under the SBD10 min condition showed greater removal of total T from skin during secondary contact, with 74.7 μg $cm^{-2}$ remaining compared with 116.6 μg $cm^{-2}$ under the DBS30 min condition $p \leq 0.05$) Fig. 7A). The DBS30 min condition did not differ significantly in total remaining T on skin from either contact at 10 min in the absence of sweat or the no-contact primary condition. Despite this, increased T absorption was observed in primary skin under the DBS30 min condition, with 43.0 μg $cm^{-2}$ of T absorbed, representing a 2-fold increase compared with 21.9 μg $cm^{-2}$ following contact at 10 min in the absence of sweat $p \leq 0.01$) Table 4). Although more T was removed from the primary skin following contact under the SBD10 min condition, T absorption was also increased, with 35.3 μg $cm^{-2}$ absorbed, representing a 1.6-fold increase compared with 21.9 μg $cm^{-2}$ following contact at 10 min in the absence of sweat. Relative to the total amount of T remaining on the skin, the proportion absorbed was significantly greater only for SBD10 min, corresponding to 49.2% compared with 18.7% for contact at 10 min without sweat $p \leq 0.01$). Consistent with these observations, both SBD10 min and DBS30 min conditions resulted in greater permeation of T across primary skin between 2 and 24 h compared with contact occurring 10 min after dosing in the absence of sweat Fig. 7B).

## 4. Discussion

This study developed and applied a novel *in vitro* model to assess the secondary transfer of a commonly used T gel. Based on conventional Franz skin diffusion cells, the model enables quantification of both the absorbed and unabsorbed fractions of T transferred to exposed 'secondary' recipient skin and the T remaining on dosed 'primary' skin following contact. Secondary transfer was modelled by bringing two excised ex vivo skin samples into contact: a dosed 'primary' skin sample representing the treated patient and an undosed 'secondary' skin sample representing the exposed individual. Contact was initiated at defined time intervals following T gel application to the primary skin, allowing investigation of factors influencing transferability under controlled conditions. Unlike previous *in vivo* secondary transfer studies, which have primarily focused on drug exposure in the recipient only following skin-to-skin contact [16–18], this model also investigated the effect of secondary transfer on the dosed individual by measuring changes in T remaining and absorbed within the primary skin after contact. The only previous investigation to consider the dosed individual assessed the effect of washing the application site on T absorption to determine whether preventative measures to reduce secondary transfer altered systemic exposure [41]. Thus, no previous study has simultaneously evaluated both primary and secondary skin following secondary transfer, and no *in vitro* investigations of this kind have previously been reported. Past literature has highlighted the absence of an *in vitro* model [14], to assess secondary transfer with expressed interest from the FDA in the development of one [31]. Separation of transference into absorbed and unabsorbed fractions provides mechanistic information relating to the process and also provides a better understanding of the risk of exposure which may include the unabsorbed fraction. The parameters influencing transferability that were investigated include the time interval between dose administration and secondary transfer

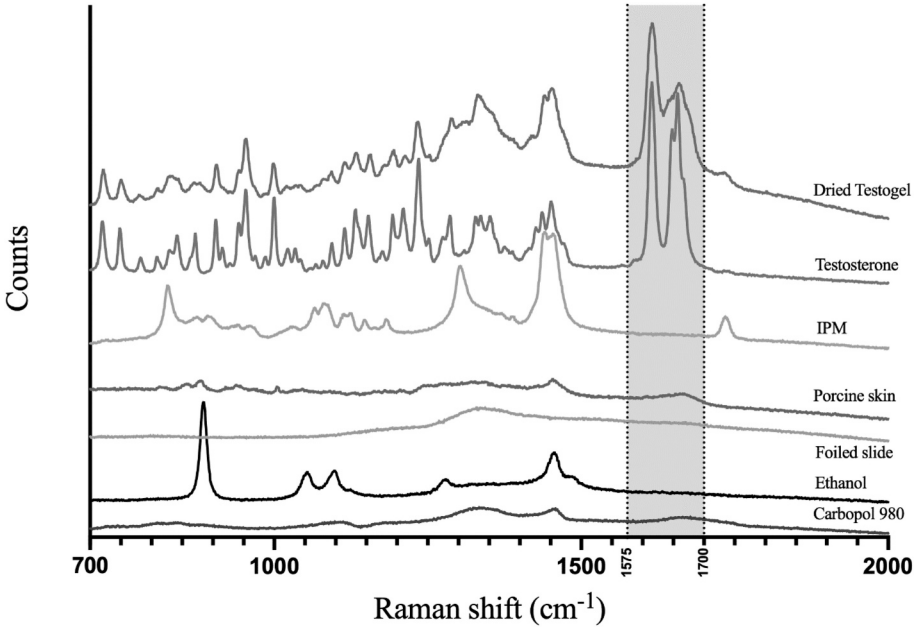



**Fig. 4.** Raman spectra obtained from all excipients of Testogel, dried Testogel, foiled slide and porcine skin acid. Spectra were obtained at x50 magnification, a laser excitation wavelength of 785 nm, three accumulations per sample and an acquisition time of 10 s.

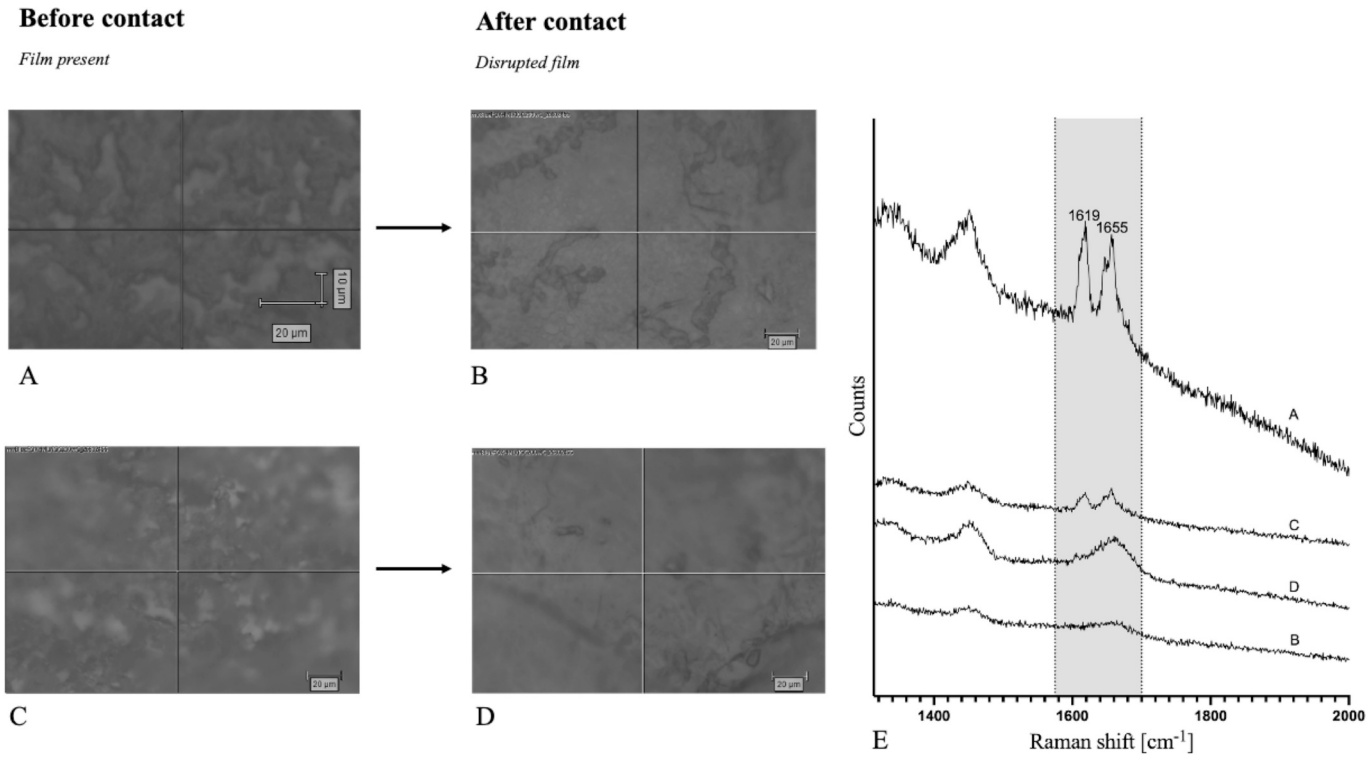



**Fig. 5.** Representative light microscopy images x20 magnification), selected from 19 acquired images, of the T gel film on porcine skin before contact at two different sites on primary skin A and C) and after contact B and D), with their respective Raman spectra shown E). Raman spectra were acquired from the centre of the imaging field, corresponding to the intersection point of the crosshairs used to align the Raman laser.

contact and the presence of sweat on the skin both prior to and following application of the T-gel dose.

For the model, a standard Franz diffusion cell experiment with a therapeutic dose of T gel where the primary skin was dosed without any subsequent contact by primary skin was used as a control condition. This allowed the effect of contact on primary skin absorption to be assessed and the exposure of secondary skin relative to a standard T gel dose to be understood. The majority of experiments were performed with porcine skin, which is widely accepted as a relevant surrogate for human skin due to its physiological and permeability similarities and is more readily available. [42,43]. Confirmation of similar trends in the data was made through comparison of select conditions using human skin. It is known that although broadly similar, porcine skin has higher permeability to drugs than human skin [38], including for T [44,45]. This study's

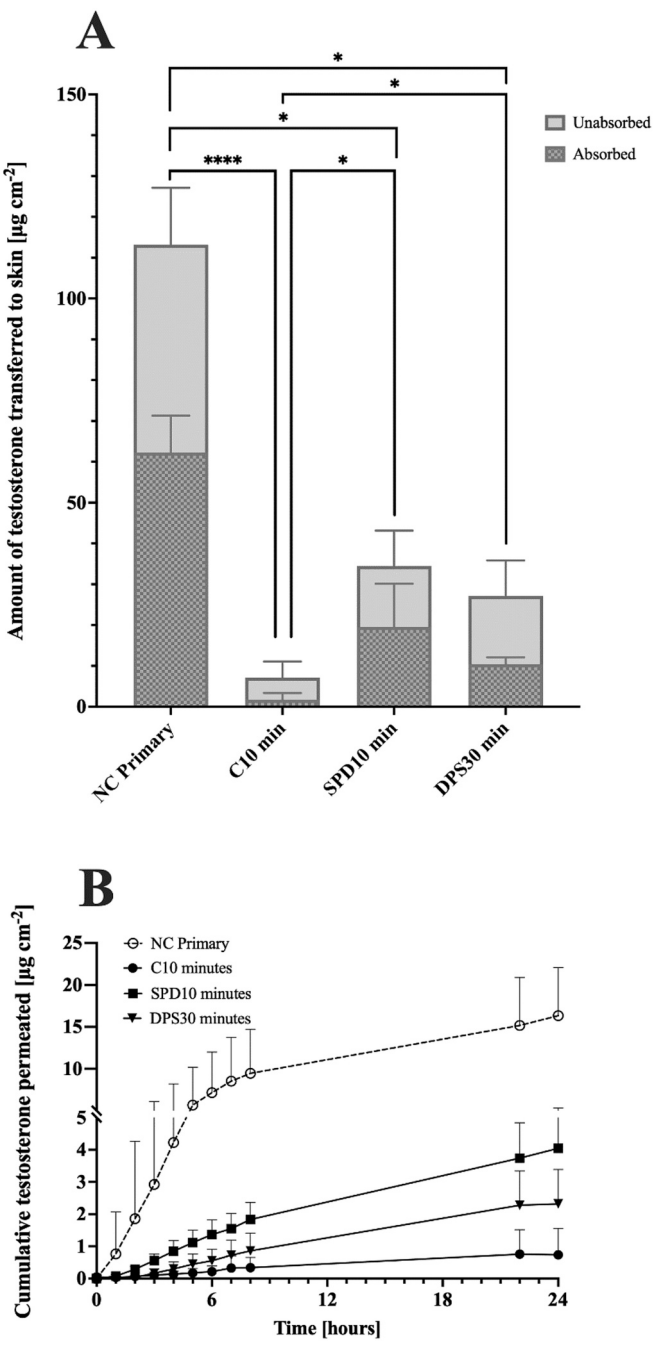



**Fig. 6.** A) Total T transferred to porcine secondary skin after 24 h following secondary contact under sweat-modified conditions. Conditions comprised no-contact primary skin NC Primary), contact 10 min after dosing without sweat C10 min), sweat applied 10 min before dosing followed by contact 10 min later SBD10 min), and sweat applied 30 min after dosing followed by contact 10 min later DBS30 min). Transferred T is presented as unabsorbed surface-associated) and absorbed within skin) fractions. B) Cumulative permeation of T into porcine secondary skin over 24 h following the conditions shown in A). Statistical comparisons were performed on total transferred T unabsorbed + absorbed) relative to the no-contact NC Primary) control. Bars and permeation profiles represent mean ± SD $p \leq 0.05$, ****$p \leq 0.0001$; $n = 6$).

findings align with this consensus across human and porcine skin experiments as although the trend in the transfer and absorption data between porcine and human skin were similar, T absorption into human skin was lower.

This *in vitro* model successfully demonstrated that transfer of T from a marketed T gel product can occur between dosed patient-model skin and exposed recipient-model skin. The data obtained at differing time intervals between dosing and secondary skin contact showed that transfer occurred at all tested time points, with greater transfer observed when contact occurred at shorter intervals after dosing. This finding aligns with previous *in vivo* studies investigating secondary transfer of hormonal gels [16–18,46] and FDA studies with AndroGel® and Testim® [15].

T gels contain high concentrations of ethanol, with the tested formulation in this study comprising approximately 72.4% ethanol by weight in the administered dose (Electronic Medicines [11]. Ethanol facilitates rapid drying **see Supplementary Material**), leading to the formation of metamorphosed dried films shortly after application [13,47]. At later contact time points, as the film becomes drier, adhesion of the formulation to the secondary skin upon transfer would be expected to be reduced, resulting in the observed lower amount of T being transferred to secondary skin. On transfer of these dry films to secondary skin, their reduced adhesive properties would be expected to limit intimate contact between the secondary skin and the transferred formulation, contributing to the observed decrease in total T absorption at later contact times.

The increased absorption of T at shorter contact times may be attributed to improved film-skin interaction because of the wetter state of the film, which could facilitate more intimate contact with the skin. Ethanol also exhibits penetration-enhancing properties [48,49]. When transfer occurs shortly after dosing, some ethanol would be expected to remain within the transferred film and be able to enhance T permeation into the secondary skin. Similarly, the non-volatile, isopropyl myristate IPM) serves as a penetration enhancer for T [50]. Over time, IPM would be expected to be absorbed into the dosed primary skin, depleting its concentration in the residual film. When transfer occurs at later time points after dosing, the formulation that is transferred would be expected to contain lower IPM content, reducing its penetration-enhancing effect and consequently T absorption into secondary skin [51].

At earlier contact time points, a lower total T content remained on the primary skin as a result of the greater amount of T being transferred to the secondary skin. This was expected because the film on the primary skin was wetter, which enabled a higher degree of formulation transfer compared to a drier film. However, this model provided new insight into the absorption profiles of T into primary skin following contact. Whilst the amount of T remaining on the primary skin was larger when contact occurred at later time points, T absorption was significantly reduced. This reduction in absorption was attributed to the disruption of the T film upon contact that was identified through light and Raman microscopy, revealing a roughened film and the loss of intimate contact between the film and the primary skin. The loss of intimate contact between the film and the skin surface, thereby reduced the film's ability to deliver T and reduced permeation over the 24-hour period. A corresponding increase in unabsorbed T on the skin surface was observed, as any T that did not transfer to the secondary skin remained on the primary skin surface.

The impact of contact on the dosed individual has not been specifically addressed in the existing body of *in vivo* secondary transfer studies, which have primarily focused on transfer to non-dosed exposed individuals and their associated clinical outcomes. Findings from the primary skin T delivery data presented here suggests that contact with the dosed site, potentially including that through garments, clothing, or backpacks, could produce contact-related disruption similar to that observed in the patient-modelled skin delivery profile. Such disruption could reduce the efficacy of T gels in alleviating hypogonadal symptoms and may also impair their performance in novel male contraceptive applications [52].

To date, no *in vivo* human study has incorporated sweat into their methodology to understand its influence on secondary transfer from direct skin-to-skin contact, or its impact on drug absorption in the dosed patient. In this *in vitro* model, a greater transfer and absorption of T occurred to the 'exposed individual' during both sweat-administered

**Table 3**
Amounts of T recovered from secondary skin under sweat conditions on primary skin and compared with contact after 10 min without sweat C10 min) and the no-contact primary skin control therapeutic dose). Data are presented as mean ± SD n = 6) or %RSD. Statistical comparisons are indicated by * $p \leq 0.05$) or *** $p \leq 0.001$). Matching numbered superscripts identify the groups included within each statistical significant comparison, with asterisks indicating the corresponding level of statistical significance. Percent of transferred T that was absorbed represents the proportion of transferred T that permeated into and across the secondary skin relative to the total transferred amount. Percent delivery of therapeutic dose represents the absorbed amount of T in secondary skin expressed as a percentage of the absorbed amount associated with the therapeutic dose.

| Contact condition | Transferred T [μg $cm^{-2}$] | Unabsorbed T [μg $cm^{-2}$] | Absorbed T [μg $cm^{-2}$] | Percent of transferred T that was absorbed. [%] | Percent delivery of therapeutic dose. [%] | Stratum corneum surface [μg $cm^{-2}$] | Epidermis [μg $cm^{-2}$] | Dermis [μg $cm^{-2}$] | Receptor fluid [μg $cm^{-2}$] |
|---|---|---|---|---|---|---|---|---|---|
| SBD10 minutes | 34.4 ± 18.0) | 14.8 ± 8.7) [1] | 19.6 ± 10.5) [1, 2] | 56.3 ± 9.1) [1] | 31.5 ± 16.9) | 1.6 ± 1.4) | 0.6 ± 0.3) | 13.3 ± 8.3) | 4.1 ± 1.3) |
| DBS30 minutes | 27.1 ± 9.3) | 16.7 ± 8.8) [1] | 10.5 ± 1.7) [1, 2] | 42.8 ± 15.2) | 16.8 ± 2.7) | 1.5 ± 0.5) | 0.5 ± 0.3) | 6.1 ± 1.1) | 2.3 ± 1.1S) |
| C10 minutes | 7.1 ± 5.3) | 5.4 ± 4.0) [1] | 1.7 ± 1.6) [1, 2*] | 25.1 ± 12.4) [1**] | 2.7 ± 2.6) | 0.4 ± 0.4) | 0.1 ± 0.1) | 0.5 ± 0.5) | 0.7 ± 0.75) |
| *NC Primary* | *N/A* | *50.9 ± 13.9) [1*]* | *62.3 ± 9.1) [1***]* | *55.5 ± 9.5) [1]* | *100.0 ± 14.5)* | *27.8 ± 8.4)* | *8.0 ± 7.7)* | *10.2 ± 3.6)* | *16.3 ± 5.7)* |

conditions, while the patient-modelled skin showed that sweat contact conditions had a smaller reduction in their T permeation and absorption profile compared to skin-to-skin contact without sweat.

The increase in T transferred to the secondary skin may be attributed to the wetter residual film that secondary skin contacts when sweat is present which likely has increased adhesive properties, causing transference to be greater. A wetter film may then also promote more intimate contact between the transferred T film and the secondary skin, increasing absorption. T has also been hypothesized to dissolve in sweat [32,33]. Although its solubilising capacity was not measured here, sweat may act as a vehicle for T remaining within the residual film, facilitating transfer. Once transferred, the presence of sweat may then enhance T absorption by providing an 'improved' vehicle from which T can partition into the secondary skin, compared with dry transferred films. Similar effects may explain the primary skin absorption data. In contrast to when skin-to-skin contact without sweat disrupts intimate contact between the dried film and the skin surface, decreasing absorption, the wetter film in the presence of sweat allows the remaining formulation to adhere more closely to the primary skin. This improved intimate contact mitigates the reduction in T absorption observed under dry contact conditions at later time points.

Two separate sweat conditions were evaluated to represent distinct physiological and behavioural scenarios relevant to T gel use. The dose before sweat condition DBS30 min), where the primary skin was dosed and the gel allowed to dry for 30 min before sweat application, was designed to assess whether sweat could increase the transferability of T from a fully dried residual gel film. This represented a post-application exercise scenario in which perspiration occurs after the formulation has dried on the skin. In contrast, the sweat before dose condition SBD10 min) involved applying sweat to the primary skin 10 min before dosing, allowing it to partially evaporate before T gel application, mimicking 'sweaty' skin. The sweat volume of 36 μL $cm^{-}2$, applied either before dosing the primary skin SBD10 min) or after dosing DBS30 min), was based on physiological sweat production rates of 1.2 mg $cm^{-2}$ $min^{-1}$ at the abdomen and shoulders during exercise [35] scaled to represent 30 minutes of steady-state exercise. Both the abdomen and shoulders are common application sites for hormonal gels. Regardless of sweat condition, the presence of sweat affected the transfer model similarly, increasing transference and absorption to secondary skin, and reducing the impact of disruption of the T gel film on absorption into primary skin. The finding that sweat increases T transfer from dried T gel films, while also enhancing re-absorption of T from the primary application site following delayed contact, has important clinical and regulatory implications and regulatory agencies such as the FDA and UKMHRA, as well as clinicians and manufacturers, could incorporate these findings into patient guidance. The model also offers opportunity to assess other situations or parameters affecting secondary transfer or drug absorption from dried films, including contact with clothing or bedding, or multiple events of contact to further elucidate safety considerations for both the patient model and other exposed individuals.

There is insufficient data available from the literature to enable comparison between the exposure measured here to thresholds that may cause adverse events in different patient groups such as children. Moreover *in vitro* model skin permeation experiments are not expected to perfectly replicate in vivo absorption. The present model therefore provides a comparative measure of secondary exposure and a methodology to investigate the factors influencing transfer and absorption, rather than a direct predictor of systemic adverse effects. However for comparative purposes, when women are prescribed testosterone off label for low sexual desire, the transdermal gel dose is typically one eighth of the dose given to men. Several of the conditions in this study including e.g. the presence of sweat showed absorption into secondary skin above one eighth of the therapeutic dose suggesting that such exposure scenarios may cause clinical effects. The data presented here evaluated a single marketed T gel, and evaluation of alternative products would be valuable. Differences in ethanol and other excipient content in different products may alter formulation drying, the physicochemical and adhesive properties of the residual film, and consequently both transferability and subsequent absorption into secondary skin. To minimise experimental variability the human skin experiments performed here used tissue from a single donor. Expanding this to include skin from additional donors including those of different age, sex, and ethnicity as well as skin from different anatomical sites would provide a fuller understanding of the variability in secondary transfer. The contact procedure used in this study was standardised to provide reproducible pressure, duration and rubbing. Real-world skin-to-skin contact will encompass a wider range of contact geometries, pressures, durations and intensities. Repeated contact with the application site may also occur in practice and could result in different patterns of transfer over time. Future experiments could evaluate these parameters to provide a fuller understanding of the secondary transfer phenomenon. The model could also be adapted to incorporate clothing which could act as a barrier to direct skin-to-skin transfer and as a surface onto which T may itself be transferred. Further adaptation of the model could include the use of transepidermal water loss TEWL) measurements of the skin mounted in the Franz cells before and after the contact event to examine whether the contact event impacts skin integrity.

Overall, this novel *in vitro* model provided new insights into the secondary transfer and absorption of topically applied T whilst also providing data that aligns with previously published *in vivo* human studies. The model demonstrated that T transfer to secondary skin decreases as the time between dosing and contact increases.The presence of sweat on primary skin was shown for the first time to be able to increase both T transfer and absorption into secondary skin. Furthermore, this study demonstrates for the first time that secondary contact following dosing of T gel that has dried to a film, can significantly reduce

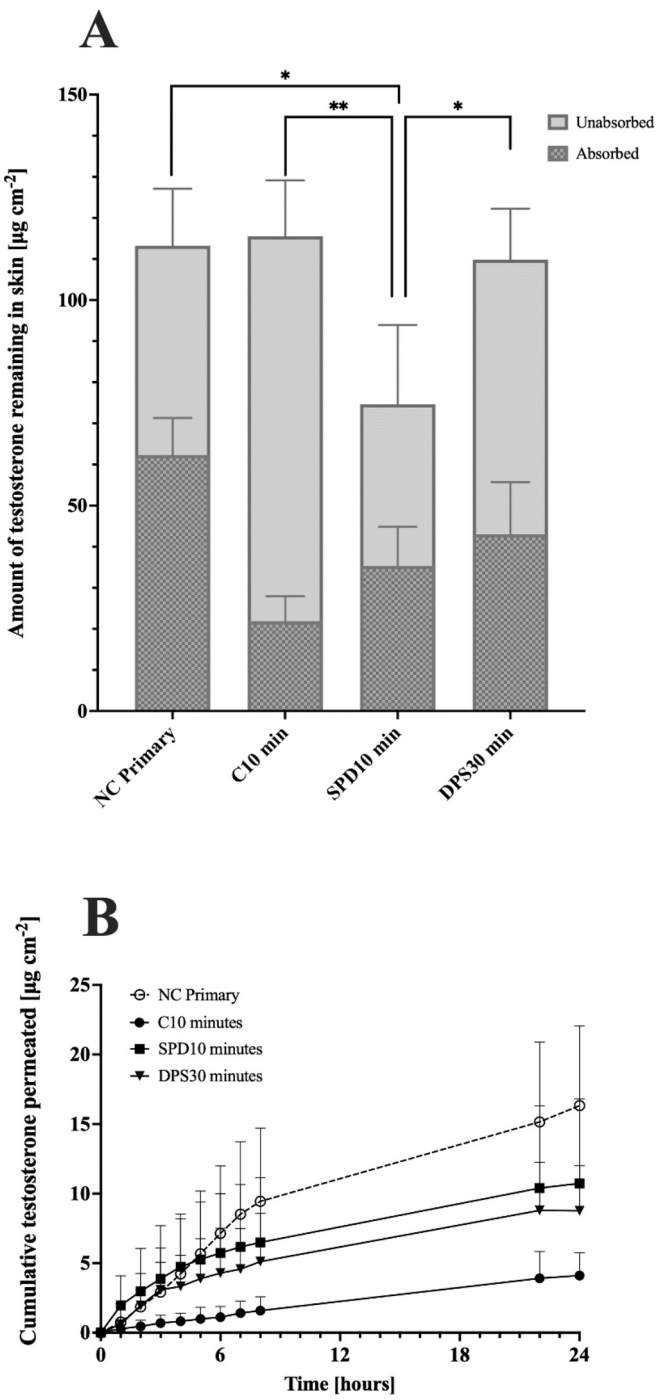



**Fig. 7.** A) Total T remaining on porcine primary skin after 24 h under sweat-modified conditions. Conditions comprised no-contact primary skin NC Primary), contact 10 min after dosing without sweat C10 min), sweat applied 10 min before dosing followed by contact 10 min later SBD10 min), and sweat applied 30 min after dosing followed by contact 10 min later DBS30 min). T remaining on primary skin is presented as unabsorbed surface-associated) and absorbed within skin) fractions. B) Cumulative permeation of T across porcine primary skin over 24 h following the conditions shown in A). Statistical comparisons were performed on total T remaining on primary skin unabsorbed + absorbed) relative to the no-contact NC Primary) control. Bars and permeation profiles represent mean ± SD $p \leq 0.05$, **** $p \leq 0.0001$; n = 6).

**Table 4**
Amounts of T recovered from primary skin under sweat conditions and compared with contact after 10 min without sweat C10 min) and the no-contact primary skin control therapeutic dose). Data are presented as mean ± SD n = 6) or %RSD. Statistical comparisons are indicated by * $p \leq 0.05$) or ** $p \leq 0.01$). Matching numbered superscripts identify the groups included within each statistical significant comparison, with asterisks indicating the corresponding level of statistical significance. Percent of remaining T that was absorbed represents the proportion of remaining T that permeated into and across the primary skin relative to the total remaining amount. Percent delivery of therapeutic dose represents the absorbed amount of T in primary skin expressed as a percentage of the absorbed amount associated with the therapeutic dose.

| Contact condition | Remaining T [µg $cm^{-2}$] | Unabsorbed T [µg $cm^{-2}$] | Absorbed T [µg $cm^{-2}$] | Percent of remaining T that was absorbed. [%] | Percent delivery of therapeutic dose. [%] | Stratum corneum surface [µg $cm^{-2}$] | Epidermis [µg $cm^{-2}$] | Dermis [µg $cm^{-2}$] | Receptor fluid [µg $cm^{-2}$] | Combined recovery [80–120 % OECD] |
|---|---|---|---|---|---|---|---|---|---|---|
| SPD10 minutes | 74.7 ± 25.7) | 39.4 ± 19.3) [1] | 35.3 ± 9.5) [1] | 49.2 ± 11.5) [1] | 56.7 ± 15.3) | 5.0 ± 3.1) | 1.5 ± 0.8) | 18.1 ± 4.4) | 10.7 ± 6.1) | 81.9 ± 13.2) |
| DPS30 minutes | 116.6 ± 18.8) | 73.6 ± 18.0) | 43.0 ± 12.7) [2] | 37.0 ± 10.2) | 69.1 ± 20.4) | 16.5 ± 11.6) | 2.5 ± 1.6) | 15.3 ± 5.1) | 8.7 ± 3.3) | 107.9 ± 11.4) |
| C10 minutes | 115.5 ± 18.9) | 93.7 ± 13.6) [1*] | 21.9 ± 6.0) [1, 2**] | 18.7 ± 2.7) [1**] | 35.1 ± 9.7) | 13.4 ± 5.1) | 1.5 ± 0.7) | 2.9 ± 1.0) | 4.1 ± 1.6) | 99.0 ± 16.8) |
| *NC Primary* | *113.2 ± 13.4)* | *50.9 ± 13.9)* [1] | *62.3 ± 9.1)* [1*] | *55.5 ± 9.5)* [1] | *100.0 ± 14.5)* | *27.8 ± 8.4)* | *8.0 ± 7.7)* | *10.2 ± 3.6)* | *16.3 ± 5.7)* | *85.0 ± 10.1)* |

T gel absorption by the dosed individual despite only a small amount of the formulation being transferred to the secondary contact. This has implications beyond secondary transfer, of T replacement products and is likely to be relevant for therapeutic efficacy of newly emerging male contraceptive hormonal gels [52,53]. These findings advance understanding of the conditions that elevate the risk of secondary transfer and provide a stronger evidence base to inform refinements in clinical guidance for patients using T gels. The in vitro model described here also offers a practical tool for formulation scientists, enabling systematic assessment of how formulation properties and external factors influence transferability and skin absorption. By directly quantifying T within both donor and recipient skin, this approach provides greater sensitivity and mechanistic resolution than in vivo clinical studies that have typically relied on serum measurements alone. In addition, the model reduces the financial, logistical, and ethical burdens associated with human transfer studies, supporting its broader application in formulation development, risk mitigation strategies, and future evaluation of transdermal T products.

## CRediT authorship contribution statement

**J.R.J. Kellaway:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **M.T. Beebeejaun:** Writing – review & editing, Supervision, Conceptualization. **R.P. Chilcott:** Writing – review & editing, Supervision, Conceptualization. **W.J. McAuley:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Funding acquisition, Formal analysis, Conceptualization.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejpb.2026.115283.

## Data availability

Data will be made available on request.

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