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## The effect of heat on skin permeability

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### Abstract

Although the effects of long exposure ( $\gg 1$  s) to moderate temperatures ( $\leq 100$  °C) have been well characterized, recent studies suggest that shorter exposure ( $< 1$  s) to higher temperatures ( $> 100$  °C) can dramatically increase skin permeability. Previous studies suggest that by keeping exposures short, thermal damage can be localized to the stratum corneum without damaging deeper tissue. Initial clinical trials have progressed to Phase II (see <http://clinicaltrials.gov>), which indicates the procedure can be safe. Because the effect of heating under these conditions has received little systematic or mechanistic study, we heated full-thickness skin, epidermis and stratum corneum samples from human and porcine cadavers to temperatures ranging from 100°C to 315°C for times ranging from 100 ms to 5 s. Tissue samples were analyzed using skin permeability measurements, differential scanning calorimetry, thermomechanical analysis, thermal gravimetric analysis, brightfield and confocal microscopy, and histology. Skin permeability was shown to be a very strong function of temperature and a less strong function of the duration of heating. At optimal conditions used in this study, transdermal delivery of calcein was increased up to 760-fold by rapidly heating the skin at high temperature. More specifically, skin permeability was increased (I) by a few fold after heating to approximately 100°C – 150°C, (II) by one to two orders of magnitude after heating to approximately 150°C – 250°C and (III) by three orders of magnitude after heating above 300°C. These permeability changes were attributed to (I) disordering of stratum corneum lipid structure, (II) disruption of stratum corneum keratin network structure and (III) decomposition and vaporization of keratin to create micron-scale holes in the stratum corneum, respectively. We conclude that heating the skin with short, high temperature pulses can increase skin permeability by orders of magnitude due to structural disruption and removal of stratum corneum.

### Keywords

skin permeability; thermal effect; transdermal drug delivery

## 1. Introduction

Transdermal drug delivery offers a non-invasive route for drug administration that offers the possibility to continuously control the delivery rate and avoid the first-pass effects of the liver

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(Prausnitz et al., 2004). These advantages have led to a multi-billion dollar market for transdermal patches used for smoking cessation (nicotine), hormone replacement (estradiol), and other indications. Despite these advantages, transdermal drug delivery is severely limited by the poor permeability of human skin; most drugs do not cross skin at therapeutic rates and fewer than 20 drugs have been approved by FDA for transdermal delivery since the first patch was introduced more than 25 years ago.

The skin's barrier properties come from the highly impermeable outer layer called stratum corneum, which is just 10 – 20  $\mu\text{m}$  thick. Drugs that cross the stratum corneum barrier can generally diffuse to deeper capillaries for systemic distribution. For this reason, most approaches to increase transdermal delivery have emphasized disruption of stratum corneum microstructure using chemical or physical methods (Cross and Roberts, 2004; Down and Harvey, 2003; Schuetz et al., 2005).

One approach to increasing skin permeability involves heating the skin (Mitragotri, 2006). Previous studies have emphasized either long exposures at moderate temperatures or very short exposures at high temperatures. As an example of extended heating at moderate temperature, exposure to 40°C for 4 h has been shown to increase human skin permeability to a hydrophobic drug (fentanyl) by 4-fold via a mechanism believed to involve stratum corneum lipid fluidization (Shomaker et al., 2000). Exposure to 80°C for 15 s showed a 12-fold increase in porcine skin permeability to another hydrophobic model drug, butanol (Flynn et al., 1982). Other studies have similarly measured increased skin permeability after heating (Jain and Panchagnula, 2003; Murthy et al., 2004). Mechanistic studies have attributed these permeability increases largely to disordering of stratum corneum lipid bilayer structures (Duzee, 1975).

In contrast, pretreatment of the skin using sub-second exposures to temperatures well above 100°C has been shown to increase skin permeability by orders of magnitude. Rapidly heating the skin surface is hypothesized to locally ablate the stratum corneum without significantly heating, or damaging, deeper tissues. Activation of an array of microheaters on the skin surface has been shown to increase insulin and vaccine delivery (Badkar et al., 2007; Bramson et al., 2003). Radio-frequency heating of skin has also been shown to increase delivery of small molecule drugs, human growth hormone, and DNA (Birchall et al., 2006; Levin et al., 2005; Sintov et al., 2003). Laser treatment of the skin has also been shown to increase skin permeability to large dextrans, among other compounds (Fang et al., 2004; Fujiwara et al., 2005; Lee et al., 2001). These studies carried out in animal models and human subjects showed that heating under these conditions was well tolerated. Phase I and II clinical trials are currently under way using this technology (see <http://clinicaltrials.gov>).

Despite these demonstrations that brief heating can increase skin permeability, there has been no systematic study of the relationship between skin permeability and the temperature and duration of exposure to heat at high temperature (> 100 °C) and short duration ( $\leq 1$  s). Moreover, the mechanism by which heating increases skin permeability is not fully understood. To address these needs, this study determined the effect of time and temperature of heating on skin permeability to a hydrophilic model drug, calcein, and on changes in stratum corneum structure measured by thermomechanical analysis (TMA), thermal gravimetric analysis (TGA), differential scanning calorimetry (DSC), brightfield and confocal microscopy, and histology. Heating was studied at temperatures ranging from 25°C to 315°C for durations ranging from 100 ms to 5 s.

## 2. Materials and methods

### 2.1. Skin preparation

Human cadaver skin was obtained from Emory University (Atlanta, GA) with approval from the Georgia Tech IRB and stored at  $-70^{\circ}\text{C}$  until use. Porcine cadaver skin was obtained from St. Joseph's Research Institute (Atlanta, GA) with approval from the Georgia Tech IACUC and stored at  $-70^{\circ}\text{C}$  until use.

To isolate epidermis, full thickness human cadaver skin was immersed in distilled water at  $65^{\circ}\text{C}$  for 3 min, after which the epidermis was mechanically separated from the dermis using a spatula (Hobson, 1991). To isolate stratum corneum, the epidermis was incubated at  $4^{\circ}\text{C}$  in a solution of 0.5% trypsin in phosphate-buffered saline (PBS; Sigma Chemical, St. Louis, MO) for 12 h; rinsed with distilled water and immersed in a fresh 0.5% trypsin solution for 3 h at  $38^{\circ}\text{C}$ ; rinsed again with distilled water and dried at room temperature under vacuum (30 kPa absolute pressure) overnight; and finally stored in  $-70^{\circ}\text{C}$  freezer (White et al., 1988).

### 2.2. Methods of skin heating

To heat the skin for a controlled time at a controlled temperature, skin samples were attached to the plunger of a solenoid, which was actuated to contact a constant-temperature heat source for a predetermined length of time. To accomplish this, samples of full-thickness skin, epidermis and stratum corneum were cut into square pieces measuring up to  $2\text{ cm} \times 2\text{ cm}$ , which were mounted on the end of a 1 cm length of silicone tubing (0.1 cm inner diameter, 0.3 cm outer diameter, Cole Parmer, Vernon Hills, IL) using adhesive (Instant Crazy Glue, Elmer's Products, Columbus, OH). To provide additional mechanical support, epidermis and stratum corneum samples were first mounted onto a  $0.7\text{ cm} \times 0.7\text{ cm}$  piece of 500  $\mu\text{m}$ -thick polydimethylsiloxane (Sylgard 184, Dow Corning, Midland, MI), which was adhered to  $0.7\text{ cm} \times 0.7\text{ cm}$  piece of 500  $\mu\text{m}$ -thick polycarbonate (Bayer Films America, Berlin, CT), which was adhered to the tubing.

After curing the adhesive for 10 min, the tubing was connected to the plunger of a solenoid (S-16-50-33H, Magnetic Sensor System, Van Nuys, CA;  $< 5\text{ ms}$  response time). The solenoid was then actuated by a digital timer (H3CA-A, OMRON, Schaumburg, IL) to contact the skin with one of two different heat sources for 100 ms to 5 s. Heating up to  $250^{\circ}\text{C}$  was accomplished using a temperature-controlled hot plate with an operating range of  $30^{\circ}\text{C}$  to  $250^{\circ}\text{C}$  (OMEGA, Stamford, CT). Heating above  $250^{\circ}\text{C}$  was accomplished using a temperature-controlled soldering iron with an operating range of  $176^{\circ}\text{C}$  to  $450^{\circ}\text{C}$  (WESD51, Weller, Wiltshire, United Kingdom). After heating, skin was removed from the central region of the heated skin sample and analyzed as described below.

Although the temperature of the heating source was precisely controlled, the temperature of the skin was not measured. This is because it is extremely difficult to measure the temperature of the skin, because the temperature varies with time (i.e., on the millisecond timescale) and with position (i.e., on the micron lengthscale for these short application times). Temperatures in the skin varied from room temperature to, possibly, hundreds of degrees Celsius depending on when and where the measurement is made (if it could be made). Therefore, there is no single temperature of the skin that characterizes an exposure. For this reason, we elected to provide the one piece of information that is certainly known, namely the temperature of the heat source. A detailed characterization of the complex, dynamic temperature profiles within the skin was beyond the scope of this study.

### 2.3. DSC, TGA and modified TMA analysis of stratum corneum

Differential scanning calorimetry (DSC 220C, Seiko Instruments, Torrance, CA) and thermogravimetric analysis (TG/DTA 220, Seiko Instruments) were used to analyze stratum corneum samples after heating. In each case, 10 mg of dehydrated stratum corneum was put into an aluminum crucible (Mettler-Toledo, Columbus, OH). DSC measurement was carried out by ramping from 25°C to 350°C at a rate of 10°C/min. TGA measurement was carried out by ramping from 25°C to 400°C at a rate of 10°C/min.

A modified thermomechanical analysis (TMA) was carried out by measuring the mechanical fracture strength of stratum corneum samples using a force-displacement test station (Model 921A, Tricor Systems, Elgin, IL). Dried stratum corneum was cut into circular pieces with a 2 cm diameter and then mounted on the surface of a 200 µm thick annular ring of polycarbonate (2 cm outer diameter, 1 cm inner diameter, Bayer Films America, Berlin, CT) using adhesive (Instant Krazy Glue). The test station then pressed an aluminum cylinder measuring 2 mm in diameter and 1 cm in length against the stratum corneum at a rate of 0.2 mm/s. The force required to press the cylinder against the skin was recorded over time. A sudden drop in the force was taken as an indication that the stratum corneum had fractured, consistent with previous findings (Davis et al., 2004). The modified TMA procedure used here involved heating skin and then testing mechanical strength afterwards (at room temperature). This contrasts with conventional TMA, which measures mechanical strength at the test temperature (Humphries and Wildnauer, 1972).

### 2.4. Histological examination of skin

To carry out histological analysis after heating, skin samples were covered with optimal cutting temperature solution (Tissue-Tek, Sakura Finetechnical, Tokyo, Japan) and frozen with liquid nitrogen in an orientation that allowed subsequent sectioning perpendicular to the skin surface. Skin samples were sectioned by a cryostat microtome (HM 560MV, Microm, Walldorf, Germany) at 15 µm (for optical microscopy) or 30 µm (for confocal microscopy) thickness. Skin samples were then examined by brightfield microscopy (IX-70, Olympus, Mellville, NY) to visualize changes in stratum corneum structure.

To image changes in stratum corneum lipid structure, cryosectioned skin samples were expanded with Sorensen Walbum buffer (0.1 M glycine, 0.1 M NaCl and 0.1 M NaOH, Sigma Chemical) for 20 min mounted on glass slide (Kleene et al., 2001); washed with de-ionized water; stained with Nile Red (Sigma Chemical), which is a red-fluorescent lipid stain (Kleene et al., 2001); and imaged using confocal microscopy (LSM 510; Zeiss, Thornwood, NY). The lipid stain was applied using a stock solution 0.05% (w/v) of Nile Red in acetone, which was diluted to 2.5 mg/ml with a 75:25 (v:v) mixture of glycerol/water before use.

### 2.5. Transdermal delivery measurements

To image transdermal delivery using histological imaging, full-thickness pig skin was heated and then exposed on its epidermal surface to a 0.4 % aqueous solution of Trypan blue (Sigma Chemical) at room temperature. After 10 h, residual Trypan blue was cleaned off the skin surface with a wet cotton swab. Skin samples were then cryosectioned and imaged, as described above.

To determine transdermal flux quantitatively, human cadaver epidermis was heated and then a 2 cm diameter circular piece of the epidermis was mounted in a Franz diffusion chamber (PermeGear, Bethlehem, PA). After filling the donor and receiver compartments with PBS and incubating for 1 hr to hydrate the skin at 37°C in a water bath (Immersible multi-stirrer, Cole Parmer, Vernon Hills, IL), the donor compartment was filled with 0.5 ml of 1 mM calcein (Sigma) and the receiver compartment was filled using approximately 5 ml of fresh PBS. After

2 h, the receptor solution was sampled for measurement of calcein fluorescence intensity using a spectrofluorimeter (QM-1; Photon Technology International, South Brunswick, NJ). A calibration curve was used to convert fluorescence intensity into calcein concentration (detection limit was  $10^{-9}$  M), which was used to calculate transdermal calcein flux.

### 3. RESULTS

#### 3.1. Effect of temperature on skin permeability

Skin permeability to a model hydrophilic molecule, calcein, was measured as a function of temperature after exposure at 25°C to 315°C for 100 ms, 1 s or 5 s. Note that these were the temperatures of the heat sources applied to the skin surface. As discussed below and in the literature (Jiang et al., 2002), exposure for 1 s or 5 s should be sufficient to bring the full thickness of the stratum corneum to the same temperature (although a temperature gradient should still exist across the viable epidermis), but the 100 ms exposure should produce a temperature gradient across the stratum corneum. Effects of the duration of exposure will be discussed further below.

As shown in Fig. 1, heating the skin to 100°C increased transdermal flux by 2.2 – 4.1 fold compared to 25 °C (Student's t-test,  $p < 0.05$ ). This indicates that a transition occurred in skin properties. We will define temperatures above this transition as Zone I. Further heating to 140°C was not significantly different from 100°C (Student's t-test,  $p > 0.05$ ). A second transition (i.e., Zone II) occurred upon heating the skin to 180°C (Student's t-test,  $p < 0.05$ ), which increased transdermal flux by 14–25 fold compared to skin permeability at 25 °C. Further heating to 260 °C was not significantly different from 180 °C (Student's t-test,  $p > 0.05$ ). A final transition (Zone III) was seen upon heating the skin to 315°C (Student's t-test,  $p < 0.05$ ), which increased transdermal flux by 660 – 760 fold.

To supplement transdermal flux measurements, we used brightfield microscopy to image penetration of a model hydrophilic dye, Trypan blue, into skin exposed to a range of different temperatures for 100 ms. As shown in Fig. 2, changes in Trypan blue staining of the skin were small for skin heated to Zone I (Fig. 2b and 2c). Heating to Zone II showed a marked change, with distinct regions of Trypan blue penetration into the skin (Fig. 2d and 2e). Finally heating to Zone III produced extensive blue staining of the skin (Figs. 3f – 3h). These imaging results are consistent with the transdermal flux analysis. Note that due to limitations of our apparatus, the heating source at 180°C (Fig. 2d) and below was a hot plate and at 260°C (Fig. 2e) and above was a soldering iron, which may explain the different skin morphologies.

To further investigate the effects of heating at the highest temperatures, skin samples were heated and exposed to Trypan blue in the same way, and then cryosectioned for histological analysis. As shown in Figure 4, dye penetration into the skin increased with increasing temperature and the greatest dye penetration was seen for skin heated to Zone III.

#### 3.2. Analysis by DSC

To further interpret these skin permeability results, we carried out differential scanning calorimetry (DSC) analysis of stratum corneum. Note that although a similar range of temperatures was used in this measurement, the temperature was ramped at a rate of 10°C/min, which means that the skin was slowly heated over the course of a few hours. This contrasts with the extremely rapid heating used in the permeability studies.

DSC analysis of stratum corneum at moderate temperatures, as shown in the inset of Fig. 4a, identified two characteristic peaks at 68–78 °C and 90–100 °C. These two peaks are known from the literature and have been associated with (i) stratum corneum lipid melting from a lamellar to a disordered state and (ii) protein-associated lipid transition from gel to liquid form,

respectively (Silva et al., 2006; Tanojo et al., 1999). We interpret these lipid rearrangements as being responsible for the modest increase in skin permeability observed in Fig. 1 upon heating to Zone I. It should be mentioned that a commonly observed, lower-temperature peak at 35 – 40°C is not easily observed (Potts et al., 1991) and an additional higher temperature peak at 105 – 120°C, which represent changes in stratum corneum protein conformation, is also not seen in our DSC thermogram, because it requires a stratum corneum water content of at least 15% (Leopold and Lippold, 1995) and our skin samples were dried before DSC analysis.

Our DSC analysis at higher temperatures (Fig. 4a) was often noisy and poorly reproducible. Nonetheless, there were some general features of those DSC thermograms that were consistently observed. For this reason, the representative thermogram shown in Fig. 4a should be viewed only as a qualitative indication of the trends. One of the reproducibly observed features was a broad transition that peaks near 150°C and then bottoms out near 200°C. We propose that the process associated with this transition is responsible for changes in skin permeability that take place in Zone II. This process is discussed in greater detail below in the analysis by modified TMA.

Finally, the DSC analysis in Fig. 4a generally showed a sharp transition beginning at approximately 250°C and extending beyond 300°C. This appears to correspond to the transition to Zone III. The very large size of this peak suggests the occurrence of a chemical reaction, such as decomposition of the stratum corneum. This possibility is further discussed below in the analysis by TGA.

### 3.3. Analysis by modified TMA

As an additional assessment of thermally induced changes in stratum corneum structure, we carried out a thermomechanical analysis (TMA) to determine the mechanical strength of stratum corneum after heating at various temperatures by measuring the force required for a metal probe to penetrate across the tissue. As shown in Fig. 4b, the failure force of stratum corneum in Zone I (i.e., 100°C and 140°C) was slightly increased, but this change was not significant (Student's t-test,  $p = 0.17$ ).

In Zone II (i.e., 180°C to 260°C), stratum corneum mechanical strength decreased (Student's t-test,  $p < 0.05$ ). This finding is consistent with a previous TMA study of stratum corneum, which determined that the mechanical strength of stratum corneum progressively weakened over a similar temperature range (Humphries and Wildnauer, 1972; Miller and Wildnauer, 1977). This transition was found to be shifted to much higher temperatures after exposure to formaldehyde. Because formaldehyde is known to crosslink keratin, these findings suggest that the transition to Zone II is associated with a breakdown of the keratin network structure. The previous TMA study also found that lipid extraction from the stratum corneum using various organic solvents did not affect this Zone II transition temperature, further indicating its association with the keratin network, as opposed to stratum corneum lipids.

We were not able to make TMA measurements above 260°C in Zone III, because the stratum corneum became too fragile to handle.

### 3.4. Analysis by TGA

We also carried out thermogravimetric analysis (TGA) to provide additional insight. As shown in Fig. 4c, the transition to Zone I below 100°C was associated with the loss of a few percent of the stratum corneum mass. This loss may be due to vaporization of a small amount of residual water in the stratum corneum that was not removed during the drying process used to prepare the stratum corneum before TGA analysis. Increasing temperature to Zone II was associated with essentially no further change in stratum corneum mass.

Above approximately 180°C there was a steep loss of mass that reached a maximum rate at approximately 290°C, which corresponds to the transition to Zone III. At 290°C, 35% of the stratum corneum mass had been lost and at 315°C, which is the maximum temperature examined in this study, 46% of the stratum corneum mass was lost. Disappearance of that much mass can only be explained by loss of keratin, since keratin makes up 75–80% of stratum corneum mass on a dry basis (Wilkes et al., 1973). Because keratin cannot be vaporized in an intact form, this loss can best be explained by chemical decomposition of the stratum corneum most likely by a combination of oxidative combustion and depolymerization of stratum corneum proteins

### 3.5. Stratum corneum histology

To provide more detailed imaging of stratum corneum, the skin was treated after heating by incubating in an alkaline solution that expanded the stratum corneum and then staining with a red-fluorescent lipid stain (Nile Red). As shown in Fig. 5a, untreated stratum corneum exhibits a highly ordered and aligned structure. Skin treated in Zone I (i.e., 100°C and 140°C) appears to have stratum corneum that is more densely packed (Figs. 6b and 6c), which could be consistent with stratum corneum lipid melting and keratin denaturation that occurs at those temperatures. Heating to Zone II (i.e., 160°C to 260°C) produced stratum corneum with a very different appearance that is highly disordered, where stratum corneum and lipid boundaries are less clear, possibly due to breaking of the stratum corneum keratin network. Finally, in Zone III (i.e., 315°C), the stratum corneum was extensively damaged, with little of its original structure remaining, probably due to chemical decomposition of the tissue.

### 3.6. Effect of duration of heating on skin permeability and histology

Although Fig. 1 shows that variation in temperature had a large effect on skin permeability, the duration of heating sometimes had an effect too (ANOVA,  $p < 0.05$ ). In Zone I (i.e., 100°C and 140°C), heating for 100 ms, 1 s and 5 s were statistically indistinguishable (Student's t-test,  $p > 0.05$ ). However, in Zone II (i.e., 180°C and 260°C), heating for 100 ms, 1 s and 5 s were all statistically different from each other, such that longer exposure times lead to greater increases in skin permeability (Student's t-test,  $p < 0.05$ ). Finally, in Zone III (i.e., 315°C), the duration of heating did not have a significant effect on skin permeability (Student's t-test,  $p > 0.05$ ).

Because the duration of heating had a strong effect in Zone II, we heated skin to 260°C for 100 ms, 400 ms, and 1 s and then applied Trypan blue. Skin samples were then cryo-sectioned, stained with hematoxylin and eosin (H&E), and imaged, as shown in Fig. 6. Unfortunately, most of Trypan blue was removed by the H&E staining process, but some remained for visualization. Compared to untreated skin (Fig. 6a), heating for 100 ms caused visible damage to the stratum corneum (Fig. 6b). Heating for 400 ms caused more extensive damage to the stratum corneum, which extended into the viable epidermis (Fig. 6c). Heating for 1 sec caused still more damage, with extensive removal of both stratum corneum and viable epidermis (Fig. 6d). These effects could explain the increased skin permeability observed in Fig. 1 after longer durations of heating in Zone II.

To provide additional information about the duration of heating in Zone II, skin was again heated to 260 °C for 100 ms, 400 ms, and 1 s and imaged after alkaline expansion and Nile Red staining of the stratum corneum, as shown in Fig. 7. Untreated skin showed an organized and intact stratum corneum structure (Fig. 7a). After heating for 100 ms, the stratum corneum became visibly damaged (Fig. 7b). After 400 ms, there was more extensive damage and removal of stratum corneum (Fig. 7c). After 1 s, stratum corneum was largely removed (Fig. 7d). These Nile Red-stained images are consistent with the H&E-stained images in Fig. 6.

To examine effects in Zone III, we heated skin to 350°C for 100 ms, 400 ms and 1 s, and then applied Trypan blue. Skin samples were then cryo-sectioned and imaged, as shown in Fig. 8. H&E stain was not applied and, as a result, the Trypan blue dye remained for visualization. At all three durations of heating, there was extensive delivery of Trypan blue into skin (Figs. 8a – 8c), which is consistent with the observation in Fig. 1 that duration of heating did not affect skin permeability in Zone III. Fig. 8 does show, however, that longer heating times increased the extent of skin damage. This suggests that shorter heating times may minimize skin damage while still effectively increasing skin permeability in Zone III.

## 4. DISCUSSION

### 4.1. Effect of heat on skin permeability

Previous studies have measured skin permeability either after long exposures to moderate temperatures (e.g., < 100°C) or after very short exposures to high temperatures (e.g., >> 100°C). For the first time, this study carried out a broad analysis of the effects of temperature and duration of heating on skin permeability. We found that there was an extremely strong dependence on temperature, such that a two-fold increase in absolute temperature (i.e., 25°C = 298 K; 315°C = 588 K; 588 K/298 K = 1.97) corresponded to a 760-fold increase in skin permeability. There was a much weaker dependence on the duration of heating over the range examined. A 50-fold variation in heating time (i.e., 100 ms to 5 s) had no effect at some temperatures and caused up to a 6-fold increase at other temperatures. This weak dependence of permeability suggests that the duration of heating can be further optimized (i.e., minimized) for transdermal drug delivery based on other factors, such as reducing collateral tissue damage and avoiding pain.

The effect of temperature on skin permeability was found to fall into three zones. Zone I corresponded to skin heated to roughly between 100°C and 150°C. At these conditions, there was a few-fold increase in transdermal flux of our model hydrophilic molecule. This increase was attributed to lipid melting in the stratum corneum, based on DSC analysis.

Zone II corresponded to skin heated to roughly between 150°C and 250°C. At these conditions, transdermal flux was increased by one to two orders of magnitude. These large increases in skin permeability were attributed to disruption of stratum corneum keratin network structure, based on DSC and TMA analysis.

Zone III corresponded to skin heated above 300°C. At these conditions, transdermal flux was increase by three orders of magnitude. This huge increase in skin permeability was attributed to decomposition and vaporization of the stratum corneum, based on TMA, TGA, and microscopy analysis.

It should be noted that measurements in this study were made after the skin was heated and allowed to cool down again. Thus, rapidly reversible effects of heating were not captured. For example, lipid fluidization or melting at Zone I temperatures may have partially or largely reversed after cooling the skin. The effects we measured were probably irreversible *in vitro*, but should be reversible *in vivo* over the timescale of normal skin healing mechanisms. Indeed, previous *in vivo* studies suggest that the effects of short, high temperature exposures are reversible (Badkar et al., 2007; Lee et al., 2002; Sintov et al., 2003).

We made measurement in this study after heating protocols were finished because it is extremely difficult to measure transdermal flux or other characteristics of skin during such short exposures to heat. However, applications of short, high temperature heating of skin envision the heating as a pretreatment (Badkar et al., 2007; Birchall et al., 2006; Bramson et



al., 2003; Fang et al., 2004; Fujiwara et al., 2005; Lee et al., 2001; Levin et al., 2005; Sintov et al., 2003). Thus, our methods are directly relevant to transdermal drug delivery applications.

At the highest temperatures, skin structure was altered on the micron scale, as seen through histological characterization. Although the mechanisms are different, these results can be compared to other physical methods to disrupt the stratum corneum barrier. Ultrasound has been shown to disrupt stratum corneum lipid structure on the nanometer length scale (Paliwal et al., 2006). Electroporation similarly makes nanometer disruptions to skin, although electroporation combined with chemical enhancers has been shown to produce micron scale holes (Ilic et al., 2001). Microneedles create micron scale holes in the skin (McAllister et al., 2003).

#### 4.2. Applications to transdermal drug delivery

Maximum skin permeability was achieved using the hottest temperature studied (i.e., 315°C). The 760-fold increase in transdermal flux of a hydrophilic molecule should be useful for a variety of drug delivery applications. Moreover, because the mechanism appears to involve removal of stratum corneum on the micron scale, this approach is likely to be broadly applicable. This is because almost all biotherapeutics and vaccines are macromolecules, viral particles and other compounds of nanometer dimensions that should readily pass through micron-scale holes in the stratum corneum.

A limitation of high-temperature ablation of the skin is that there can be collateral effects on neighboring tissues, such as damage to the viable epidermis and excitation of painful nerve sensation. To minimize these effects, heat can be applied to the skin for very short periods of time, such that the surface of the stratum corneum gets extremely hot, but there is not enough time for heat to propagate across the stratum corneum to neighboring tissues before the heating is stopped. This study specifically addressed thermally induced changes to the stratum corneum and did not address possible changes to the viable epidermis, dermis, sweat glands or hair follicles, which would be important to assessing safety.

This approach has been used in a number of previous studies (Badkar et al., 2007; Birchall et al., 2006; Bramson et al., 2003; Lee et al., 2001; Nelson et al., 1991; Sintov et al., 2003) and is the subject of commercial development by a few companies (e.g., Altea Therapeutics, TransPharma Medical). Despite this progress in the field, there remains uncertainty about the mechanisms by which these short, hot pulses increase skin permeability. The present study suggests that the mechanism may involve decomposition and vaporization of the stratum corneum, which removes tissue to generate micron-scale holes. This contrasts with the mechanisms of chemical, electrical (iontophoresis and electroporation), and ultrasonic enhancement of transdermal transport, which each involve structural rearrangements of stratum corneum on the molecular or nanometer scale (Cross and Roberts, 2004). It also differs from microneedles, which similarly make micron-scale holes in the skin, but do so by cutting a pathway without removing tissue (Prausnitz, 2004).

Although the greatest effects were seen in Zone III, transdermal flux increased by one to two orders of magnitude at lower temperatures in Zone II. This level of increased skin permeability may be sufficient for many drugs. By operating at lower temperatures, it may be easier to protect neighboring tissues for collateral thermal damage. Moreover, a lower temperature device may be easier to design and less expensive to manufacture. Heating skin to Zone II was also shown to dramatically weaken stratum corneum mechanical strength. Pretreatment of the skin in this way could be followed by applying a minor mechanical force to fracture the stratum corneum and thereby increase skin permeability further.

Overall, this study provided the first measurements of the effect of heat on skin permeability over a broad range of temperatures and durations of heating. Skin permeability was found to depend very strongly on temperature and less strongly on the duration of heating. At optimal conditions examined in this study, transdermal delivery of calcein was increased by 760 fold. Structural changes in the skin associated with stratum corneum lipid order were found to increase transdermal delivery a few fold at temperatures in the range of approximately 100°C – 150°C (Zone I). At temperatures of approximately 150°C – 250°C (Zone II), transdermal flux was increased by one to two orders of magnitude, which was associated with disruption of stratum corneum keratin network structure. Above 300°C (Zone III), transdermal flux increased by three orders of magnitude, which was associated with decomposition and vaporization of keratin to create micron-scale holes in the stratum corneum. Although the duration of heating had a lesser effect on skin permeability, longer heating times should lead to greater heating of neighboring tissues, which can cause collateral damage and pain. We therefore conclude that heating the skin using extremely hot, short thermal pulses should provide dramatically increased skin permeability localized to the stratum corneum.

### Acknowledgements

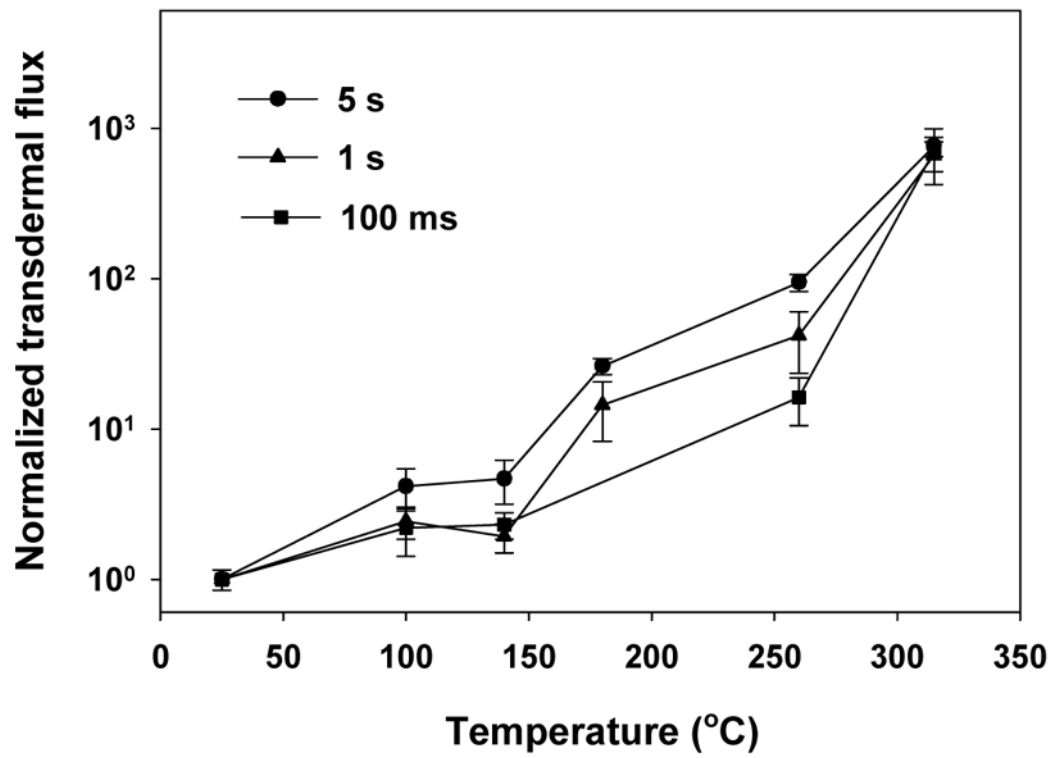
We acknowledge Yong-Kyu Yoon, Jin-Woo Park, Seong-O Choi, Yeun-Ho Jung, Rachna Kamath and Mark G. Allen for helpful discussions and laboratory assistance. This work was supported in part by the National Institutes of Health. Mark Prausnitz is the Emerson-Lewis faculty fellow. This work was carried out in the Center for Drug Design, Development and Delivery and the Institute for Bioengineering and Bioscience at the Georgia Institute of Technology.

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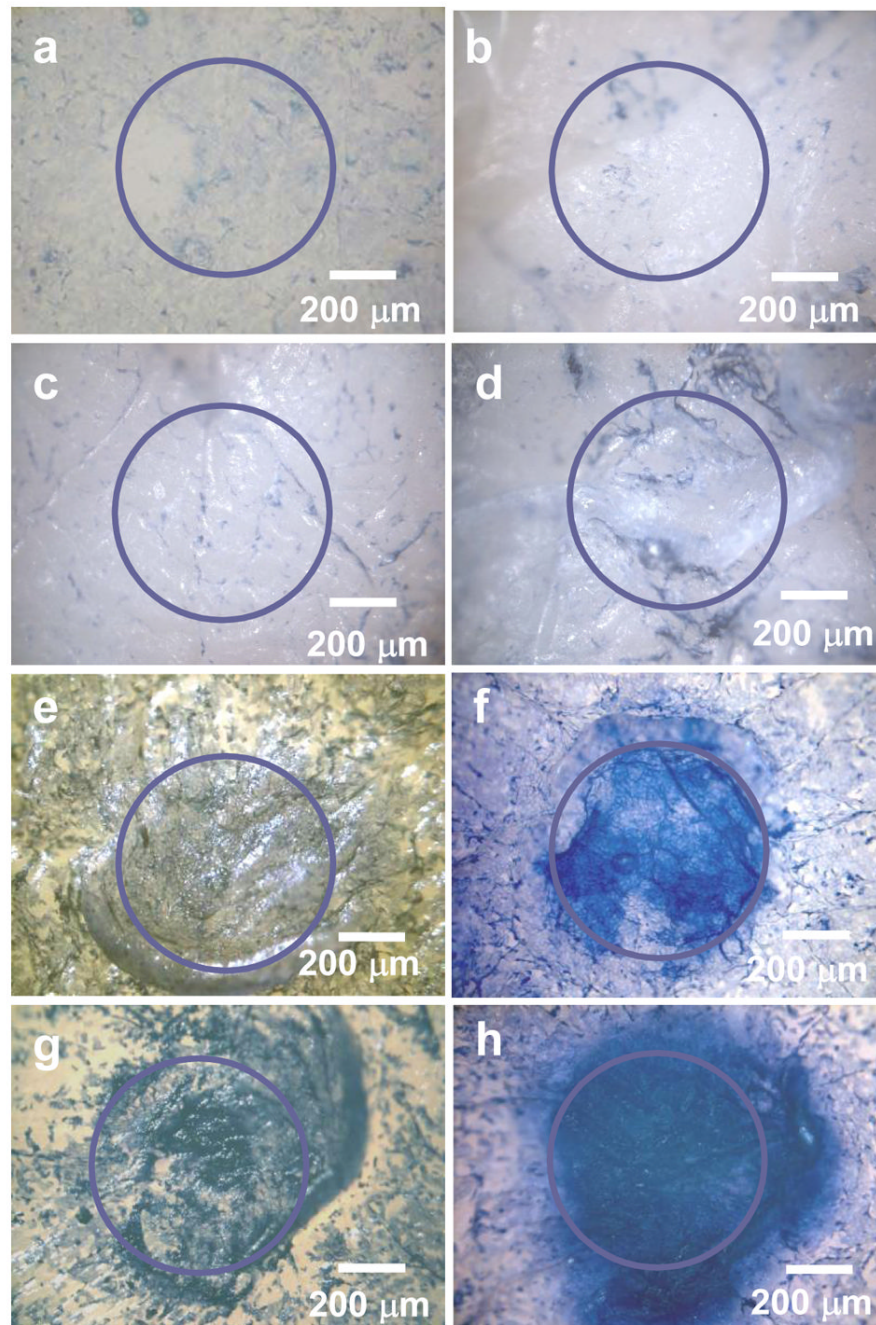
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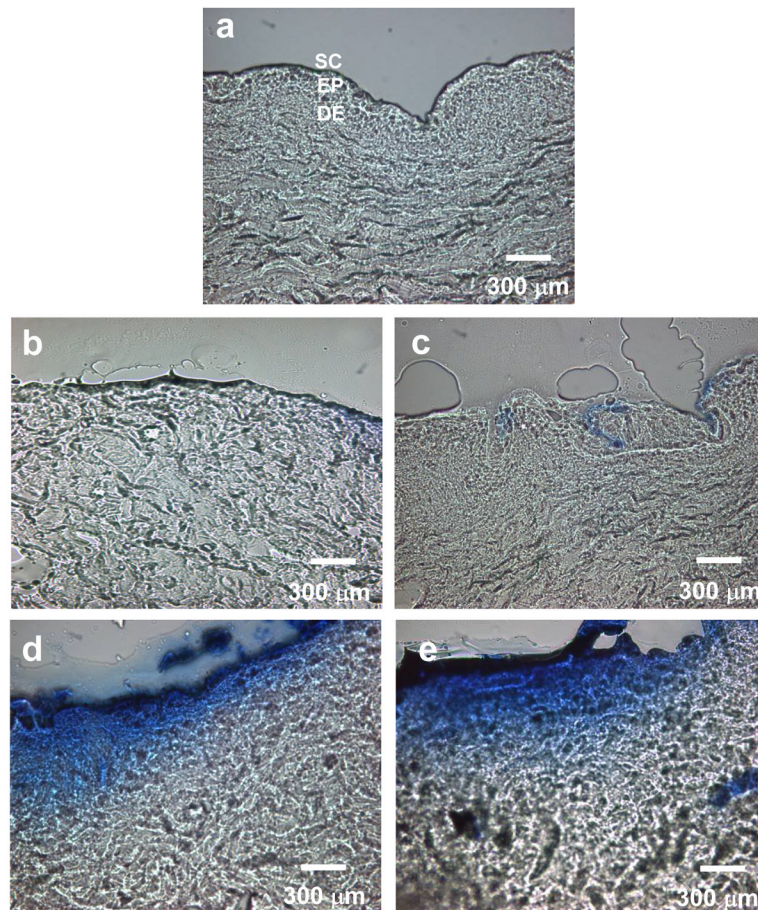
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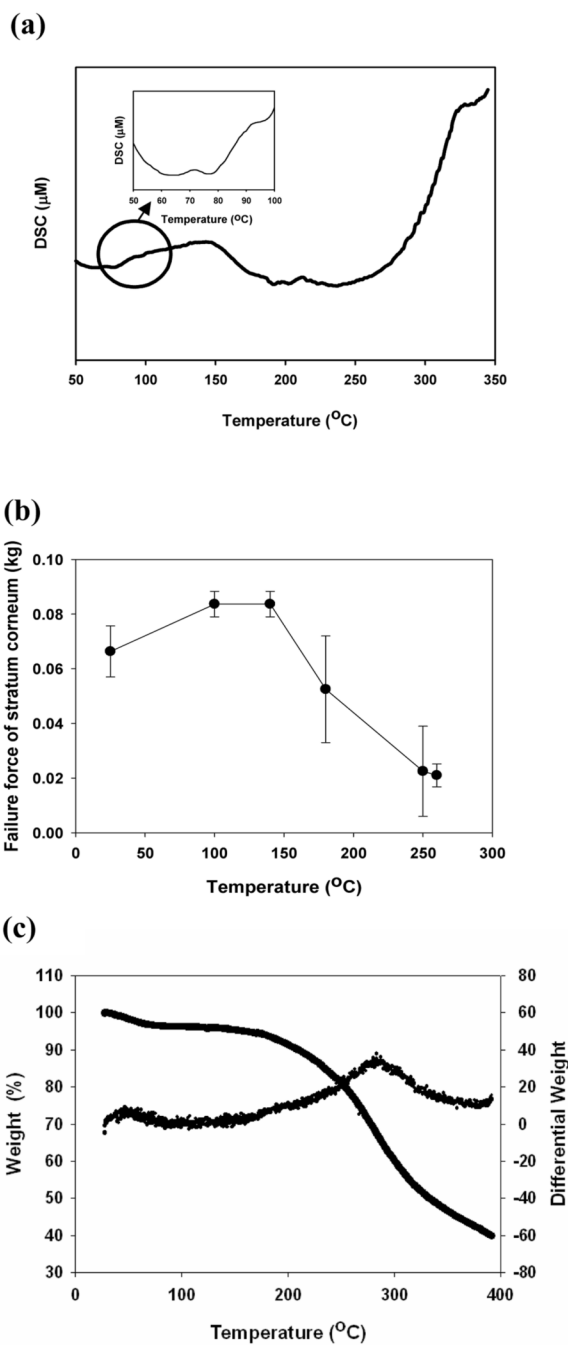
**Fig. 1.** Effect of temperature and heating time on the flux of calcein across human epidermis. Skin permeabilities are normalized to the average skin permeability at 25°C. Data represent the average and standard deviation error bars determined from n 5 samples.



**Fig. 2.** Representative brightfield microscopy images of the surface of human skin showing the extent of Trypan blue dye penetration into the skin after a 100-ms exposure to different temperatures: (a) no treatment, (b) 100, (c) 140, (d) 180, (e) 260, (f) 315, (g) 360, and (h) 415 °C

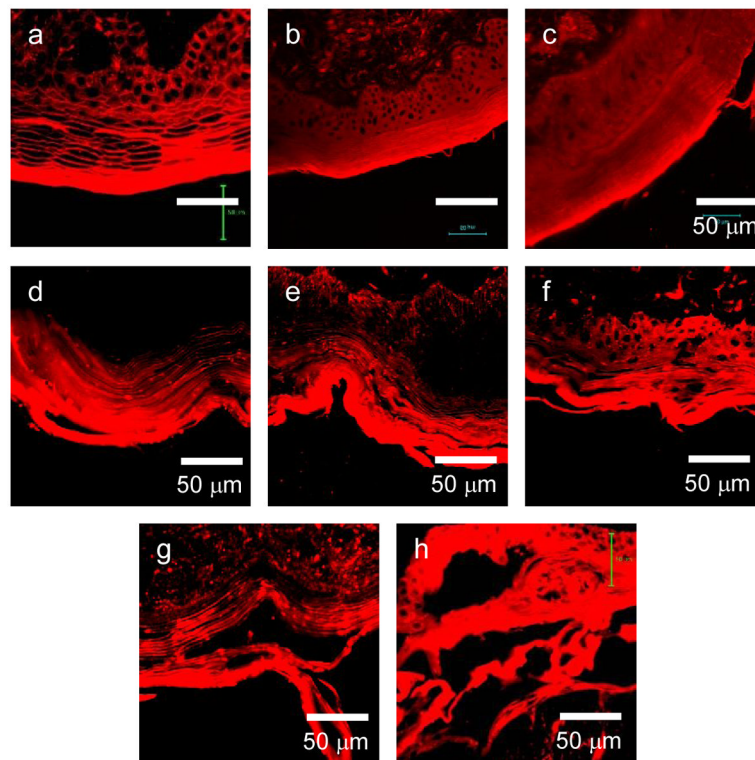


**Fig. 3.** Representative histological sections of human skin imaged by brightfield microscopy after a 100-ms exposure to different temperatures: (a) no treatment, (b) 260, (c) 315, (d) 360, and (e) 415 °C. The skin surface was exposed to Trypan blue as a marker of increased skin permeability and then stained with H&E.

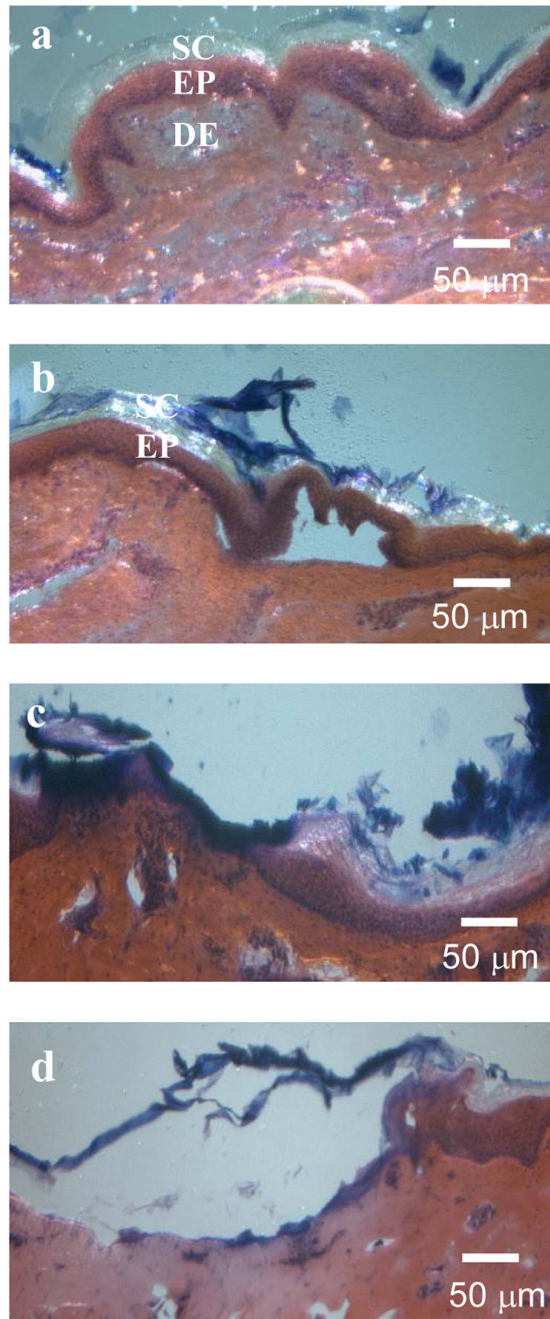


**Fig. 4.** Thermal analysis of skin by DSC, modified TMA, and TGA. (a) Representative DSC thermogram. (b) Modified TMA showing the change in mechanical strength of stratum corneum after a 1-s exposure to different temperatures. Data represent the average and standard deviation error bars determined from  $n \geq 5$  samples. (c) Representative TGA analysis showing the weight (black data points) and the differential change in weight (white data points) as a function of temperature.

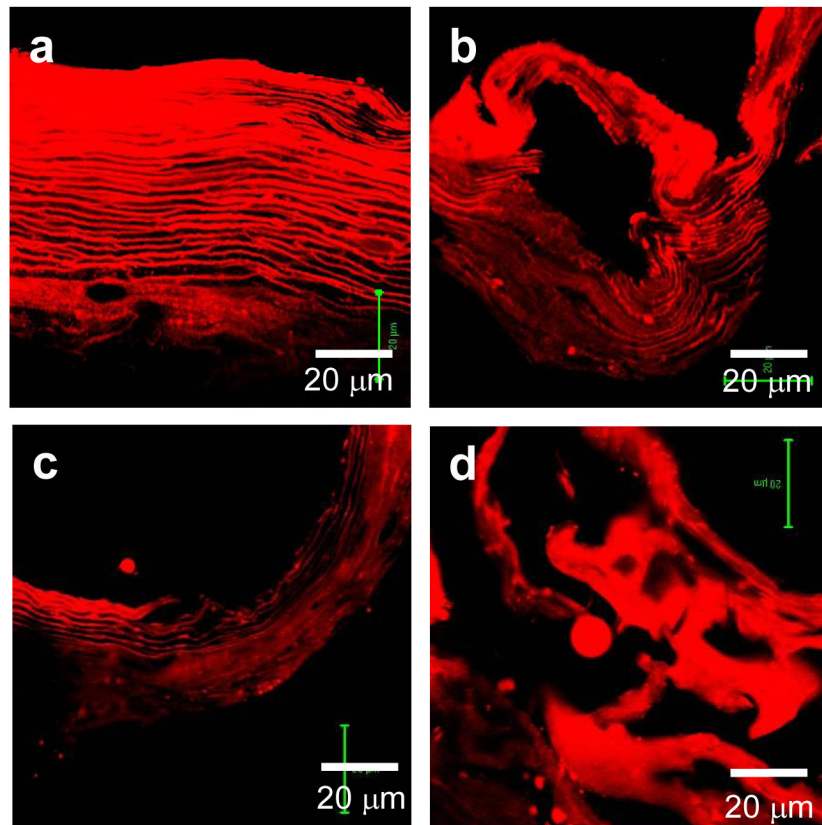




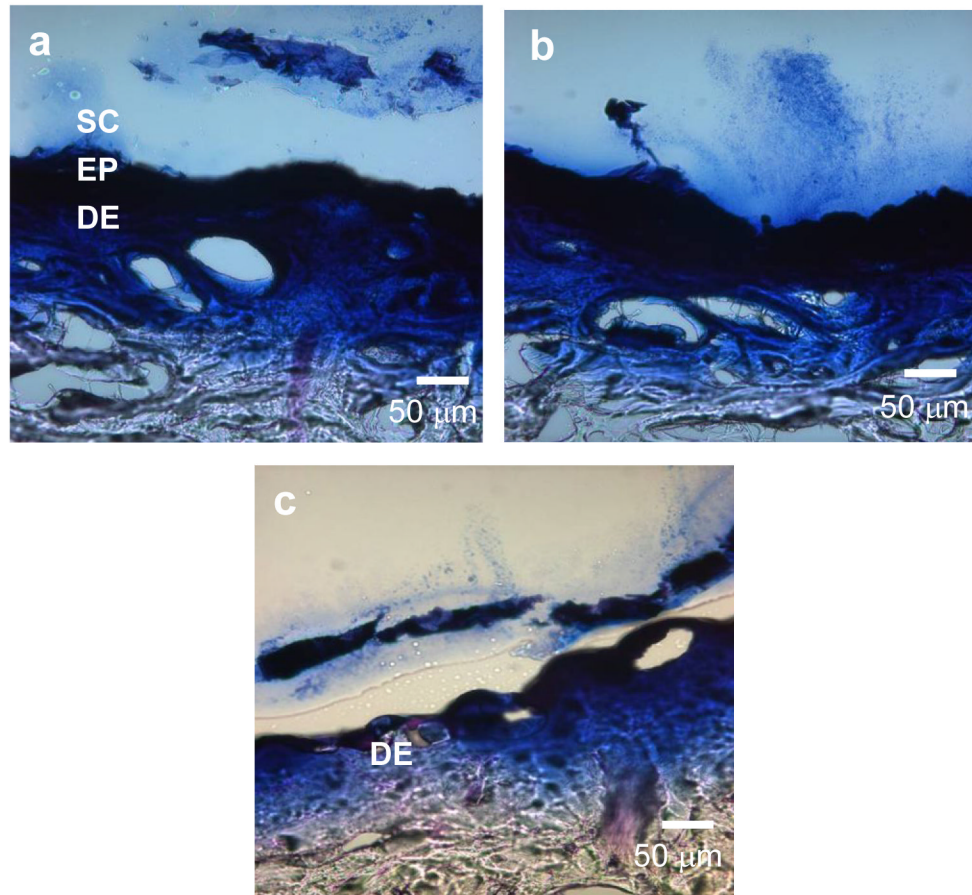
**Fig. 5.** Representative histological sections of human skin imaged by confocal microscopy after a 1-s exposure to different temperatures: (a) no treatment, (b) 100, (c) 140, (d) 160, (e) 180, (f) 200, (g) 260, and (h) 315 °C. After treatment, stratum corneum was first expanded using Sorensen Walbum buffer and the lipids were then stained with Nile Red.



**Fig. 6.** Representative histological sections of porcine skin imaged by brightfield microscopy after exposure to 260 °C for (a) no treatment, (b) 100 ms, (c) 400 ms, and (d) 1 s. The skin surface was exposed to Trypan blue as a marker of increased skin permeability and then stained with H&E.



**Fig. 7.** Representative histological sections of porcine skin imaged by confocal microscopy after exposure to 260 °C for (a) no treatment, (b) 100 ms, (c) 400 ms, and (d) 1 s. After treatment, stratum corneum was first expanded using Sorensen Walbum buffer and the lipids were then stained with Nile Red.



**Fig. 8.** Representative histological sections of porcine skin imaged by brightfield microscopy after exposure at 350 °C for (a) 100 ms, (b) 400 ms and (c) 1 s. After treatment, stratum corneum was first expanded using Sorensen Walbum buffer and the lipids were then stained with Nile Red.