Unravelling drug 'metamorphosis' in the skin using microCT

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Aims

Drug delivery to the skin is challenging because of the unique composition of the outer layer of the skin – the stratum corneum. Much research has focused on efforts to overcome this skin barrier with different formulation approaches and yet effective drug delivery to this organ remains low or inefficent. The hypothesis of drug reservoir formation in the skin has now been confirmed and drug crystallization or 'metamorphosis' in the skin has also been proposed as the explanation for "stranding" of drug in the skin. This phenomenon restricts continuous drug transport into the skin, resulting in poor drug delivery. To examine drug crystallization in the skin, our recent investigations including spectroscopic and localised nano-thermal and synchrotron SAXS/WAXS analyses provided new evidence of this phenomenon, however, confirming the precise location of crystals remains challenging. In addition, most approaches used to date required disruption of the membrane by tape stripping, with crystal detection limited only to the superficial skin layers. Therefore, the present work aims to resolve the spatial resolution of drug crystallization in the skin using microCT after application of a saturated drug solution to porcine skin.

Method

An *in vitro* permeation study was performed using Franz-type diffusion cells (diffusional area = ~1 cm^2) with porcine ear skin at 32ºC. The donor chamber was loaded with 10 µL/cm^2 of a saturated solution of diclofenac sodium (DF Na) in propylene glycol (PG) for 24 h. PG was selected to ensure high loading and thermodynamic activity of the drug substance in the solvent to ensure supersaturation and ultimately drug crystallization. After 24 h, the Franz-type diffusion cells were disassembled and the skin membrane was carefully removed. The skin sample was wrapped with Parafilm™ to avoid tissue dehydration and mounted onto a plastic holder for the microCT scan.

A benchtop cone-beam microCT system (Nikon Metrology, X-Tek, UK) equipped with a microfocus X-ray tube (12 µm focal spot, 50 kV) was used for all experiments. The source to detector distance was fixed at 688 mm. The number of projections was 1026 with an average of 64 frames for each projection. CT image reconstruction was carried out using the in-built X-Tek software (Nikon Metrology, X-Tek, UK). The microCT scan was repeated with a control skin sample. The analysis of the images was performed using MicroView Version 2.6.0-3 (GE Healthcare Biosciences) to visualise the skin samples as 2D and 3D maps (resolution: 23 µm) for different areas of the skin and drug crystals.

Drug distribution in the skin was evaluated using confocal Raman spectroscopy (CRS) (Model 3510 Skin Composition Analyzer, River Diagnostics, Rotterdam, The Netherlands) in an *ex vivo*

permeation study applying the same DF Na formulation in PG on the skin for 30 min. Because of the high drug thermodynamic activity of the saturated drug solution, the permeation was conducted over a shorter time period than usual to allow precise observation of drug distribution without affecting drug crystals during CRS analysis. Data were recorded using RiverIcon® software Version 3.0 and analyzed using Skin Tools® software Version 2.0 (River Diagnostics, Rotterdam, The Netherlands).

Results

Figure 1 and Figure 2 show selected 2D maps of the skin sample slicing at the x and z axis after the 24 h permeation study. The grey zones represent the skin sample while the dark grey zone corresponds to the sample holder and Parafilm™ which was confirmed by analysis of a control skin sample (data not shown). In addition, the white areas located at the top of the skin sample reflect the drug crystals. This represents drug penetration into the skin followed by drug precipitation as separate crystal clusters in the skin. The vertical section view of the skin sample is shown in Figure 3 where drug crystals were found to extend into the skin layers. Interestingly, Figure 3 also showed that drug crystal growth occurred along the lateral junctions between the penta- or hexagonal-shaped corneocytes and the appendages such as hair follicles. It might be speculated that these regions enriched with drug crystals may be “hotspots” to trigger further drug crystallization in the inner skin layers.

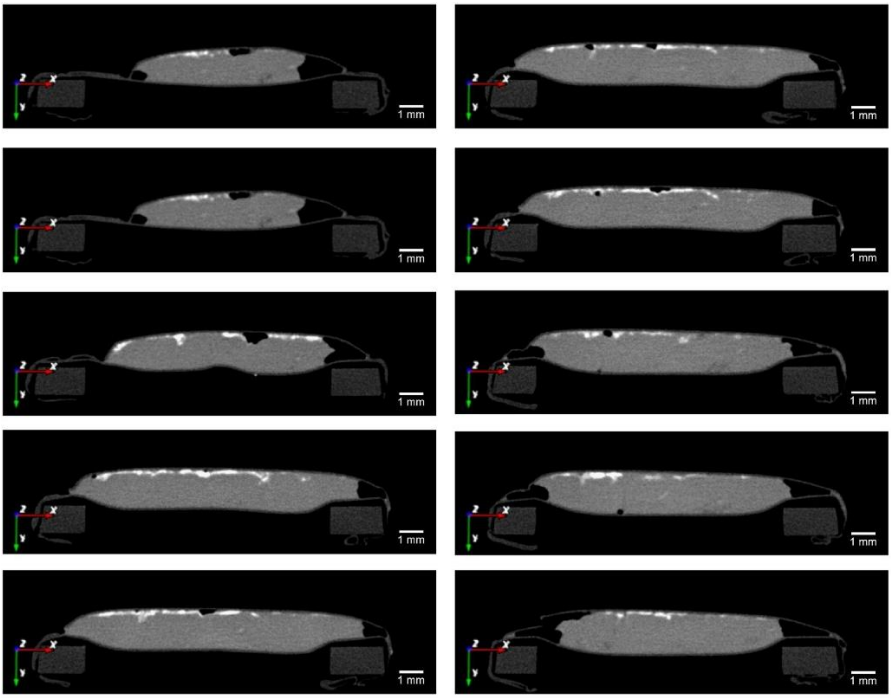

Figure 1: 2D microCT images of skin sample slicing at the z axis after 24 h permeation study

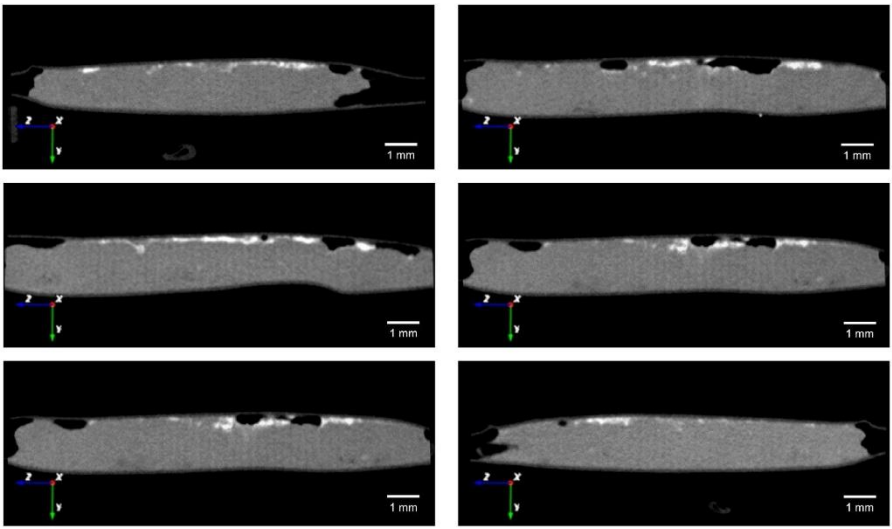

Figure 2: 2D microCT images of skin sample slicing at the x axis after 24 h permeation study.

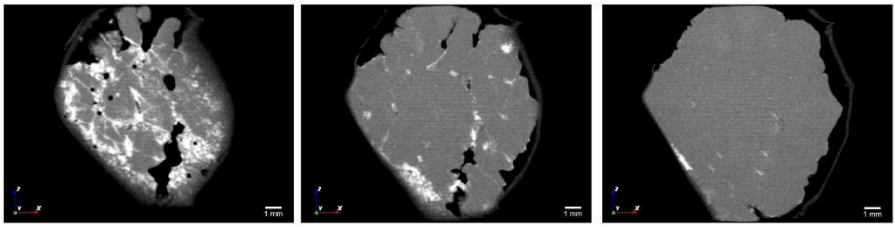

Figure 3: 2D microCT images of skin sample slicing at y axis after 24 h permeation study (left to right: top to inner skin layers).

However, it is difficult to differentiate isolated drug crystals and protruding drug crystals from the skin surface using the 2D maps. Therefore, 3D maps were reconstructed to determine crystal distribution in the skin. Following careful examination, it is evident that there are several individual drug crystal clusters (highlighted with the red circle) that are isolated from the drug crystal layer on the skin as shown in Figure 4 and Figure 5. The images in Figure 4C – D and Figure 5C – D are vertically flipped to show the drug crystal clusters embedded in the inner skin layers. These observations confirm the formation of isolated drug crystals in the skin rather than extensions of crystal growth from the skin surface.

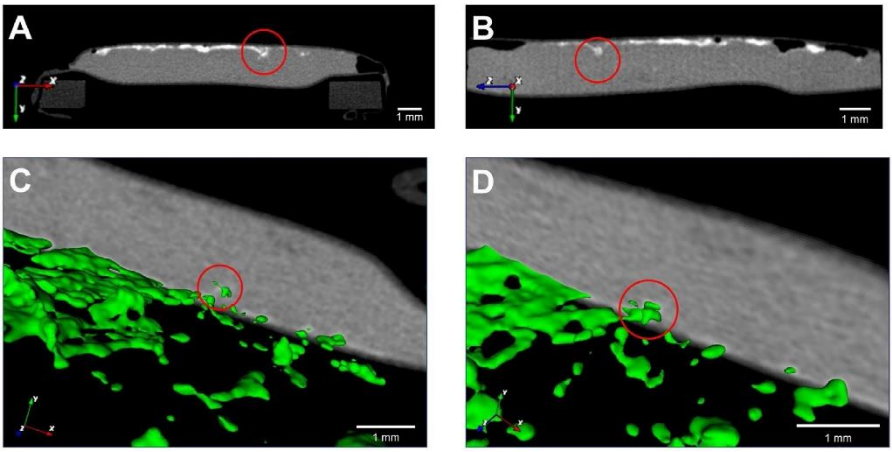

Figure 4: 2D microCT images of skin sample slicing at (A) x and (B) z axes and (C – D) 3D maps (vertically flipped) showing drug crystals (green colour) in different projections after 24 h permeation study. The isolated drug crystal cluster is highlighted in the red circles.

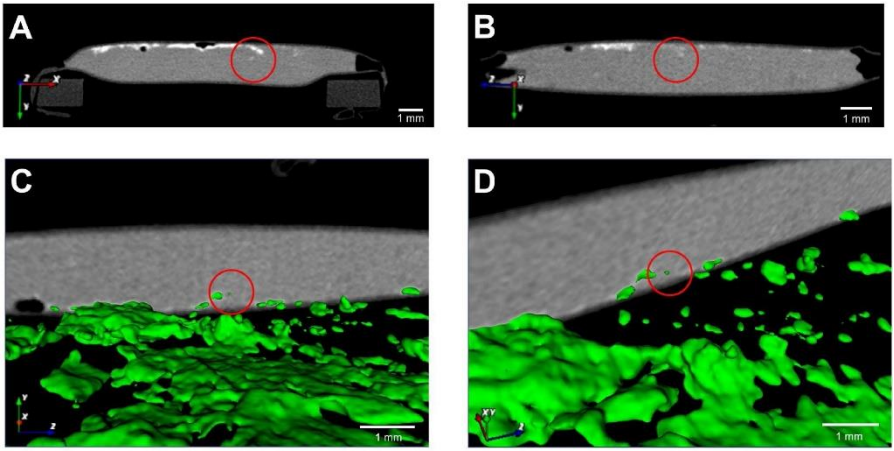

Figure 5: 2D microCT images of skin sample slicing at (A) x and (B) z axes and (C – D) 3D maps (vertically flipped) showing drug crystals (green colour) in different projections after 24 h permeation study. The isolated drug crystal cluster is highlighted in the red circles.

When the same DF Na formulation in PG was applied to the skin, CRS analysis (Figure 6) showed that DF Na penetrated up to ~20 µm in the skin which is beyond the porcine stratum corneum (thickness: ~10 µm). In the current study, it is interesting to note that drug crystals appeared as isolated clusters embedded in the skin structure at a depth of up to 0.2 – 0.3 mm. Our previous work with SAXS/WAXS analysis only recorded the formation of drug crystals up to 20 – 25 µm. Considering that microCT only allows a spatial resolution within the micrometre

range (23 µm in this work), any drug crystals with sizes smaller than a few microns would not be resolved.

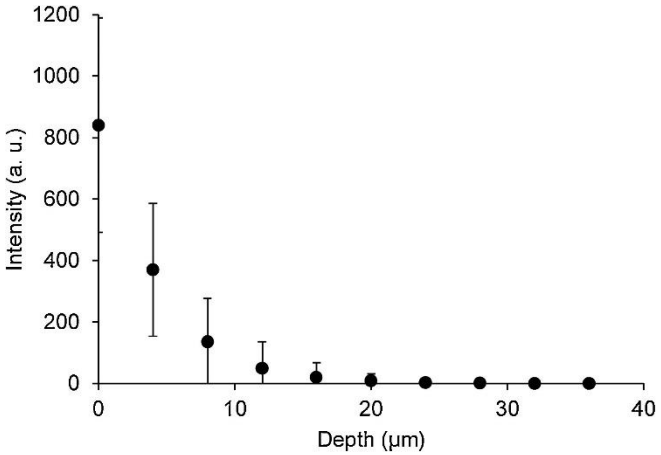

Figure 6: 2D microCT images of skin sample slicing at (A) x and (B) z axes and (C – D) 3D maps (vertically flipped) showing drug crystals (green colour) for different projections after 24 h permeation study. The isolated drug crystal cluster is highlighted in the red circles.

Conclusion

This is the first report of the use of microCT to detail the composite structure of skin samples with entrapped drug crystals.The spatial resolution capability of microCT allows the detection of drug crystals *in situ* in deeper skin layers which has not been reported to date. This imaging technique also enables the differentiation of isolated drug crystal clusters in the skin from the bulk drug crystal layer on the skin. The findings represent a significant advance in understanding drug crystallization in biological membranes and are expected to facilitate a more rational formulation design approach in topical and transdermal delivery.

References:

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