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Quantitative Penetration Profiles of Free and Nanocarrier-Bound Dexamethasone in Human Skin Studied by Soft X-Ray Spectromicroscopy

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The penetration of topically applied drugs in skin is known to be enhanced by drug nanocarriers [1]. However, quantitative drug uptake studies with high spatial resolution in which the exposure time and drug formulation are systematically varied are lacking to date. We have investigated the uptake of the anti-inflammatory drug dexamethasone by human skin using soft X-ray spectromicroscopy.

Chemical selectivity of this approach is gained by excitation of the O 1s→π^*-transition of dexamethasone at 530.65 eV, which is suitable to suppress the background absorption of fixed human skin as well as drug nanocarriers (core-multi-shell nanocarriers (CMS)), which were loaded by 5% dexamethasone. In addition, we used two different exposure times (4 h and 16 h) and varied the drug formulation, where either ethanolic solution or HEC gel (drug concentration: 0.5%) were used, respectively. Any cross sensitivities from absorption of untreated skin were subtracted, so that exclusively the drug concentration was monitored.

The experiments were performed at the BL4U beamline at UVSOR III using a scanning X-ray microscope (STXM), similar to previous work, in which the feasibility of this approach was explored [2].

Fig. 1 shows a comparison of depth profiles of dexamethasone in different skin samples along with an optical micrograph, in which the skin regions are labeled (stratum corneum (SC), viable epidermis (VE), and dermis (D)). Fig. 2 shows the integrated drug concentration for the samples under study, which facilitates to derive quantitative information regarding the drug distribution, if the exposure time and drug formulation are varied. Specifically, Fig. 1(a) indicates that after 4 h most of the drug is found in the SC, whereas a lower fraction is found in the VE (cf. Fig. 2). No drug is observed in the dermis (D). This situation changes, when 16 h of drug exposure in HEC gel are used (cf. Fig. 1(b) and Fig. 2). The intense maximum in the SC is weaker and the drug concentration in the VE is enhanced. If drug nanocarriers in HEC gel are used instead of the neat drug (cf. Fig. 1(c)), no drug signal was observed after 16 h in the SC rather than in the VE. This underscores the expected drug transport into deeper skin layers by nanocarriers. A drop in local drug concentration is observed for all samples in the dermis (D). This indicates that in this region rapid clearance is possible, so that no enhanced drug concentration is observed.

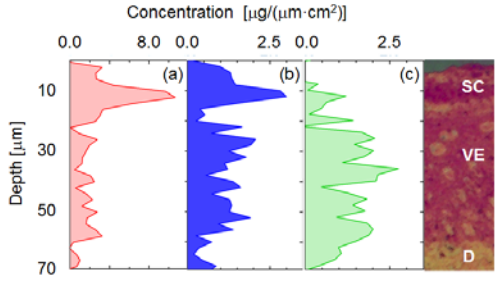

Fig. 1. Distribution of dexamethasone in the top skin layers (right hand side: typical optical micrograph, see text for details): (a) dexamethasone in ethanol: exposure time: 4 h; (b) dexamethasone in HEC gel: exposure time: 16 h; (c) CMS nanocarriers in HEC gel, exposure time: 16 h.

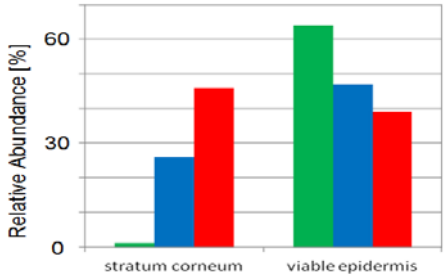

Fig. 2. Relative abundance of dexamethasone in the stratum corneum and viable epidermis (green: drug- loaded CMS nanocarriers in HEC gel, exposure time: 16 h; blue: dexamethasone in HEC gel: exposure time: 16 h; red: dexamethasone in ethanol: exposure time: 4 h) compared to the dose applied to skin.

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The effect of carbon bombardment of ZnO probed by scanning transmission x-ray microscopy

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In the present work, the effect of carbon bombardment of ZnO nanowires (NW) and nanocactus (NC) has been studied using X-ray-based microscopic and spectroscopic techniques. Figures 1(a-d) display the O *K*-edge scanning transmission X-ray microscopy (STXM) stack mappings of randomly selected sample regions of ZnO NW and NC before and after carbon bombardment (C:NW and C:NC). The stack mappings are decomposed into blue, yellow, red and green maps (right panels), which correspond to the regions that are associated with different thickness and spectroscopic variations of the samples. Overall, the divisions of the mappings into thick (green), thin (red), carbon lacing (yellow) and background (blue) regions were generated via principle component analysis (PCA) for cluster analysis based on spectroscopic differences. The O *K*-edge STXM-XANES (X-ray Absorption Near-Edge Structure) spectra in the Figs. 1(e) and 1(f) are the sum of the corresponding XANES spectra of the thick (green) and thin (red) regions of ZnO NW and NC before and after C bombardment, respectively.

As displayed in Figs. 1(e) and 1(f), according to the dipole-transition selection rule, the near features at ~535-550 eV in the O *K*-edge STXM-XANES spectra are attributed to the electron excitations from O 1*s*-derived states to $2p_{\sigma}$-derived (along the bilayer) and O $2p_{\pi}$-derived (along the ***c*** axis) states, which are approximately proportional to the density of the unoccupied O 2*p*-derived states. The O *K*-edge STXM-XANES spectra clearly reveal that the intensities of near features in the thin regions are higher than those in the thick regions of ZnO NC/NW and C:NC/C:NW. The enhanced intensities of the near features in the O *K*-edge STXM-XANES spectra can result in a higher population of defects in the thin or surface/edge regions because of the non-stoichiometric chemical composition or dangling bonds.[1]

Additionally, the intensities of near features of ZnO C:NC/C:NW samples (after C bombardment) are also higher than those in ZnO NC/NW samples (before C bombardment), further suggesting that the bombarding C atoms may play an important role to enhance density of the unoccupied O 2*p*-derived states.

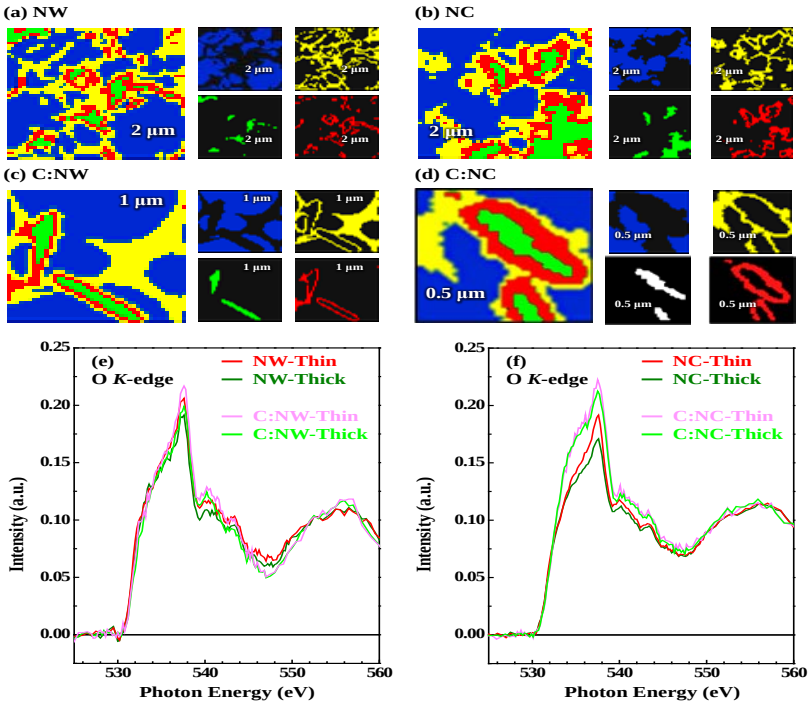

Fig. 1 (a)-(d): O *K*-edge scanning transmission X-ray microscopy (STXM) stack mappings of randomly selected sample regions of ZnO NW and NC before and after carbon bombardment (C:NW and C:NC). The spatial distribution of background (blue), carbon lacing (yellow), thick (green) and thin (red) regions of samples. (e) and (f): The O *K*-edge STXM-XANES spectra are the sum of the corresponding XANES spectra between the thin (red) and thick (green) regions of ZnO NW and NC before and after C bombardment, respectively.

References

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Observation of Morphology of Functional Polymers by Using a Humidity Control Sample Cell for STXM

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One of the advantages of a scanning transmission X-ray microscopy (STXM) is tolerance for atmosphere of a sample. Due to high transmittance of the X-ray, the STXM does not require vacuum condition and enables to observe the samples in air pressure or in water. Moreover, a focusing optical element of the STXM, a Fresnel zone plate, has long working distance and long focal depth. These features enable to perform *in-situ* observation, which is impossible for an electron microscopy, so that various sample cells are developed widely [1]. Then, control of humid atmosphere around the sample is a promising *in-situ* observation method [2]. For example, as an application, a fuel cell works under high humid atmosphere and high temperature. In this study, we have developed the humidity control sample cell and performed a test measurement.

The humidity control sample cell (shown in Fig. 1) is a small chamber consisted of two silicon nitride membranes (thickness of 100 nm) as windows. A small sensor to measure humidity and temperature (SHT7x, Sensirion AG) is set inside of the chamber. The cell has three ports for inlet/outlet of gas flowing and two of them were used in this study. The inlet and outlet were connected to a feedthrough of the STXM chamber with stainless tubes and dry/humid helium gas is flowed into the cell to control the humidity. Humidity in the gas is added by bubbling pure water in a bottle and its flow rate is controlled by using a needle valve manually. Then, the inside of the STXM chamber was transferred by helium gas until air pressure. As a performance test, we could change the humidity for from 16.1 to 80.4% at 29°C, where the temperature inside of the STXM chamber was higher than RT.

As test observation by using the cell, morphological change of thin sections of a functional polymer fixed on Formvar membrane was used as a sample. 2-dimensional distributions of fluorine in the polymer were obtained by using K-absorption edge of fluorine (687 eV). X-ray transmission images of below and above of the edge (682 and 692 eV) were obtained. After conversion to optical density images, the distribution of fluorine was obtained by subtracting these images. The distribution of fluorine of the humidity at 16% and 8% are shown in Fig. 2. In these images, bright area shows high concentration of fluorine and dark area like a crack is seen on the center. By changing the humidity from 16% to 80%, 11% of the dark area decreased. This change of the area was caused by swelling of water vapor of the polymer.

As a further application of this humid cell, observation of living biological samples, such as cells and bacteria, will soon be performed. The temperature control and auto-control (i.e. feedback of the humidity and temperature) systems are now under consideration.

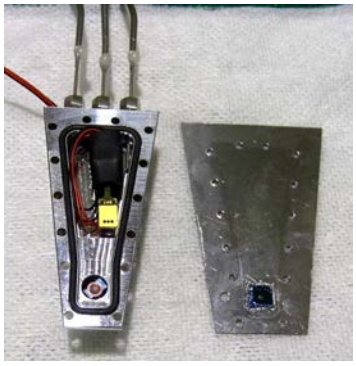

Fig. 1. Photo of the main chamber (inside) and the base plate of the humidity control sample cell.

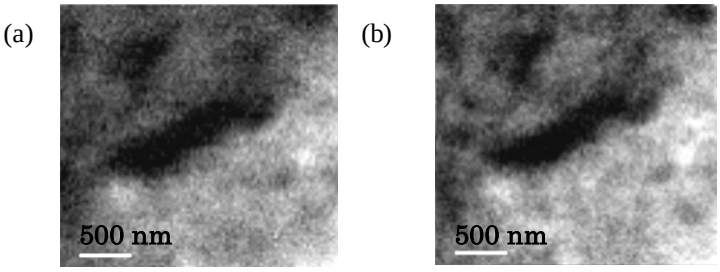

Fig. 2. Distribution of fluorine in the polymer (Bright pixel shows high concentration). The humidity of the cell were at (a) 16% and (b) 80%.

References

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