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Dermal Drug Delivery Probed by Scanning Soft X-Ray Spectromicroscopy

Human Skin, Skin Diseases, and Topical Therapy Concepts

Skin is the largest human organ. It has multiple functions, among them a few are highlighted: Skin serves as a flexible barrier that separates the body from the environment and protects humans from mechanical, biological, and chemical impact and regulates the hydration balance. A vertical section of human skin is shown in Figure 1, where an optical micrograph and a simplified cartoon of the top skin layers are depicted. Skin consists in a simplified view of the following layers starting at the dashed white line in Figure 1, the basal membrane, separating the dermis from the epidermis. Blood vessels reach into the dermis, as indicated by red lines in Figure 1(b). The viable epidermis consists mostly of keratinocytes besides a smaller number of immune cells. The keratinocytes grow steadily from the basal membrane and convert into corneocytes of flat shape without a cell nucleus. This growth process ensures a steady renewal of skin. The top layer is the stratum corneum, consisting of about 20 layers of corneocytes indicated by brown color in Figure 1(b) which are separated by thin layers of ca 100 nm of lipids (green color). Numerous skin diseases are known which affect the barrier function of skin. Among them are inflammatory skin diseases, such as psoriasis or atopic dermatitis, which have a high prevalence in the population. Treatment of such diseases can be done systemically, which can lead to severe side effects. Preferably would be a topical therapy leading to the directed drug delivery by minimizing side effects of potent drugs. This implies to have suitable formulations for topical treatment from the skin surface to promote directed drug delivery. The penetration paths from the skin surface are short, of the order of 100 µm, to get to the site of action, where the drugs are needed for therapeutic success. This makes skin from the physico-chemical point of view an attractive object for spatially and time resolved studies. However, the quantitative probing of the success of drug penetration as a function of depth and preferably with high spatial resolution remain a challenge since therapeutic concentrations are often too low to probe locally the drugs and formulations on their path to the site of action for probing therapeutic success.

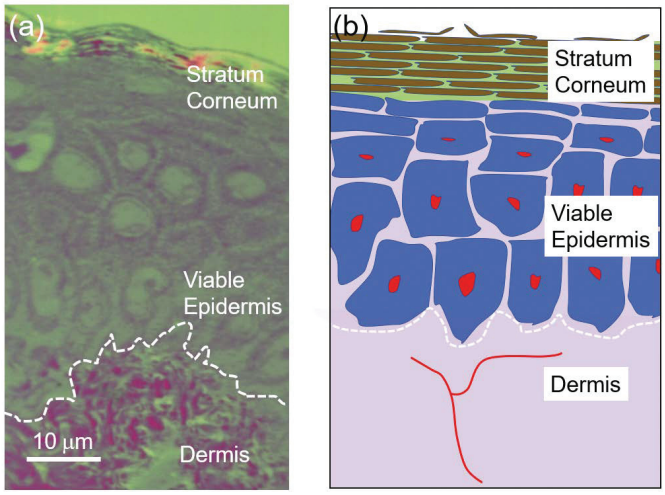

Fig. 1: (a) Optical micrograph of a human skin section and (b) cartoon of the outermost skin layers. Keratinocytes are marked by blue color with nuclei (red), corneocytes (brown), and lipids (green), blood vessels in the dermis are indicated by red color. The dashed white line indicates the basal membrane.

Label-Free Spectromicroscopy: Scanning X-Ray Microscopy

Probing highly dilute drugs and drug formulations in skin samples *ex vivo* requires sensitive techniques providing a spatial resolution that is preferably below the diffraction limit, corresponding the $\approx\lambda/2$, where λ is the wavelength of the incident radiation, to gain sub-cellular details of the transport and penetration processes. There is the possibility to probe even single molecules by highly sensitive techniques which often requires specific labels. For example, single molecule detection is known for fluorescence, also coupled with spatial superresolution, i.e., well below the diffraction limit [1]. However, most drugs and drug formulations do not fluoresce and cannot be probed in this manner. One might wish to label the drugs or formulations with fluorescence probes, but this bears the inherent problem, that the pharmacological and transport properties of such labeled species change and lose their efficacy. This is explained by the different modes of action of, e.g., anti-inflammatory drugs that are often inhibitors. As a result, any labeling is preferably avoided. It is rather attempted to use the molecular properties of drugs and formulations to probe sensitively by spectroscopic techniques their time-dependent penetration and localization in biological matter, such as skin. This is called label-free detection and, combined with spatially-resolved spectroscopy methods, this yields label-free spectromicroscopy [2]. There are different experimental approaches yielding sufficient contrast by probing spectroscopically molecular properties: Here, we focus as one simple and quantitative approach on absorption spectroscopy using the Beer-Lambert-law. Tunable soft X-rays received from

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synchrotron radiation are advantageous for element-selective excitation via inner-shell absorption, i.e., absorption of the 1s electrons of light elements [3]. Distinct chemical shifts reflecting the local chemical surroundings of the absorbing atom are used to excite a specific molecular site. This is of importance for gaining chemical contrast, which is especially favorable for probing actives in biological matter. Spatial resolution is achieved for monochromatic soft X-rays by a Fresnel zone plate yielding focused radiation with sizes between 10 and 100 nm, where the absorbing skin sample is placed. This yields 2-dimensional maps of the spatial distribution of the absorber of interest, which is, e.g., the drug or the drug formulation topically applied to skin. Then, one can choose a pair of photon energies, where the species of interest, e.g., the drug, shows a change in absorption cross section compared to the other contained species. This is sufficient for the quantitative detection, according to the Beer-Lambert law. However, often the substances of interest do not show the required chemical shifts of resonant transitions. Then, more advanced techniques are required to be used: One takes in each pixel that is part of the 2-dimensional map of the skin sample the entire spectroscopic information, for example the near-edge spectra of the elements of interest (see Figure 2(c, d)). Then, one can distinguish different shapes of inner-shell absorption spectra and their relative weights following from a singular value decomposition analysis [4].

Topically Applied Drug Formulations: Quantitative Depth Profiles

Selected results on topical drug delivery to human skin probed by scanning X-ray microscopy are shown in Figure 2, taken from ref. [5]. The goal of this research was to use different formulations including core-multishell nanocarriers (CMS) as versatile drug delivery polymer particles (with sizes below 20 nm, see Figure 2(a)) for the anti-inflammatory drug dexamethasone (DX, $C_{22}H_{29}FO_5$, see Figure 2(b)).

Figures 2(c) and (d) show typical spectroscopic results as a prerequisite for gaining chemical selectivity by scanning X-ray microscopy. The experiments were performed at the UVSOR III synchrotron radiation facility (Institute for Molecular Science, Okazaki, Japan). Here, the X-ray absorption of the drug nanocarrier (CMS) and the drug (DX) are shown in the O 1s-excitation regime. Both spectra consist of a low-lying resonance, which is due to the O 1s → π*-transition and a higher-lying O 1s → σ*-transition. It is assumed that the drug is transported in the nonpolar section of the nanocarriers, which is indicated by green color in Figure 2(a). Figures 2(e)-(g) show how sensitive the drug formulation is for the vertical drug penetration profiles. These profiles are derived from the depth dependent integration of the drug concentrations, which are derived from 2-dimensional maps by selectively probing the drug, as described in earlier work [5, 7]. These profiles contain absolute concentrations in the unit µg dexamethasone per cm^2 and µm depth, indicating the sensitivity of the approach, since it is possible to probe of the order of femtograms of the drug per $µm^3$. Supplementary high spatial resolution studies indicate that the drug is found in the lipophilic regions of the stratum corneum [8], as indicated in Figure 1 by green color. These ca. 100 nm wide sections promote drug penetration by a drop in free energy upon skin uptake, but represent at the same time a diffusion barrier, as model calculations indicate [9]. This situation is similar, if hydroxyethyl cellulose (HEC) gel, a frequently used formulation, is applied for 16 h penetration time (Figure 2(f)), besides a somewhat larger local drug concentration in the viable epidermis due to the extended penetration time. A significantly different result is observed for core-multishell nanocarriers (Figure 2(g)). They transport the drug efficiently through the stratum corneum so that this top skin layer is not acting as a drug reservoir. Rather, enhanced drug concentrations are found in the viable epidermis. This means that nanocarriers increase the local drug concentrations in the skin layer, where it is needed for therapeutic success, which underscores the importance of their use. Nanocarriers are, as supplementary studies indicate, also transported via the lipid layers deep into the stratum corneum where they release the drug. Nanocarriers cannot enter the viable epidermis, which is ascribed to the tight junctions located at the lop layers in the viable epidermis.

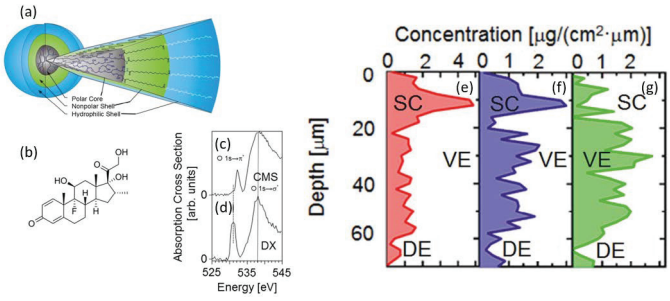

Fig. 2: (a) Schematic diagram of (a) core-multishell (CMS) nanocarriers (from ref. [6]); (b) structure of dexamethasone; (c) O 1s-absorption spectrum of core-multishell nanocarriers (CMS) and (d) O 1s-absorption spectrum of dexamethasone (DX); (e) penetration profiles of dexamethasone in human skin *ex vivo* after 4 h exposure to dexamethasone dissolved in ethanol (cf. ref. [7]); (f) 16 h exposure to dexamethasone in HEC gel; (g) dexamethasone released from CMS nanocarriers after 16 h exposure time. This Figure is taken with permission from Elsevier B.V. (*J. Control. Release* 2016, 242, 64-72).

Conclusions and Outlook

Label-free spectromicroscopy is a powerful approach for probing quantitatively dermal drug delivery, which is illustrated here by scanning X-ray microscopy. There are other and similarly potent approaches that could not be covered in detail, such as atomic-force microscopy-based spectromicroscopy. It makes use of tunable infrared laser sources, such as quantum cascade lasers. They can be operated in a wide wavenumber regime, either in a pulsed mode or in a continuous wave mode, respectively. This allows either for photothermal expansion [10] or scattering optical near-field spectromicroscopy [11]. These approaches are complementary, as they are bulk- and surface-sensitive, respectively. The spatial resolution ranges between 10 nm and 100 nm, depending on the sample, with a similar sensitivity as scanning X-ray microscopy. Further work also covered other drugs of higher molecular weight that are only penetrating skin if properly formulated or the skin barrier is disrupted [4, 8]. Finally, advanced methods of data acquisition including mathematical approaches [12, 13], and data reduction, such as singular value decomposition [4] or principal component analysis, play increasingly a crucial role for the efficient and sensitive detection of drugs in biological matter.

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Eckart Rühl is since 2006 Full Professor of Physical Chemistry at Freie Universität Berlin. Since then, he collaborated with research groups from life sciences in the field of nanoparticle interactions with cells and skin partially related to drug delivery. He coordinated in this interdisciplinary research field projects in Priority Programs and a Collaborative Research Center funded by DFG. His research interests also cover fundamental research, developments in methodology including instrumentation of large scale facilities, such as synchrotron radiation and free electron lasers, and applied research. His work is documented in more than 280 publications. Earlier career stages of Eckart Rühl were: Professor of Physics at Johannes Gutenberg Universität Mainz, Full Professor for Environmental Physics at Universität Osnabrück, and the Chair of Physical Chemistry I at Julius-Maximilians-Universität Würzburg. He studied chemistry at Freie Universität Berlin and received from there his doctoral degree and his habilitation in physical chemistry, where his mentor was Helmut Baumgärtel. Postdoctoral stays brought him to France (Orsay, Meudon), UK (Oxford), Canada (Hamilton), and U.S.A. (Boulder). Later, he spent sabbaticals in Okazaki (Japan), Berkeley (U.S.A.), and Catania (Italy). He served in several review boards of synchrotron radiation and free electron laser facilities, as well as in their scientific advisory committees. For DFG he served as a member and head of a review board. Since 2008 he is head of the Steering Committee of the Russian-German Laboratory at the synchrotron radiation facility BESSY II and since 2009 he is the scientific coordinator of the Russian-German Center of Excellence “German-Russian Interdisciplinary Science Center” funded by DAAD and the Federal Foreign Office. For the German Bunsen Society he served as a member of “Ständiger Ausschuss” and the head of “Themenkommission”. He is since more than 12 years active in the European Chemical Society (EuChemS), where he founded the Division of Physical Chemistry and he is since more than five years the Treasurer of the European Chemical Society.