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Applications of Raman spectroscopy in skin research — From skin physiology and diagnosis up to risk assessment and dermal drug delivery☆

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ABSTRACT

In the field of skin research, confocal Raman microscopy is an upcoming analytical technique. Substantial technical progress in design and performance of the individual setup components like detectors and lasers as well as the combination with confocal microscopy enables chemically selective and non-destructive sample analysis with high spatial resolution in three dimensions. Due to these advantages, the technique bears tremendous potential for diverse skin applications ranging from the analysis of physiological component distribution in skin tissue and the diagnosis of pathological states up to biopharmaceutical investigations such as drug penetration kinetics within the different tissue layers.

This review provides a comprehensive introduction about the basic principles of Raman microscopy highlighting the advantages and considering the limitations of the technique for skin applications. Subsequently, an overview about skin research studies applying Raman spectroscopy is given comprising various in vitro as well as in vivo implementations. Furthermore, the future perspective and potential of Raman microscopy in the field of skin research are discussed.

Contents

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1. Introduction

1.1. State-of-the-art analytics in skin research

The skin forms the outermost biological barrier between the human body and the external environment. It consists of different layers exhibiting individual composition and physiology. The main barrier function is constituted by the stratum corneum as the outermost skin layer, comprising cornified cells which are surrounded by a coherent lipid matrix. Deeper skin layers are constituted by the viable epidermis with the basal membrane as well as by the dermis, where appendices like sweat glands and hair follicle are located. Due to the complex assembly and diverse functions of the skin, skin related research is focused on a variety of different aspects. The scientific interest ranges from the elucidation of the biochemical composition and physiological functions up to the diagnosis of pathological skin states. Based on such basic research studies, the scientific interest proceeds towards the discovery of strategies to influence and manipulate physiological functions and processes in the skin. In this context, skin absorption and permeation processes are in the focus of interest. On the one hand, this interest stems from the necessity to evaluate the absorption of harmful substances for risk assessment and safety studies. On the other hand, skin absorption processes can be used to deliver substances via the skin for cosmetic, protective or therapeutic purposes. While the cosmetic delivery is mainly based on local treatment, the therapeutic application aims either for local delivery to the skin as a target organ or for systemic delivery by crossing the skin as a barrier to reach the systemic blood circulation. Rational development of drug delivery systems for targeted application of molecules to the skin requires accurate knowledge of absorption mechanisms and transport kinetics in skin tissue. Thus, for investigation and evaluation of novel skin therapeutics, suitable analytical techniques are essential prerequisites.

The in vivo evaluation of rate and extent of the penetration behavior of a substance into the skin is usually performed by tape stripping. After application of a substance to the skin, the tape stripping procedure includes a layer-by-layer removal of the stratum corneum by means of adhesive tapes with subsequent extraction and quantification of the applied substance most commonly achieved by HPLC. In contrast, the in vivo skin permeation of a substance in vivo can directly be determined by its serum concentrations in the systemic blood circulation. However, this approach does not elucidate information about metabolism and permeation pathways within the skin tissue. Furthermore, in vivo studies with human volunteers are limited to therapeutic substances and are not suitable for risk assessment.

Moreover, as in vivo diagnosis of skin related diseases solely based on visual inspection is often challenging, diagnostic procedures frequently depend on the removal of biopsies of the affected skin areas. Most commonly, the final diagnostic decision is made after subsequent histological in vitro analysis.

For in vitro penetration studies, excised skin samples are subjected to tape stripping in combination with further tissue segmentation and extraction of the deeper skin layers allowing depot assessment of an applied substance or monitoring substance degradation in the viable epidermis and dermis. For in vitro permeation experiments, the Franz-diffusion cell is the most common analytical setup. The cell consists of a donor and an acceptor compartment which are separated by an excised skin sample. Substance permeation can be analyzed by investigating the donor compartment, whereas the remaining substance can subsequently be extracted from the excised skin sample.

However, all these techniques are destructive as well as labor intense and therefore restricted. Additionally, the supply of excised mammal skin is limited and at the same time high inter- and intraindividual variability in skin samples demands high numbers of experiments.

Against this background, there is a strong need for non-destructive analytical techniques to reduce sample size in vitro as well as to allow more comprehensive in vivo analysis. In this context, non-destructive optical methods with immediate analytical readout, such as fluorescence, Infrared (IR) and Raman spectroscopy show the potential to fill this scientific gap.

1.2. Optical methods in skin research

Great progress in optics, computer sciences and electronic engineering facilitated the rise of optical methods in all fields of research. The immediate analytical readout and the contact-free implementation gave rise to the transfer of these techniques to skin research. Since the late 90s, several optical methods have been implemented in pathophysiological skin diagnosis, physiological tissue evaluation as well as permeation and penetration studies in vitro and in vivo [1,2].

In this context, optical coherence tomography (OCT), an interferometric technique based on infrared light scattering, provides images of the skin with micrometer resolution and has already widely been used in diagnosis of skin diseases [3,4]. However, the inadequate spatial resolution and the restriction to solely visualize the macroscopic skin structure constrain OCT.

Recently, microscopy based techniques like confocal laser scanning microscopy (CLSM) and multiphoton microscopy (MPM) have been used to track substances within the skin and to explore in vivo and in vitro skin physiology [5,6]. These techniques provide information about dermal penetration depth and molecular substance interaction with the skin tissue with high spatial resolution. Although, MPM showed good results by monitoring the skin's autofluorescence and represents a powerful tool in dermatological imaging [7], most tracking procedures require labeling of the substances of interest with fluorescent dyes.

Unfortunately, the labeling is prone to change the physicochemical properties of the substances, thus affecting transport within and interaction with biological tissues. Therefore, transferability of such data to the in vivo situation in the human body is impaired.

In contrast, vibrational spectroscopy techniques can provide direct molecular information of a sample without labeling. Infrared spectroscopy (IR), an optical technique based on laser light absorption is the widest spread technology in this particular field. In skin research, near-IR spectroscopy has successfully been applied in vivo [8,9] and in vitro [10,11] to track substances within the skin by chemically selective depth profiling and mapping. Unfortunately, the resolution of IR measurements is limited to 5–10 μm and water interferes with IR