Raman Microscopy and Imaging: Applications to Skin Pharmacology and Wound Healing

Carol R. Flach, Guojin Zhang, and Richard Mendelsohn

Abstract The utility of confocal Raman microscopy to study biological events in skin is demonstrated with three examples. (i) monitoring the spatial and structural differences between native and cultured skin, (ii) tracking the permeation and biochemical transformation in skin of a Vitamin E derivative and (iii) tracking the spatial distribution of three major skin proteins (keratin, collagen, and elastin) during wound healing in an explant skin model.

15.1 Introduction

Vibrational microspectroscopy provides a unique means for molecular level structure characterization of a variety of biological processes associated with skin. For the past several years, this laboratory has utilized Raman and IR spectroscopy, microscopy, and imaging to monitor the biophysics of the skin barrier, mechanisms of drug permeation and metabolism in intact tissue, and, more recently, the complex events that transpire during wound healing in an ex vivo skin model [1–6].

This chapter is organized as follows: We initially provide a brief overview of the basic elements of skin structure. Next, a validation of confocal Raman measurements to monitor skin processes and structure is given. Finally, we demonstrate the utility of the approach with three examples taken from our own work: (i) monitoring the spatial and structural differences between native and cultured skin; (ii) tracking the permeation and biochemical transformation in skin of a vitamin E derivative; and (iii) tracking the spatial distribution of three major skin proteins (keratin, collagen, and elastin) during wound healing. Our approach tends to emphasize the molecular structure information inherent in the spectra, obviously a unique advantage of the technology. Although the primary focus of this chapter is to introduce the technology and to highlight efforts from this laboratory, we of course recognize the efforts of other groups around the world. We refer to and comment on pertinent aspects of their work and apologize at the outset for any oversights.
15.2 Preliminaries

15.2.1 Skin Microanatomy

Figure 15.1 presents a photomicrograph of a human skin section. The heterogeneity of the tissue is immediately evident. The main barrier to permeability resides in the highly specialized outermost layer of the epidermis, the stratum corneum (SC). A major function of this layer is to maintain water homeostasis, although many other SC functions have been described [7]. This 10–20 μm thick layer consists of anucleated keratin-rich corneocytes embedded in a highly ordered lamellar layer comprised of fatty acids, ceramides, and cholesterol derivatives. The standard “bricks and mortar” representation of SC structure in which the lipids act as the “mortar” holding the corneocyte “bricks” in the appropriate geometric array is depicted in Fig. 15.2a. Several other models exist in the dermatology literature. In particular the detailed “domain mosaic” model of Forslind [8] has gained popularity. As shown in the atomic force micrograph in Fig. 15.2b, corneocytes are highly asymmetric cells, ~0.5 μm thick along the “z”-direction (perpendicular to the plane of the skin) and ~50 μm in the other two dimensions. The lipid constituents organize themselves into non-covalent supramolecular membranous sheets which constitute the primary barrier to permeability. Some possible packing motifs available to the lipids, namely orthorhombic, hexagonal, and liquid crystalline, are schematically depicted in two ways in Fig. 15.2c. The top figure depicts standard bilayer structures which become thinner with looser

Fig. 15.1. Photomicrograph of a human skin section. The thickness of the stratum corneum (SC) shown ranges from about 15 to 20 μm. Scale bar = 20 μm