Electrochemical Imaging of Molecular Transport in Skin

by Henry S. White

The delivery of drugs into the body, for therapeutic treatment, at a continuously controlled rate, is a general goal of the medical community. One means of achieving controlled release is by molecular transport across the skin, commonly referred to as transdermal drug delivery. Transdermal drug delivery systems may be passive, in which a molecular species diffuses across the skin, driven by a concentration gradient (*e.g,* nicotine patch); or active, in which an electric potential gradient across the skin accelerates transport via migration and/or electroosmosis. Active transport is referred to as iontophoresis, which has been explored by the medical community for over a century.[1] Figure 1 shows a schematic diagram of an iontophoretic device in contact with the skin. The drug of interest is contained in a thin layer of solution separating an electrode from the outer layer of skin, the *stratum corneum*. An electrical current, typically a few hundred microamperes per square centimeter, is forced across the skin, driving the molecule into the underlying tissues and the circulatory system.

Passive and active transdermal drug delivery systems avoid difficulties associated with traditional methods of drug administration. Many drugs cannot be administered orally due to extensive metabolism in the gastrointestinal tract or weak absorption into the bloodstream. Advances during the past decade in transdermal drug delivery suggest that many drug molecules, including delicate peptides,[2] proteins,[3] and oligonucleotides[4,5] can be delivered across skin at a precisely controlled rate and in an essentially non-invasive fashion. Iontophoretic transport of lidocaine and fentanyl is currently being employed for local and systemic anesthetic administration in clinical trials.[6] Reverse iontophoretic transdermal transport, in which molecules in the blood stream are transported across the skin for analytical detection, is being developed for the noninvasive monitoring of blood glucose levels by diabetic patients.[7]

The success of iontophoretic systems depends largely on a fundamental understanding of transport pathways and mechanisms in skin, a highly complex biological membrane. Over the past decade, our laboratory has developed scanning electrochemical microscopy (SECM) to investigate and quantify transport in artificial and biological membranes, including both hairless mouse[8] and human skin.[9] Spatially-resolved measurements of molecular transport using SECM,[10-12] with micrometer and submicrometer resolution,[13] provides a means to identify microscopic structures in skin that are associated with molecular transport paths and mechanisms.[14] SECM has been used to quantify the individual contributions of diffusion, migration, and convective to localized fluxes in skin[15] and other membranes.[16] This capability has been especially useful in characterizing transport mechanisms in skin, where the molecular flux varies significantly as a function of spatial position and as a function of the chemical nature of the molecule being transport-

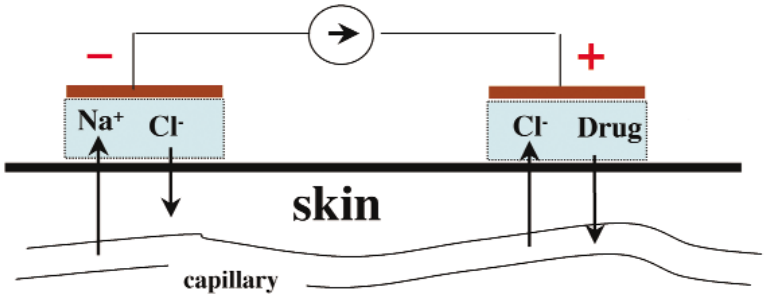

FIG. 1. Schematic diagram of drug delivery from an iontophoresis cell placed on the skin.

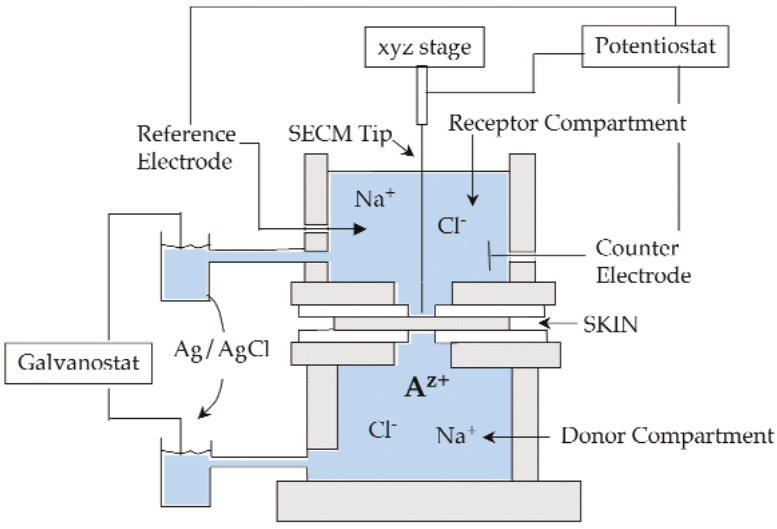

FIG. 2. Schematic diagram of the iontophoresis cell and scanning electrochemical microscope used for imaging fluxes across hairless mouse skin.[24]

ed. A brief summary of the use of SECM for imaging biological membranes, and some results obtained in our studies of iontophoretic transdermal transport, are presented in the following sections.

Electrochemical Visualization of Molecular Fluxes in Biological Membranes

Figure 2 shows the iontophoresis cell and key components of the SECM. In investigations of transdermal transport, the SECM tip (a small carbon fiber) is typically rastered across the surface of a skin sample at a tip-surface separation of a few micrometers using three piezoelectric (x, y, z) inchworm motors. In many of our experiments, the skin sample is excised from 7 week-old male hairless mice immediately after euthanasia by CO_2 asphyxiation. Hairless mouse skin (HMS) is a useful model for understanding transport in human skin. The fact that HMS contains very few hairs is advantageous in SECM studies, as hairs protruding from the skin would interfere with the motion of the SECM tip. However, in understanding the results presented below, it is important to note that the hair follicles in HMS are intact, offering low resistance pathways for transport across the *stratum corneum*.

In SECM studies of membrane transport, the sample (*e.g.,* HMS) separates a donor solution, which contains an electroactive species (A^{Z+}) at millimolar concentrations, from a receptor solution in which the redox species is absent. Both solutions contain a supporting electrolyte, typically 0.1 M NaCl. In the conventional imaging mode of SECM, the SECM tip is placed in the receptor compartment and is poised at a potential such that the electroactive molecule is oxidized or reduced as it emerges from pores in the membrane (Fig. 2). The magnitude of the tip current measured by the SECM tip is proportional to the local concentration of electroactive species above the pore opening. In addition, the local concentration of the redox species above the pore opening is proportional to the species flux within the membrane. Thus, a plot of the SECM tip current versus spatial position provides a direct means to visualize transport paths in the membrane. The resulting images are images of molecular flux that directly reflect transport rates associated with physiological structures in the skin. To study iontophoretic transdermal transport, a constant electrical current, i_{app}, is applied between two Ag/AgCl electrodes located on opposite sides of the skin sample (Fig. 2). This current can be continuously varied, allowing transport rates to be studied over a wide range of iontophoretic conditions.

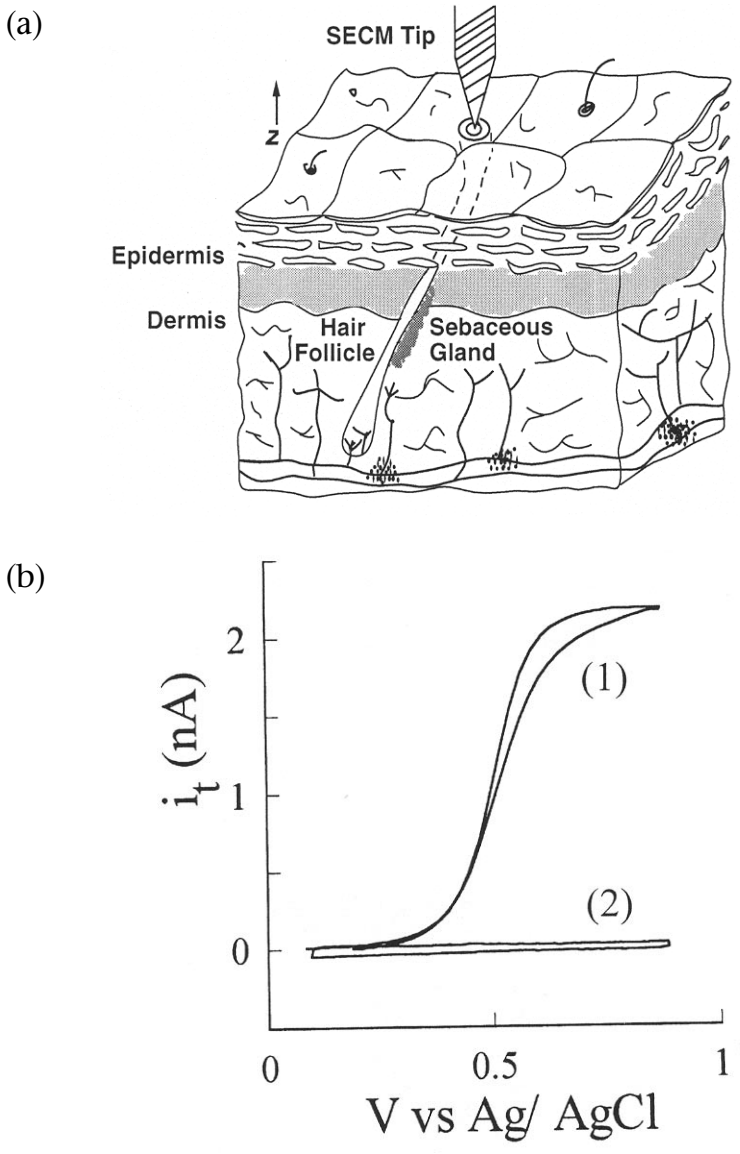

FIG. 3. (a) Schematic drawing illustrating the SECM tip positioned directly above the opening of a hair follicle. (b) Voltammetric response of a 2.7 µm-radius SECM tip positioned directly above a hair follicle opening in hairless mouse skin (curve 1) and 150 µm away from the opening (curve 2). The voltammetric current corresponds to oxidation of HQ that has diffused through the hair follicle.[17]

Localized Transport Pathways in Skin

A key issue in transdermal drug delivery is the identification of transport pathways that allow molecules to pass through the skin. To address this question, we use SECM to image molecules emerging from skin as they are transported across the sample by either passive or active transport. For example, Fig. 3 shows the steady-state voltammetric response of the tip when it is positioned directly above a hair follicle opening (curve 1) and at a lateral distance of ~150 µm from the opening (curve 2).[17] In this experiment, the redox-active molecule hydroquinone (HQ) is transported across HMS by diffusion alone and is detected by oxidation at the SECM tip. The sigmoidal shaped voltammetric wave recorded above the hair follicle (curve 1) corresponds to oxidation of HQ molecules that have diffused across the skin sample through the hair follicle. The magnitude of the tip voltammetric current is proportional to the local rate at which HQ permeates the skin. (Transport rates can be readily quantified from SECM tip approach curves).[16] The SECM tip current decreases to background levels (curve 2) when the tip is moved away from the pore opening,

demonstrating that the diffusive flux of HQ is localized to the hair follicle. The sites of high diffusive flux are independently identified as hair follicles using a dye staining technique in which colloidal Prussian blue is precipitated at the opening of the follicle. Similar experiments using small organic and inorganic redox species, with different charges (z = +1, 0, and –1) indicate that hair follicles in HMS act as the primary route for transdermal transport.

Titration of Electroosmotic Fluxes within Individual Hair Follicles

A number of studies during the past decade have demonstrated that an applied electrical current can also enhance the rate of transport of electrically neutral molecules.[18-20] This result suggests that iontophoresis can induce an electroosmotic flow of solution across skin tissues. Analogous to electroosmotic flow in capillary electrophoresis and synthetic membranes (*e.g.,* Nafion), electroosmosis in skin results from a fixed ionic charge in the epidermal layers.

The three SECM images presented in Fig. 4 demonstrate that electroosmotic transport of HQ is operative inside the hair follicle when a constant iontophoretic current, i_{app}, is applied across the skin sample. The SECM images were recorded by rastering the SECM tip at a fixed height above the opening of a hair follicle while detecting HQ at the tip. The middle image was obtained at $i_{app} = 0$, corresponding to diffusion of HQ. The bottom image, obtained at $i_{app} = 50$ µA, clearly demonstrates that a positive iontophoretic current enhances the rate of molecular transport through the hair follicle. Because HQ is electrically neutral at pH = 6.0, its transport is not directly influenced by the applied current. Rather, the enhancement in molecular transport must result from electroosmotic flow of solution inside the hair follicle.

In our experiments, the application of a positive i_{app} corresponds to the upward migration of electrolyte cations (Na^+) from the donor to the receptor solution and/or the downward migration of anions (Cl^-), *i.e.,* the anode is in the donor compartment. The direction of electroosmotic flow that is induced by the current depends upon the permselective properties of the skin sample. HMS, which exhibits a net negative charge at pH = 6.0,[21] displays cation-selective membrane properties. Thus, the majority charge carrier in skin at pH = 6.0 is Na^+. The cation permselectivity of HMS may arise from protein chemistry similar to that of human skin; it is reported that the observed permselectivity results from a larger number of carboxylate ($-COO^-$) than ammonium ($-NH_3^+$) groups (associated with protein amino acid residues) that reside in epidermal and dermal tissues.[22] The increase in HQ flux at positive i_{app} (Fig. 4) is consistent with the reported cation-selectivity of HMS.

When the direction of the current is reversed, *i.e.,* when i_{app} is negative, the directions of Na^+ migration and electroosmotic flow are also reversed. In this case, electroosmotic flow should oppose the diffusion of HQ through the hair follicle, resulting in a decrease in the total flux of HQ. SECM images recorded at negative i_{app} (top image, Fig. 4) show that the HQ transport rate is indeed reduced when electroosmotic flow opposes the diffusion of HQ.

The direction of electroosmotic flow is determined by the acid-base properties of the epidermal and dermal tissues. The isoelectric point, pI, of skin, *i.e.,* the pH at which skin is electrically neutral, has been measured for bulk samples to be between 4.5 and 4.6.[23] HMS is negatively charged at pHs above the pI and, thus, exhibits cation selectivity at pH = 6.0, as demonstrated by the transport studies described above. At pHs below the pI, skin is expected to have a net positive electrical charge, and should exhibit anion permselectivity. Thus, it should be possible to reverse the direction of electroosmotic flow by lowering the pH of the contacting solutions from a value above the pI to a value below the pI.

We repeated the iontophoretic experiments shown in Fig. 4 at pHs above, below, and near the pI of HMS to determine if the electroosmotic transport could be reversed in the hair follicle by simply adjusting the pH of the contacting solution. The dependence of electroosmotic flow on pH may be quantified using the enhancement factor, *E*, which is defined as the ratio of the fluxes of HQ under iontophoretic (N_{iont}) and diffusive (N_{diff}) conditions, *i.e.,* $E = N_{iont}/N_{diff}$. Figure 5 shows *E* as a function of pH for i_{app} = -50 and 50 µA. Figure 5 demonstrates that *E* is equal to unity at pH ~ 3.5, indicating that electroosmotic flow is not operative near the reported pI of HMS. This finding, in addition to the sigmoidal shape of the *E* vs. pH curves, suggests that the direction

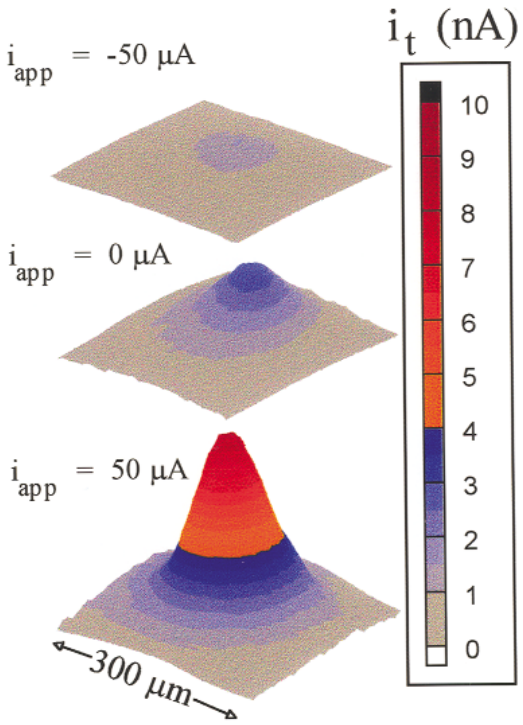

FIG. 4. SECM images of HQ emerging from the opening of a hair follicle as a function of the applied iontophoretic current at pH = 6.0. The center image corresponds to diffusion of HQ from the donor to the receptor solution. The lower image corresponds to positive current (i_{app} = 50 µA), inducing electroosmotic flow in the same direction as diffusion. The upper image corresponds to a negative current (i_{app} = -50 µA), resulting in electroosmotic flow opposing diffusion.[17]

and magnitude of electroosmotic flow in the hair follicle is determined by the acid-base equilibria of the protein amino acid residues located in the epithelial cells comprising the hair follicle structure.

Toward *In Vivo* Imaging of Drug Transport

We are currently developing new SECM methodologies for investigating transdermal transport *in vivo*.[24,25] In this experiment, it is necessary to have both the solute molecule and SECM tip positioned in the solution that is contacting the exterior surface of the membrane or tissue being imaged. We refer to this operation of SECM as reverse imaging mode (RIM)—the tip is scanned on the donor side of the membrane in order to observe molecules entering into the membrane (Fig. 6). In SECM-RIM, the diffusion of molecules into the pore depletes solute in the donor solution immediately adjacent to the pore entrance, resulting in a decrease in the tip current as the tip is rastered across above pore. Analogous to the increase in tip current measured in conventional SECM images (Fig. 4), the decrease in current in RIM signifies a local region in the membrane where the flux is large. In principle, SECM-RIM has an inherently lower sensitivity than conventional imaging, because the signal is measured relative to a large background. However, SECM-RIM provides a means to image molecular transport into biological membranes. This capability is necessary for monitoring the uptake of molecular species into single cells and biological tissues.

We have succeeded in obtaining SECM-RIM images of both synthetic membranes and HMS samples. For example, Fig. 6 shows an SECM-RIM image of a 16 µm radius hair follicle in excised HMS. The image corresponds to the diffusion of acetaminophen into the hair follicle (from a 5 mM solution), demonstrating the first step toward *in vivo* imaging of transdermal transport.[24] A quantitative theory of SECM-RIM imaging has also been recently developed, which allows electroosmotic velocities and molecular fluxes to be quantified directly from the images.[25] ■

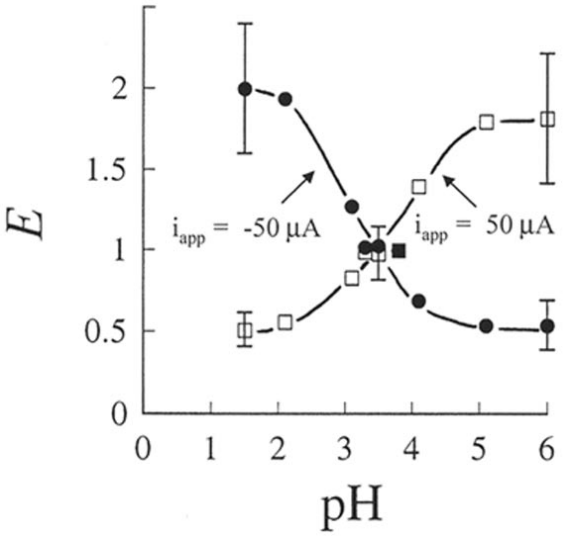

Fig. 5. Flux enhancement factor, E, at positive and negative iontophoretic currents as a function of pH. E is equal to unity at pH ~ 3.5, corresponding to the pH of the epithelial cell layers comprising the hair follicle structure.[17]

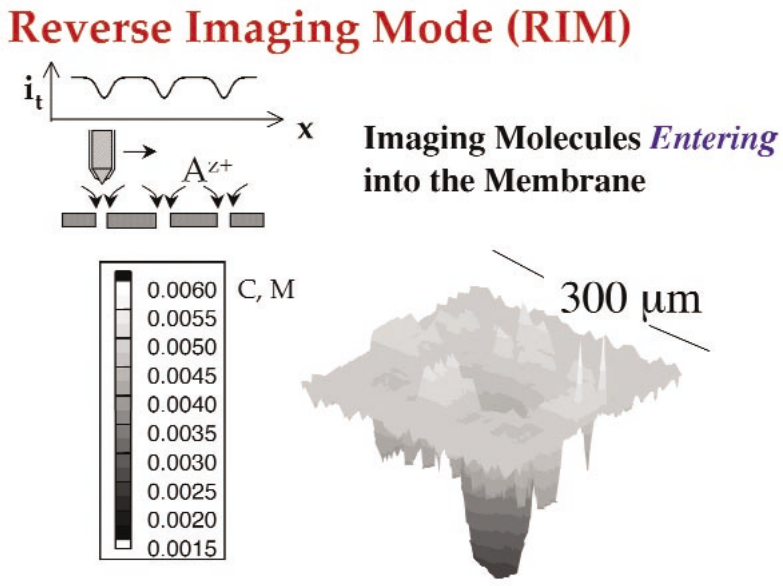

Fig. 6. (Top) Schematic of the reverse-imaging mode (RIM) used to image molecule entering the skin. The arrows represent the direction of transport of an electroactive molecule, Az+, into the skin tissue. Qualitative sketch of the tip current as it is scanned above the pore. (Bottom) SECM image of acetaminophen above a 16.2 mm radius hair follicle in hairless mouse skin. The donor compartment contained 5 mM acetaminophen.[24]

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About the Author

Henry S. White *is a Professor in the Department of Chemistry at the University of Utah. In addition to studies of iontophoresis across skin, his research interests include electroanalytical chemistry, magnetic field-induced transport, oxide films and corrosion, and electrochemical phenomena at electrodes of nanometer dimensions. He received the 2002 Faraday Medal from the Electrochemical Group of the Royal Society of Chemistry (London) and is an Associate Editor of the* Journal of the American Chemical Society. *He may be reached at white@chem. utah.edu.*