Insight into how pharmaceutical solvents diffuse through a human nail

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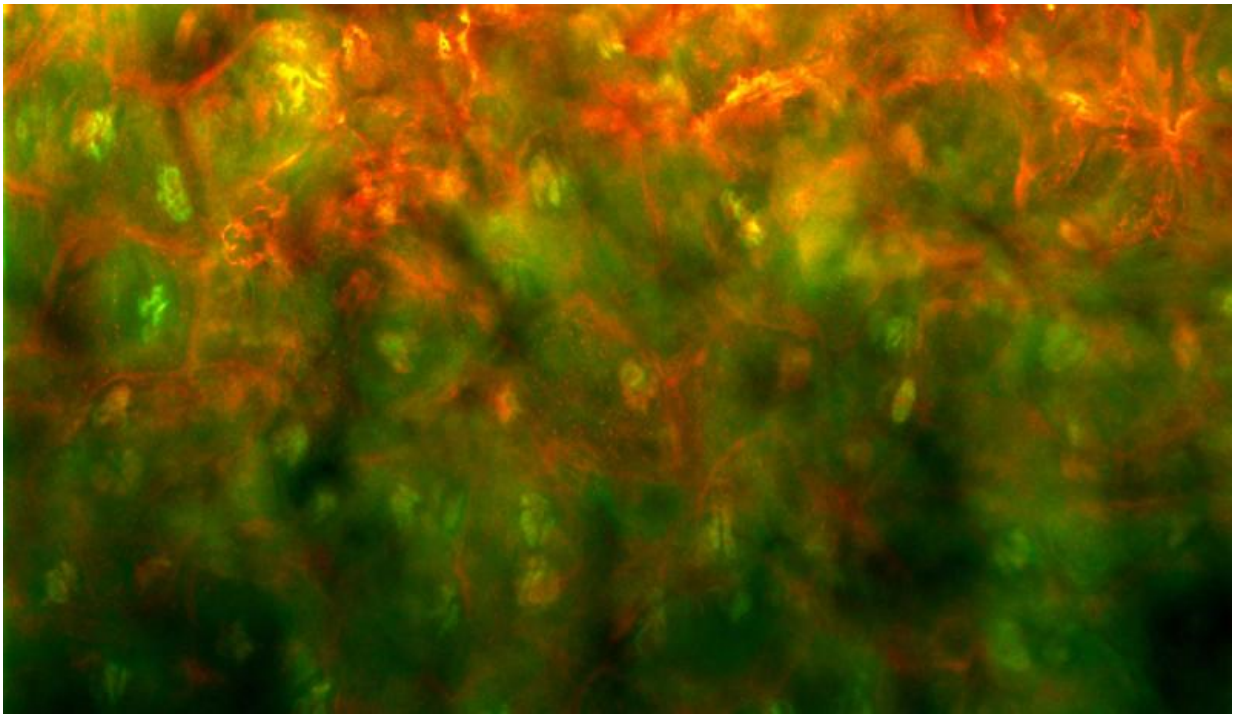

Compact structure of human nail revealed using red and green fluorescent dyes. Credit: Dr. Wing Sin Chiu.

(Phys.org)—One of the biggest difficulties in treating nail disease is finding a topical drug that adequately penetrates through the nail. While some improvements in nail drug delivery have been made, they have been slow-going and still pose difficulties in treatment. A better

understanding of drug delivery and solvent diffusion is needed.

In a recent study, Wing Sin Chiu, Natalie A. Belsey, Natalie L. Garrett, Julian Moger, M. Begoña Delgado-Charro, and Richard H. Guy from the University of Bath and the University of Exeter developed a method to acquire real-time semi-quantitative data on solvent diffusion through a human nail using stimulated Raman scattering microscopy. Their research appears in the *Proceedings of the National Academy of Sciences*.

Human skin, hair, and nails are made of a hard, fibrous protein called keratin. Keratin provides a protective barrier keeping unwanted compounds from easily entering the body. However, keratin's ability to protect the body also makes it an obstacle for topical drug delivery. Up to now, it has only been possible to obtain time- and position-dependent data on the movement of chemicals through the outermost 20μm of the nail. In an effort to better understand the diffusion of key pharmaceutical solvents through a nail, Chiu et al. used stimulated Raman spectroscopy (SRS) to trace the movement of dimethylsulfoxide (DMSO), propylene glycol (PG), and water through several human nail samples.

Stimulated Raman spectroscopy is an imaging technique that was first reported in 2008 for detecting small amounts of chemicals, such as pharmaceuticals or metabolites, within biological systems without having to use fluorescent labels. Since SRS is based on matching laser frequencies to chemical vibrational frequencies, it is able to neglect the environmental background, making it a good technique for non-invasive biomedical studies. Furthermore, SRS scan time is fast enough for real-time measurements.

Keratin has a characteristic $-CH_2$ stretch at 2,855 cm^{-1} that Chiu et al. used to normalize SRS signals from the nail samples. Studies were conducted using deuterated solvents to easily distinguish the solvent

vibrational bands from those of the nail. Concentration is linearly related to SRS signal allowing for a semi-quantitative measurement of solvent diffusion. This technique is "semi-quantitative" because of signal attenuation from light scattering as sample depth increases. In this case, signals from deeper within the nail would underestimate the amount of chemical present because the signal is slightly weakened with increasing depth.

For D_2O, the Raman wavelengths were tuned to the O-D stretching band at 2,500 cm^{-1}. Signals were taken as a function of time (every 2.7 minutes after t=10 minutes). After about thirty-five minutes, D_2O had diffused ~100μm into the nail. The authors report that this is the first time that this kind of rapid, real-time transport of water through a nail has been visualized.

Studies with deuterated DMSO and PG showed that they took much longer to diffuse through the nail. While water took less than an hour to penetrate 100μm, after a day, DMSO and PG only penetrated 40-50μm into the nail.

Data analysis showed that the solvents deviate from classical diffusion behavior as the time of diffusion increases. Quantitative analysis was performed by accounting for several factors, including nail curvature, sample movement, and solvent depletion at the surface. Scans were normalized to keratin's $–CH_2$ frequency and analyses showed no significant solvent depletion at the nail surface. For each of the solvents, as diffusion time increased, the concentration profile deviated from classical behavior (Fick's Second Law). Based on calculations using experimental results, this behavior is characteristic of a time- and concentration-dependent diffusion coefficient. Additionally, the rapid diffusion of water compared to the other two solvents indicates that there is a likely a strong molecular size dependence on diffusion across the nail. Finally, SEM imaging confirmed that the solvents loosen the

nail structure and increase nail roughness, indicating that the nail is weakened when exposed to the solvents for a long period of time, which may lead to increased solvent diffusion over time. Additional studies of the nanostructure of the nail may provide further insight as to why solvent diffusion into a human nail deviates from classical behavior.

This paper reports for the first time real-time solvent behavior as it is absorbed into a human nail. These results elucidate certain factors in drug solvent and nail bed uptake that will help in drug discovery and the development of topical medicines for nail disease.

More information: "Molecular diffusion in the human nail measured by stimulated Raman scattering microscopy" *PNAS*, DOI: 10.1073/pnas.1503791112

Abstract
The effective treatment of diseases of the nail remains an important unmet medical need, primarily because of poor drug delivery. To address this challenge, the diffusion, in real time, of topically applied chemicals into the human nail has been visualized and characterized using stimulated Raman scattering (SRS) microscopy. Deuterated water (D2O), propylene glycol (PG-d8), and dimethyl sulphoxide (DMSO-d6) were separately applied to the dorsal surface of human nail samples. SRS microscopy was used to image D2O, PG-d8/DMSO-d6, and the nail through the O-D, -CD2, and -CH2 bond stretching Raman signals, respectively. Signal intensities obtained were measured as functions of time and of depth into the nail. It was observed that the diffusion of D2O was more than an order of magnitude faster than that of PG-d8 and DMSO-d6. Normalization of the Raman signals, to correct in part for scattering and absorption, permitted semiquantitative analysis of the permeation profiles and strongly suggested that solvent diffusion diverged from classical behavior and that derived diffusivities may be concentration dependent. It appeared that the uptake of solvent

progressively undermined the integrity of the nail. This previously unreported application of SRS has permitted, therefore, direct visualization and semiquantitation of solvent penetration into the human nail. The kinetics of uptake of the three chemicals studied demonstrated that each altered its own diffusion in the nail in an apparently concentration-dependent fashion. The scale of the unexpected behavior observed may prove beneficial in the design and optimization of drug formulations to treat recalcitrant nail disease.