Monitoring the Transdermal Delivery of Fluorescent Nanoparticles Using Multiphoton Fluorescence Microscopy

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Abstract

An interesting area of applying multiphoton fluorescence microscopy is in investigating the delivery of fluorescent nanoparticles (quantum dots) across biological barriers such as the skin. Fluorescence nanoparticles are nanometer in size and understanding the delivery mechanisms of these materials across the skin can be important in understanding the delivery of important biological macromolecules for therapeutic purposes. In addition, pathological diagnosis can be performed with the successful delivery of fluorescent nanoparticles.

Keywords: transdermal delivery, quantum dots, multiphoton microscopy

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1. Introduction

1.1 Multiphoton fluorescence microscopy

Since the introduction of two-photon fluorescence microscopy in 1990, the field of microscopic biological imaging benefited enormously from this powerful technique. Based on the simultaneous absorption of intrinsic or extrinsic fluorescent molecules, the non-linear excitation process provides a number of distinct advantages compared to the conventional one-photon excitation microscopy. To begin with, the high incident photon necessary for sample excitation limits the excitation volume to the focal spot. As a result, confocal-like imaging quality can be achieved without the use of confocal apertures. In addition, the limited focal volume limits sample photobleaching to the focal spot. This feature allows prolonged biological imaging to be achieved. Furthermore, the near-infrared photons used for sample excitation are absorbed and scattered less by tissue components than the one-photon excitation sources. As a result, imaging using multiphoton microscopy can achieve greater penetration depths than one-photon microscopy.^{1,2} In bioimaging applications, multiphoton microscopy has been successfully applied to areas such as neurobiology, developmental biology, and tissue imaging.^{3,4,5} Recently, multiphoton microscopy was used to imaging fluorescent nanoparticles in biological specimens.⁶

1.2 Fluorescent nanoparticles

In recent years, a new class of fluorescent materials based on semiconductor nanotechnology has appeared and has attracted considerable attention. Based on the principles of quantum confinement effect, the nanometer-sized core-shell structure may be constructed to emit tunable fluorescence. A number of distinct advantages are associated with this novel class of fluorescent materials. First, they have broad excitation spectra. A single excitation wavelength in the ultraviolet can excite fluorescence from the differently colored quantum dots, from blue into the red part of the spectrum. Secondly, the fluorescence spectra of the nanoparticles are size-dependent. The emission of smaller quantum dots are blue shifted compared to that of larger quantum dots. Finally, compared to conventional fluorescent molecules, the quantum dots are more photo-resistant. In recent years, a number of interesting applications using quantum dots have appeared and commercial fluorescent nanoparticles with selected surface chemistry are now available.^{6,7,8} An interesting area of bioimaging applications using these nanoparticles is in investigating their delivery properties across biological barriers such as the skin.

2. Experimental Method

The multiphoton fluorescence microscope in our laboratory is similar to systems described elsewhere.² A titanium-sapphire laser (Tsunami, Spectra Physics, Mountain View, CA) pumped by a 10W diode laser (Millennia, Spectra Physics) is used as the excitation source. The output of the titanium-sapphire laser is guided to a microscope system with modified beam path. An x-y scanner system (Cambridge Technology, Cambridge, MA) is used to scan the laser spot across a 2-D section across the sample. Prior to being guided to the microscope objective for focusing, the laser spot is

beam-expanded at the entrance of the microscope to ensure overfilling of the objective's back aperture. This is done to ensure optimal focusing at the multiphoton focal spot. The expanded beam is reflected by a dichroic mirror (Chroma Technology, Brattleboro, VT) into the objective's back aperture. The multiphoton excited fluorescence is collected by the objective, passes through the dichroic and if necessary, additional optical filters prior to reach the photomultiplier tube. In our lab, we use the single-photon mode of detection (R7400P, Hamamatsu, Japan). The basic instrument of our microscope is shown in Fig. 1. The multiphoton microscope described above can be used to image the labeled skin specimens.

Figure 1 Multiphoton microscopic instrumentation

3. Results

The quantum dots can be synthesized and they exhibit the size-dependent spectral emission effects as shown in Fig. 2. The smaller size quantum dots are more blue-shifted in their emission wavelength properties while the larger quantum dots are more red-shifted.

Figure 2 Size-dependent effects on quantum dot emission wavelength

These quantum dots may be used to label skin structures. The transport properties of the quantum dots across the skin may be investigated by placing the fluorescent nanocrystals on the skin surface and their distributions analyzed using multiphoton fluorescence microscopy.

4. Conclusion

Fluorescent nanocrystals or quantum dots represent an exciting development in fluorescent materials. Their fluorescent properties are size-dependent with the smaller quantum dots display blue shifted fluorescence while the larger quantum dots are red shifted in emission. These fluorescent nanocrystals are useful in investigating the transdermal delivery properties of nanometer-sized materials.

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REFERENCES

1. W. Denk, J. H. Strickler, and W. W. Webb, "Two-photon laser scanning fluorescence microscopy," *Science*. **248**, pp. 73-76, 1990.
2. P. T. C. So, C. Y. Dong, CY, B. R. Masters, and K. M. Berland, "Two-photon excitation fluorescence microscopy," in *Annual Review of Biomedical Engineering*, volume 2, pp. 399-429. Annual Reviews, Palo, Alto, 2000.
3. J. M. Squirrell, D. L. Wokosin, J. G. White, and B. D. Bavister, "Long-term two-photon fluorescence imaging of mammalian embryos without compromising viability," *Nat. Biotech.* **17**, pp. 763-767, 1999.
4. K. Svoboda, K, W. Denk, D. Kleinfeld, and D. W. Tank, "In vivo dendritic calcium dynamics in neocortical pyramidal neurons," *Nature*. **385**, pp. 161-165, 1997.

5. P. T. C. So, H. Kim, and I. E. Kochevar, "Two-photon deep tissue ex vivo imaging of mouse dermal and subcutaneous structures," *Opt. Exp.* 3:339-350, 1998.
6. P. R. Larson, W. R. Zipfel, R. M. Williams, S. W. Clark, M. P. Bruchez, F. W. Wise, and W. Webb, "Water-soluble quantum dots for multiphoton fluorescence imaging in vivo," *Science* 300: 1434-1436, 2003.
7. W. C. W. Chan and S. Nie, "Quantum dot bioconjugate for ultrasensitive nonisotropic detection," *Science* 281: 2016-2018, 1998.
8. M. Bruchez Jr., M. Moronne, P. Gin, S. Weiss, A. P. Alivisatos, "Semiconductor nanocrystals as fluorescent biological labels," *Science* 281: 2013-2016, 1998.