

***In vivo* visualization of collagen transdermal absorption by a combined second-harmonic generation and two-photon excited fluorescence method**

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ABSTRACT: The transdermal administration of collagen is an important method used for wound healing and skin regeneration. However, due to the limitations of previous approaches, the process and degree of collagen transdermal absorption could only be quantitatively and qualitatively assessed *in vitro*. In the present study, we introduced a novel approach combining second-harmonic generation with two-photon excited fluorescence to visualize the dynamics of collagen transdermal absorption *in vivo*. The high resolution images showed that the exogenous recombinant human collagen permeated through the epidermis, reached the dermis and formed reticular structures in real time. We also validated these findings through traditional *in vitro* skin scanning and histological examination. Thus, our approach provides a reliable method of measurement for the real-time evaluation of collagen absorption and treatment effects.

Tracking the transdermal absorption of iFluor-R-hc using SHG-TPEF imaging *in vivo*

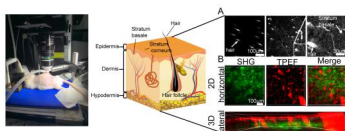


Figure 1

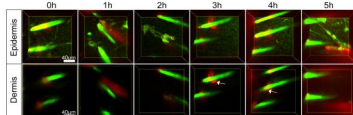


Figure 3

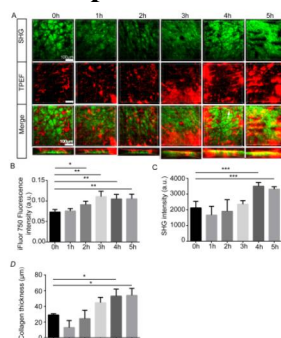


Figure 2

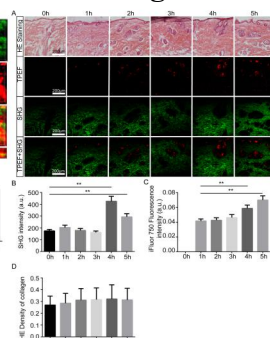


Figure 4

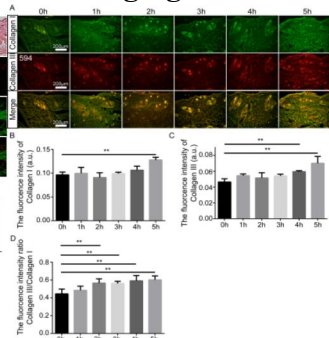


Figure 5

Figure 1: Schematic illustration of skin imaging. Left panel: *in vivo* SHG-TPEF

Figure 2: High-resolution images illustrated the process of iFluor-R-hc absorption by *in vivo* SHG-TPEF imaging.

Figure 3: SHG-TPEF imaging of iFluor-R-hc around hair follicle. Green: hair shaft and stratum spinosum/stratum basale ($\lambda=750\text{nm}$); Red: iFluor-R-hc ($\lambda=790\text{nm}$); White arrow: iFluor-R-hc in hair follicle.

Figure 4: Histological examination of skin after iFluor-R-hc administration.

Figure 5: Quantification of collagen

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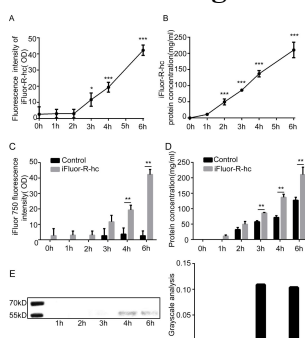


Figure 6: *In vitro* examination of iFluor-R-hc Transdermal absorption.

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