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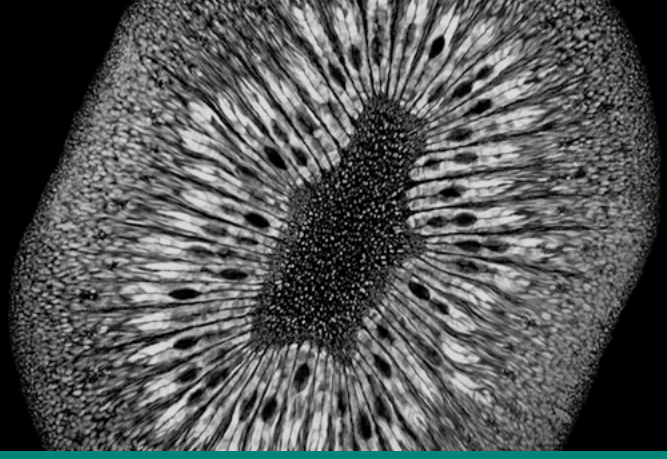
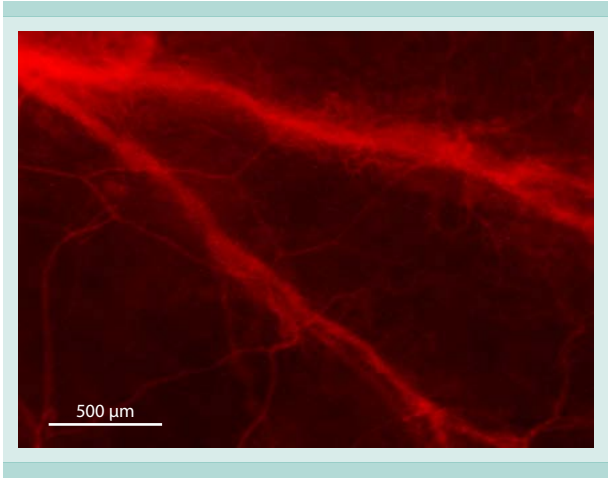


Fluorescent blood pool imaging agent



Optical imaging of vascular morphology and integrity using NiraWave™ M

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Introduction

Structural and functional changes of blood vessels have been associated with various pathological conditions such as chronic inflammation and cancer[1]. Since the ability to visualize such changes potentially allows for early detection of disease as well as monitoring of therapeutic interventions, a strong focus is currently being placed on the development of non-invasive diagnostic methods that enable blood vessel visualization.

Due to its multiple advantages including its simplicity, high sensitivity and low-cost, optical imaging is a rapidly emerging field with widespread applications[2]. In this study we use optical imaging and test the feasibility of NiraWave™ M, an innovative indocyanine green (ICG)-based imaging agent, to non-invasively visualize vascular architecture in mouse skin. Besides imaging of healthy murine vessels, we use a mouse model of cutaneous contact hypersensitivity (CHS) to test whether the employed optical imaging method allows detection of pathological alterations of blood vessels in skin associated with chronic inflammation.

Materials and methods

To determine the blood half-life of NiraWave™ M (Viscover™, *nanoPET Pharma* GmbH, Berlin, Germany), in comparison to that of the conventional optical imaging agent ICG (ICG-PULSION, PULSION Medical Systems AG, Munich, Germany), female nude mice (n = 4 per group) were anaesthetized and injected with either imaging agent at a dose of 0.4 mg ICG/kg body weight. *In vivo* intravital microscopy was performed on the animals using an AxioImager Z1 fluorescence microscope (Carl Zeiss, Jena, Germany), equipped with a 100 W halogen lamp and a 2.5x EC Plan-Neofluar objective. Mice were positioned under the microscope and the ear was fixed under the objective using adhesive tape[3]. The time-dependent fluorescence of the blood in the vessels of the skin was monitored for a period of 90 min after imaging agent injection using a near-infrared (NIR) filter set (670-750 nm band-pass excitation filter and 780-850 nm band-pass emission filter). Fluorescence microscopy images were obtained every 2 s for the first 5 min and every minute for the following 85 min using an AxioCam MRm CCD camera and its AxioVision software (Carl Zeiss, Jena, Germany). For each image, regions of interest (ROI) were placed in the blood vessels as well as in the surrounding tissue (for image intensity normalization) and the time-dependent fluorescence intensities were evaluated using SimplePCI software (Compix Inc. Imaging Systems, Hamamatsu Photonics, Japan).

To detect vascular changes associated with inflammation, experiments were performed on a mouse model of cutaneous contact hypersensitivity (CHS), where skin inflammation is induced by exposing the skin to a dermal

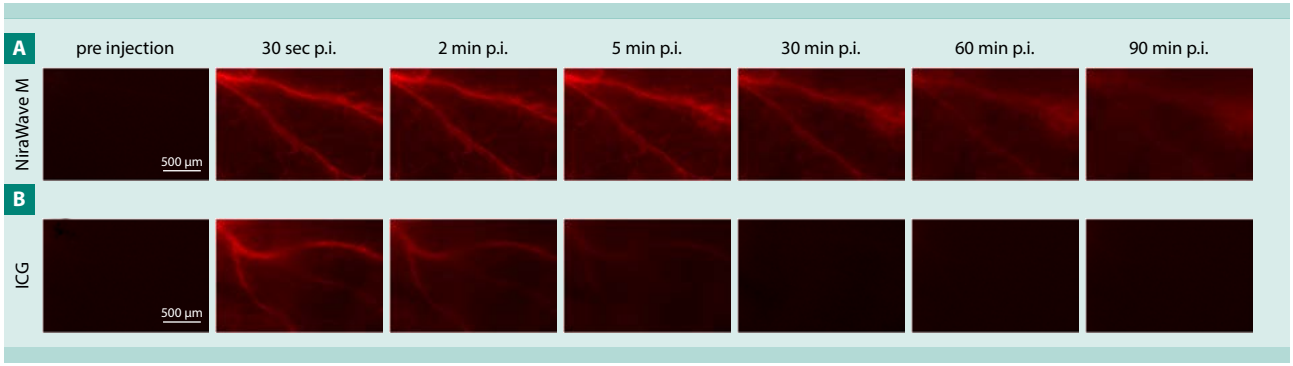

Figure 1: Fluorescence microscopy images of blood vessels in the murine ear obtained before and after injection of NiraWave M and ICG.
Images at various timepoints after injection of **(A)** NiraWave M and **(B)** conventional ICG show that NiraWave M provides a significantly higher fluorescence signal that persists for a longer period of time.

sensitizer. Briefly, the mice (n = 3) were first sensitized to 2,4,6-trinitrochlorobenzene (TNCB) and, subsequently, repeatedly challenged with TNCB on the right ear to elicit chronic skin inflammation while the left untreated ear acted as control[4]. 12 h after completion of the TNCB challenge, the animals were injected with NiraWave M at a dose of 0.4 mg ICG/kg body weight. Optical imaging was performed at 10 min post injection using an Aequoria Darkbox and a C4880 Dual Mode cooled CCD camera (Hamamatsu Photonics Deutschland GmbH, Herrsching, Germany) equipped with a NIR filter set (680 nm excitation filter and 700 nm emission filter). To locate the ears, a bright-field image without the emission filter was recorded. Image analysis was performed using Wasabi Imaging Software (Hamamatsu Photonics Deutschland GmbH, Herrsching, Germany).

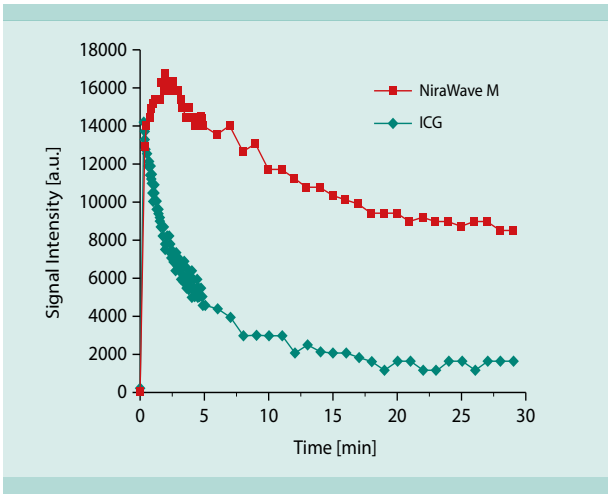

Figure 2: Time course of the fluorescence intensity of blood vessels in the murine ear over the first 30 min after injection of NiraWave M compared to ICG.

Results and discussion

In order to visualize the signal-enhancement properties of NiraWave M *in vivo*, as compared with the conventional agent ICG, healthy mice underwent intravital fluorescence microscopy following injection of either agent. The fluorescence microscopy images clearly show that, after administration of NiraWave M, the structure of blood vessels can be visualized with unprecedented clarity over a much longer time period (Fig. 1). During the first 5 min post injection of NiraWave M, the high fluorescent signal in the blood persists throughout, but evidently declines in the blood vessels of control mice injected with conventional ICG. Indeed, at 30 min post injection of NiraWave M, the blood vessels are still noticeably visible whereas in the case of ICG, the vessels are barely distinguishable at this timepoint.

In order to estimate the pharmacokinetics of both agents, the fluorescence intensity of the blood was measured over an extended time period of 90 min. Following injection of ICG, the fluorescence intensity of the blood rapidly decreases due to fast hepatic elimination (Fig. 2). The blood half-life of ICG, determined from a bi-exponential fit of the time curve, was found to be ~4 min, in accordance with literature values[5]. In comparison, NiraWave M remains within the vessels for a prolonged period of time (Fig. 2). Due to its complex pharmacokinetics, a standard mono- or bi-exponential fit could not be applied to the curve. Subsequently the blood half-life was estimated graphically and found to be ~1 h[3].

Since NiraWave M was found to enable efficient imaging of the blood in healthy vessels, the study was extended with the aim of evaluating the agent's suitability for detecting vascular changes associated with inflammation. To this end, TNCB-induced CHS mice were imaged 10 min after injection of NiraWave M. The optical images revealed strongly enhanced fluorescence signals in the right inflamed ear tissue compared to the left untreated (control) ear and most certainly pertain to elevated perfusion and vascular leakage in areas of inflammation (Fig. 3)[6].

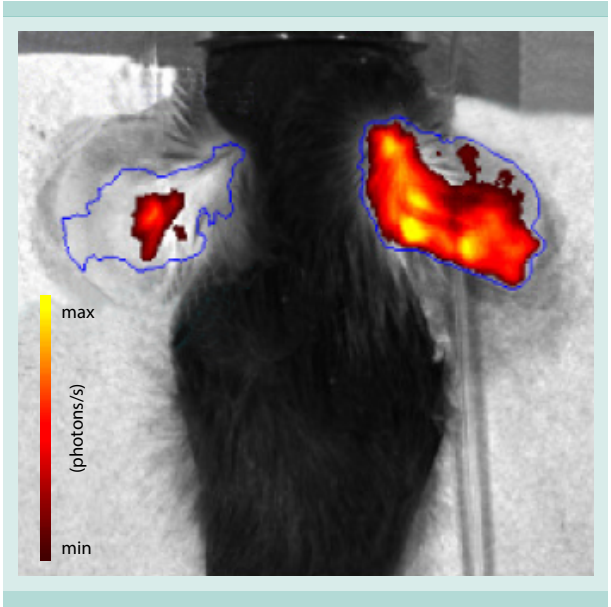

Figure 3: Optical image of a TNCB-induced CHS mouse 10 min after injection of NiraWave M.
The image reveals an enhanced fluorescence signal in the right inflamed ear tissue compared to the left control ear due to elevated perfusion and vascular leakage.

Conclusion

Due to its strong fluorescence intensity and prolonged blood half-life, NiraWave M enables improved visualization of blood vessel morphology as well as detection of increased vascular permeability. The innovative imaging agent thereby serves as a diagnostic tool for identification of pathologies with vascular involvement as well as for monitoring of therapeutic interventions.

Viscover™ Product	Order No.
NiraWave™ C, 1 x 5 injections	130-095-154
NiraWave™ C, 5 x 5 injections	130-095-155
NiraWave™ M, 1 x 5 injections	130-095-156
NiraWave™ M, 5 x 5 injections	130-095-157
NiraWave™ Rocker, 1 x 5 injections	130-095-158
NiraWave™ Rocker, 5 x 5 injections	130-095-159
NiraWave™ nano 780, 1 x 5 injections	130-095-695
NiraWave™ nano 780, 5 x 5 injections	130-095-693

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