Editorial

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Optical biopsy: a promising approach for real-time liver steatosis grading

Optical biopsy is a diagnostic approach exploiting the photophysical phenomena arising from the interaction of ultraviolet-visible-near infrared radiations with matter to provide real-time information on the morpho-functional properties of a biological substrate, in the absence of sample removal.

Among the photophysical phenomena, considerable attention is paid to autofluorescence or light-induced fluorescence, which is the fluorescence arising from a biological substrate upon excitation at a suitable wavelength, in the absence of exogenous markers, because of the presence of endogenous biomolecules acting as fluorophores. The endogenous fluorophores may be involved both in cell metabolic processes (for instance, pyridine coenzymes, flavins and lipofuscins) and in tissue histological organization (for instance, collagen and elastin). Because emission properties – such as amplitude and spectral shape – depend on the nature, amount, physicochemical state, intratissue distribution and microenvironment of fluorescing molecules, in close relationship with the morphological and metabolic conditions of the biological substrate, autofluorescence represents an intrinsic tissue diagnostic parameter (1, 2). The occurrence of either physiological changes or pathological conditions gives rise to alterations of the tissue morpho-functional state, resulting in changes of autofluorescence emission properties suitable for diagnostic purposes, provided that both endogenous fluorophores' photophysical characteristics and tissue optical properties are defined.

At least two major advantages emerge in the characterization of biological substrates through autofluorescence analysis: the widespread occurrence of endogenous fluorophores, allowing an extension of its application to various bio-medical fields, and the technological progress as to excitation sources, light delivery and detection system of fluorescence signals, allowing the high sensitivity of the fluorometric techniques to be fully exploited.

In bulk tissues, autofluorescence analysis is widely considered for *in vivo* diagnostic purposes for both the detection of pathologies and the monitoring of organ functionality under normal, physiologically or purposely altered conditions (3).

As to pathologies, optical biopsy diagnostic applications concern mainly the neoplastic growth [as reviewed by references (4, 5)]. The occurrence of a neoplasia alters the autofluorescence properties, acting on multiple factors, such as biochemical composition, histological organization and optical properties of the tissue. The optical properties, in particular, can affect the propagation of both excitation and emission light, thus influencing the amplitude and the spectral shape of the signal collected by the measuring probe, depending on the presence of non-fluorescent absorbers and scatterers within the tissue (6).

As to the monitoring of organ functionality, works have been performed to assess the response to ischaemic conditions and the subsequent recovery ability. A device has been developed, for example, that combines the collection of the autofluorescence amplitude signal attributable to NAD(P)H as a marker of the mitochondrial redox state, with the monitoring of both microcirculatory blood flow and blood volume, to respond to the necessity of a real-time survey of organs' vitality in clinics (7). The organ transplantation practice is a promising field of application (8, 9). In the case of the liver, to date, studies have mainly focused on the optimization of the preservation procedures and the prediction of organ viability, through a selective analysis of NAD(P)H and flavin autofluorescence as a probe of the energetic metabolism (10–13).

The variety of additional endogenous fluorophores occurring in the liver depending on the multiple metabolic pathways carried out by the organ (such as fatty acids, lipopigments, vitamins and biliary salt derivatives) provides interesting perspectives for an extension of optical biopsy to the diagnosis of diseased conditions. A marked contribution is made by vitamin A to the liver overall emission because of both its relatively high quantum efficiency and its noticeable amount. When undesired – such as in the case of energetic metabolic studies based on NAD(P)H emission analysis – vitamin A contribution can be removed by a preliminary sample irradiation, exploiting its strong photolability.

Among the altered morpho-functional states of liver, steatosis currently deserves particular attention because of its many implications for the outcome of liver transplantation. The efficacy of this therapeutic approach to end-stage hepatic insufficiency has led to a continuously increasing demand for organs. At present, steatosis occurs in about one-third of the livers becoming available for grafting. Organs with mild-moderate steatosis belong to the category of marginal livers, currently accepted despite the direct relationship between the presence of lipids and the enhancement of the risk of ischaemia reperfusion injuries, the cause of severe graft dysfunction (14). Currently, a precise diagnosis of steatosis requires a tissue histological examination, which provides an exact estimation of the lipid accumulation degree of the donor liver, but is time consuming and is generally available after several hours of cold ischaemia (15). Optical biopsy, in principle, can represent a good approach to overcome this limitation, through real-time detection and grading of steatosis in a donor liver. In fact, the accumulation of lipid droplets in hepatocytes' cytoplasm is expected to modify the photophysical properties of liver tissue involving both its biochemical composition and its structural organization. The alteration of biochemical composition will affect the contribution of the endogenous fluorophores, influencing the autofluorescence spectral profile and signal amplitude; the alteration of the histological organization will affect the optical properties and therefore the light migration within tissue.

In spite of this premise, only very recently optical biopsy has been considered as a suitable approach for the diagnosis of steatosis. In this view, the work by de Oliveira *et al.* (16) in this issue of *Liver International* is particularly appreciable. It refers mainly to the effect induced on the light migration in liver

tissue by the presence of lipid droplets, with particular attention to the back-scatter signal. It is known that a difference of the refractive index at the interface between lipid droplets and the surrounding medium affects light propagation inside the tissue, resulting in an increase in the back-scatter signal at the front of the measuring probe. De Oliveira and colleagues, taking advantage of an animal model consisting of rats exhibiting lipid accumulation to a different extent, found that there is a diagnostic factor (steatosis fluorescence factor, SFF) that varies linearly with steatosis, classified as absent, mild, moderate and severe: in particular, the higher the fat concentration, the higher the factor. SFF is defined as the ratio between the back-scatter amplitude at the excitation wavelength and the maximum fluorescence amplitude, for each normalized spectrum. Measurements were performed by means of an optic-fibre spectroscope using a Nd^{3+}:YAG laser (532 nm) as an excitation source. At the excitation wavelength used, however, most of the endogenous fluorophores involved both in metabolic activity and in tissue structural organization are not fluorescent or give rise to a weak fluorescence signal. In a clinic pilot study, Castro e Silva *et al*. (17), using the same experimental apparatus, observed a variation of the autofluorescence signal amplitude at wavelengths longer than 550 nm during the phases of liver transplantation, that is, before and during cold ischaemia, at the back table and after reperfusion. This variation was related to the metabolic condition of the liver graft through its course, although whether the autofluorescence signal can be attributed to a specific endogenous fluorophore remains to be investigated. In spite of this blind spot, the work by de Oliveira and colleagues can be considered an important effort to set up an effective approach for the real-time diagnosis of steatosis, combining data related to both histological (back-scatter signal) and biochemical (autofluorescence) tissue aspects. It is our opinion that an optimization of autofluorescence measurement conditions – mainly as to the involvement of specific fluorophores – can provide additional parameters leading to an enhancement of the efficacy of this diagnostic proposal. Studies performed by our group on an animal model evidenced a relationship between the occurrence of fat and both spectral shape and signal amplitude of the autofluorescence under excitation at 366 nm (18). These evidences are ascribed mainly to vitamin A (emission peak at 488 nm), which was found to be directly related to lipids in terms of amount and distribution. In particular, the strong photolability of vitamin A results in marked changes of the signal amplitude upon irradiation, which can be exploited to detect small differences of fat amount even $< 10\%$. A relationship between the photofading rate of autofluorescence and the fat amount in liver tissue was already observed in human biopsy samples (19).

In conclusion, lipid accumulation induces alterations of the tissue photophysical properties that can be evaluated by means of back-scatter and autofluorescence signal measurements. This provides the basis for the development of an optical biopsy approach for a non-invasive, real-time diagnosis of the degrees of steatosis, which can be a promising powerful alternative to conventional diagnostic methods in surgery and transplantation.

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