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Lanthanide light for biology and medical diagnosis

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ABSTRACT

Optical imaging emerges as a vital component of the various techniques needed to meet the stringent requirements of modern bioanalysis and bioimaging. Lanthanide luminescent bioprobes (LLBs) have greatly contributed to this field during the past 35 years because they have definite advantages such as little or no photobleaching and, thanks to time-gated detection, high sensitivity. The review summarizes the numerous tools offered by LLBs under their various forms, coordination compounds, nanoparticles, upconverting nanoparticles and their bioconjugates. It then focuses on biosensing, including point-of-care analysis, and then on both *in vitro* and *in vivo* bioimaging with visible and NIR light. The last section compares the performances of LLBs *versus* those of other commonly used bioprobes (organic dyes, quantum dots, and transition metal complexes). It is concluded that although LLBs will not replace all of existing bioprobes, they add invaluable new specific technologies to the biologist and medical doctor toolboxes. A good deal of improvements are achieved through nanotechnologies, which demonstrates that progresses in biosciences depend on the intersection of different disciplines, photophysics, chemistry, biochemistry, nanotechnology, and materials science.

1. Introduction

Understanding the structure and functional properties of cells, organs, and living organisms represents a key challenge in modern biology and medicine that inspires the development of smart analytical and imaging techniques. The present trend in medical diagnosis is to avoid lengthy procedures – in which fluid samples (blood and urine) are sent to an outsourcing laboratory for investigation – by implementing point-of-care (or bed-side) analyses. The latter are simple tests on urine, blood, saliva, or feces that can be conducted at the medical practice, by the patient itself, or by a police officer; examples are pregnancy and blood glucose tests or drug-of-abuse (including alcohol) screening. Imaging is equally central in therapy, namely for diagnosis and assessment of the treatment efficiency. The development of diagnostic imaging techniques such as positron emission tomography (PET), X-ray computed tomography (CT, or computed axial tomography, CAT), X-ray angiography, magnetic resonance imaging (MRI), ultrasound imaging (USI), or optical medical imaging (OMI) and their associated contrast/enhancing agents has had an impressive impact on the advancement of medicine during the past 30 years, particularly in the case of tumor detection and cure. Nevertheless, substantial improvements are still needed with respect to sensitivity, spatial resolution, penetration depth, and specificity.

Optical imaging, generally luminescence imaging, emerges as a vital component of the various techniques needed to meet the stringent requirements of modern medicine and biology [1]. Indeed, light can easily reach regions of chemical or biological edifices not accessible to molecular messengers. Additionally, light carries a substantial amount of energy that can be transmitted to the surroundings, inducing specific triggering or switching effects. Moreover, depending on its wavelength, light can penetrate deeply within biological tissues and its detection is highly sensitive since single-photon detection is achievable. Another advantage is that photons interact with electrons at the molecular level and detection of molecular interactions, *e.g.* DNA hybridization, is feasible with techniques such as Förster resonant energy transfer (FRET). Besides, depending on the lifetime of the excited state generating light emission, time-domain information can be gained thanks to time-gated detection. Finally, since quantum yields are temperature dependent, recording of luminescence intensity variations within cells or tissues results in thermal imaging. Owing to the large sensitivity of optical probes, they are usually provided as labels linked to antibodies or other relevant biomolecules, ensuring minimum invasion with high selectivity. Several classes of luminescent probes are available:

(i) Organic dyes are well documented. They are highly emissive and single-molecule detection is at hand [2]. Easy derivatization

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makes them specific, *e.g.* cell penetrating agents localizing in given organelles or bioconjugated chromophores for antigen detection. With respect to imaging, resolution down to 15–20 nm is obtained in the focal plane thanks to special techniques such as stimulated emission depletion microscopy [3]. The organic probes are fluorescent molecules, which translates into very short excited state lifetimes, on the order of nanoseconds or shorter. Time-resolved detection (TRD) that considerably enhances signal-to-noise ratio by eclipsing autofluorescence from endogenous biomolecules therefore necessitates sophisticated and expensive experimental setups. Moreover, organic chromophores are commonly subject to intense photobleaching and some of them are only operative during a few seconds, which is a rather short time for collecting valuable information.

(ii) A second class of luminescent bioprobes entails semi-conductor quantum dots (QDs) and their bioconjugates. They consist in nanocrystals with 2–10 nm diameters and made up of groups 12–15 elements (*e.g.* ZnS, CdS, CdSe, PbSe, InAs, or InP). When the size of the nanoparticles becomes smaller than a critical value known as the exciton Bohr radius (typically 10 nm), 3D-confinement of charge carriers occurs, limiting the number of energy states in the valence and conduction bands and resulting in optical properties that can be tuned by varying the particle size or the internal chemical composition. Quantum dots are highly luminescent with fairly sharp emission bands covering the visible [4] and NIR [5] spectral ranges: fwhh ~20–100 nm in the range 400–1000 nm [6] and ~100–200 nm in the range 1–4 μm [7]. Their photostability is better than that of organic chromophores. On the other hand, they suffer from flaws such as (i) flickering of the emission when there are only a small number of QDs in the target material, and (ii) concern about their long-term toxicity, for instance through the release of toxic cadmium, arsenide or selenide ions [8].

(iii) In view of the possible toxicity of QDs, analytical chemists have been investigating the potentiality of carbon nanodots (CND or C-dots, [9]) and nanotubes (CNT, [10]). The field is fairly new, C-dots having been discovered in 2004 and quantitative and comparative data are still scarce, but the carbon particles appear to be biocompatible, non-toxic, easily derivatizable, and their fluorescence properties can be tuned by modifying their size and/or surface. Moreover the 2-photon cross section of C-dots is quite large so that they can be conveniently excited at 800 nm, opening the way to their use in drug release and photodynamic treatment (PDT) of cancer [11], as well as in cell imaging [12]. Carbon nanoparticles can also be involved in FRET processes and are able to sensitize the luminescence of Ln^{III} ions [13].

(iv) Transition metal complexes. A large number of d-block luminophores have been used in cell imaging, mainly d^6 complexes (Re^{I}, Ru^{II}, Ir^{III}), as well as Pt^{II} and Au^{I} compounds [14]. Broad luminescence bands mostly arise from metal-to-ligand charge-transfer (^{3}MLCT) or metal-to-metal charge-transfer (^{3}MMCT) states. Initial excitation occurs on ^{1}MLCT or ^{1}MMCT states often mixed with intra-ligand (IL) charge-transfer (CT) states. Emission from d-states is often less useful in view of its weak intensity compared to CT states. The energy of the CT states heavily depends on the chemical environment of the metal ions and the nature of the ligand–cation bonding, resulting in emission wavelengths varying considerably from one system to the other. In addition, overlap with ligand emission bands is not unusual so that this luminescence is rarely relied upon in bioanalysis. Finally, some photobleaching may occur.

(v) In many specific cases pertaining to both bioanalysis and bioimaging, materials based on trivalent lanthanide ions, Ln^{III}, represent valuable substitutes to the previously mentioned luminophores. Their remarkable optical properties enable easy spectral and time discrimination of their emission bands that extend from UV through NIR spectral ranges depending on the ion [15]. Another noteworthy and decisive advantage of lanthanide complexes is their low propensity to photobleaching: inorganic materials are usually not at all prone to photobleaching and for complexes with organic ligands luminescence is sensitized via the ligands with energy transfer from the chromophore to the Ln^{III} ion being fast enough to avoid substantial photodegradation of the ligand. The use of lanthanide luminescence for bioanalyses started at the end of the 1970s with the aim of replacing highly sensitive radio-analyses and is now ubiquitous for a wealth of practical immunoassays. In these assays, lanthanide polyaminocarboxylates or macrocyclic complexes are conjugated to specific antibodies and luminescence from the emitting ion is detected after the biochemical reaction is completed, either in a two-step procedure (heterogeneous assays or dissociation-enhanced lanthanide fluoroimmunoassays DELFIA®) or with a one-step, FRET-based protocol (homogeneous assays) [16]. As the usefulness of lanthanide luminescent bioprobes and bioconjugates (LLBs) for immunoanalyses unfolded, attempts to apply them to imaging purposes were a logical extension [16,17]. First experiments were simply taking advantage of continuous-mode lanthanide emission but since lanthanide complexes and/or their bioconjugates are cell permeable, luminescence microscopy and more recently time-resolved luminescence microscopy (TRLM) images started to be performed with the help of LLBs. The availability of nanoparticles doped with luminescent lanthanide ions or complexes on one hand and of up-converting nanoparticles (UCNPs) on the other hand is presently adding new dimensions to this area of research by expanding it to NIR–NIR imaging, drug delivery and photodynamic therapy of cancer.

It is noteworthy that sophisticated multiplex analyses or imaging experiments may require combining two classes of luminophores, *e.g.* in some FRET experiments, QDs can function as donors [18] or acceptors [19] for LLBs In this review, we attempt to give a broad overview of the applications of lanthanide optical probes in bioanalysis and bioimaging. Main current applications of lanthanides in biology and medicine are in magnetic resonance imaging (MRI), as contrast agents (almost exclusively Gd^{III} complexes), and luminescent assays (immunoassays, protein staining), with bioimaging and drug delivery starting to gain momentum. In addition, lanthanide ions are used in radioactive treatment of cancer and they are known to have anticoagulant and antimicrobial properties, but the corresponding uses remain minor. The importance and emergence of lanthanide-based optical probes is reflected in the literature that grows at an exponential pace. As a rough measure, consider reviews, book chapters, or whole books devoted to lanthanide bioprobes. Until 2005, about 5 reviews per year were appearing in chemistry, biosciences and medicine journals, 30% of them devoted to optical probes. In the time span 2006–2008 these numbers grew to 10 per year and 40%. Since 2009, about 30 reviews are published every year including in materials journals, 70% of them devoted to luminescent bioprobes! Due to space constraint, the present review is not comprehensive but, rather, problem-oriented and, furthermore, only very selective literature is cited, mainly review or perspective articles as well as recent original contributions.

2. Lanthanide luminescent probes: the toolkit

Following the initial uses of diketonate and polyaminocarboxylates complexes for DELFIA® analyses, a wealth of lanthanide materials have been developed for bio-application purposes. In the follow-