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Review

Nanomaterial-based fluorescent probes for live-cell imaging

Qing Li, Lin Liu, Jin-Wen Liu, Jian-Hui Jiang *, Ru-Qin Yu, Xia Chu *

State Key Laboratory of Chemo/BioSensing and Chemometrics, College of Chemistry and Chemical Engineering, Hunan University, Changsha 410082, PR China

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ABSTRACT

Nanomaterial-based fluorescent probes represent a significant approach to intracellular detection with high spatiotemporal resolution. We review the properties of various nanomaterials that can be used for intracellular nanosensors in terms of the sensor design and the approaches to delivery of nanosensors based on engineering their surfaces. We also review general strategies for these nanosensors based on the transduction mechanisms of the fluorescence signal.

Contents

1. Introduction

Visualization and monitoring molecular and physical events in live cells represent a key approach to understanding cell biology and have a profound influence on progress in biomedical sciences. There is a constant need for bioanalytical and biomedical sensors that can achieve quantitative, selective signals for biological molecules or physical variables in live cells with high spatiotemporal resolution. To this end, fluorescent spectroscopic techniques offer sensing for intracellular signaling and intracellular analysis due to their non-invasiveness and high sensitivity. A number of organic dyes and fluorescent proteins have been developed as powerful molecular probes. However, these molecular probes may have limitations in making reliable intracellular measurements. Their poor photobleaching resistance may limit their use in long-term tracking in many applications. Their broad emission spectra and largely-shifted excitation bands may hinder practical application for multiplexing of different fluorophores with the requirement for multiple excitation wavelengths [1]. Also, their possible chemical

* Corresponding authors. Tel./Fax: +86 731 88664085 (J.-H. Jiang); Tel./Fax: +86 731 88821916 (X. Chu).
E-mail addresses: jianhuijiang@hnu.edu.cn (J.-H. Jiang), xiachu@hnu.edu.cn (X. Chu).

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interactions or steric hindrance with biomolecules may cause biotoxicity or perturbation to the systems being investigated [2].

The development of nanotechnology has fueled the possibility of designing fluorescent nanomaterials for intracellular sensors. Nanomaterial-based fluorescent probes have certain superior performance that makes them a promising alternative to organic dyes or fluorescent proteins for intracellular sensors. These nanomaterials have dimensions typically 1–200 nm. Larger particles generally have much lower efficiency of uptake by the live cells and may be quickly cleared from circulation by macrophages and eventually destroyed in animal experiments [3]. Some nanomaterials show tunable, narrow-absorption and luminescence peaks, with size-controlled synthesis generating diverse optical properties [4], and some display electron-accepting or energy-accepting properties that can act as signal-transduction components [5]. On the other hand, organic dyes can also be encapsulated with a large amount of nanomaterials to overcome optical instability and bio- toxicity.

Besides the superior optical properties, including strong emission, high photostability and improved multiplexing capability, the nanomaterials can also be modified by flexible surface chemistry to give excellent biocompatibility. Furthermore, surface functionalization is able to increase selective delivery of the nanosensors to specific cells or even to subcellular organelles to realize effective intracellular imaging. Typically, the construction of nanomaterial-based fluorescent biosensors starts with the synthesis of the nanomaterials in one of three ways – top down, bottom up and a combination of top down and bottom up [6] – followed by designed surface-chemistry modifications. The precisely-controlled preparation of size, shape, chemical composition, crystal structure, and surface chemistry of the nanomaterial-based biosensors is a key step to obtaining unique properties and high performance in intracellular imaging applications.

In this article, we first discuss the properties of various nanomaterials and classify them based on their applications in fluorescent live-cell imaging. Second, we outline the existing intracellular delivery strategies and the mechanisms of transport into cells or organelles. Finally, we focus discussion on the design of nanomaterial-based biosensors for fluorescent live-cell imaging and overview how they are used for measurement of different intracellular analytes. In this context, this review may provide new directions for future development and some perspective to the intracellular sensing field.

2. Nanomaterials used in intracellular fluorescent biosensors

A wide variety of nanomaterials have been used for the construction of fluorescent biosensor platforms for live-cell imaging, which can be classified into three types:

- Type 1 includes nanomaterials that possess intrinsic fluorescent properties and can serve as fluorescence reporters for live-cell imaging;
- Type 2 comprises intrinsically fluorescence-quenching nanomaterials that can be designed as quenchers in imaging applications; and,
- Type 3 are non-fluorescent, non-quenching nanomaterials that serve as a matrix for immobilizing fluorescent probes on the surface or encapsulating fluorescent dye in their interior.

It is important to note that this classification is applied to the elementary nanomaterials only. In biosensor development, two or more nanomaterials of these three types can be combined into nanocomposites to enhance the performance for live-cell imaging.

2.1. Type 1 nanomaterials with intrinsic fluorescent properties

Type 1 nanomaterials possess intrinsic fluorescent properties and can deliver fluorescence signals in intracellular detection. Typically, such fluorescence-reporting nanomaterials include transition-metal semiconductor quantum dots (QDs) [7], silicon dots [8], carbon materials {e.g., carbon dots (CDs) [9], nanodiamonds [10], graphene QD [11] and near-infrared (NIR) single-walled carbon nanotubes (SWCNTs) [12]}, metal nanoclusters (NCs) (e.g., Au, Ag, Cu, and Pt) [13,14], lanthanide-doped nanocrystals [e.g., Eu(III), Sm(III), Tb(III), and Gd(III)] [15,16], and dye-encapsulated silica [17] or polymer nanoparticles (NPs) [18]. Because these fluorescent nanomaterials are predominant in imaging applications, Table 1 summarizes their properties.

QDs exhibit the quantum-confinement effect due to their physical dimensions being smaller than the exciton Bohr radius. The most significant properties of transition-metal semiconductor QDs include high quantum yield (QY), broad absorption allowing single-wavelength excitation, narrow, symmetric size-dependent emission bands, and high photostability [7,20]. QDs have been widely used for labeling cells or tissues, and real-time cell tracking [23–25]. For example, QDs can be used for simultaneous labeling of multiple cells for long-term multicolor imaging, and the cells can remain stably labeled for over a week as they grow and develop [23]. Moreover, specific peptides or antibodies can be modified on the surface of QDs, which can be specifically recognized and internalized into the cells. It was reported that QDs functionalized with EGF cell-surface receptors could be used to investigate receptor-mediated signal transduction in different cancer cell lines, and they were demonstrated to be brighter and more photostable than organic dyes [25].

However, the excellent performance of QDs in bioimaging applications is hindered because they suffer from some significant shortcomings, such as photoblinking and toxicity [21]. The phenomenon of photoblinking can decrease QY during measurement. The biological toxicity of QDs originates from the diffusion of heavy metals, such as Cd, from the core of the QDs [7].

To address the toxicity issue, carbon QDs [carbon dots (CDs)], silicon QDs (silicon dots) and graphene QDs have been developed and they promise to exhibit undisputed cyto-compatibility [8,11,21,22]. CDs have been demonstrated in successful uses in fluorescence imaging of cells, taking advantage of their fluorescence brightness at the individual dot level, and their high photostability. They can also be used at different excitation wavelengths [26]. For example, polyethylenimine-functionalized CDs were shown to be water soluble and taken into cells, displaying tunable fluorescent emission at various excitation wavelengths, suggesting their potential in gene delivery and bioimaging [27]. More importantly, CDs are very brightly multi-photon fluorescent, with two-photon cross-sections in the NIR (800–900 nm) orders of magnitude larger than those in the benchmark organic dyes. It was reported that aminopolymer-functionalized CDs could be taken up by cells, and the internalized CDs exhibited bright fluorescence emissions under two-photon excitation with a femtosecond pulsed laser at 800 nm [28]. CDs also exhibit non-blinking, size- and excitation-wavelength-dependent photoluminescence, presumably because of the quantum effect and the different emissive traps on the surface of CDs. Their QY is still not comparable to QDs and highly dependent on the surface passivation [9]. Some of particular fluorescent carbon materials such as nanodiamonds can be luminescent in a region spanning visible and IR wavelengths due to their nitrogen vacancy center, and SWCNTs can have very large Stokes shift and bright NIR emission fluorescence, which may be very attractive for high-resolution images [20].

To apply silicon dots to bioimaging, extensive efforts have been undertaken to realize their aqueous dispersibility, because most such silicon dots are covered with hydrophobic moieties (e.g., styrene, alkyl and octene) on their surfaces and not well dispersible in water. These silicon dots can be modified to become well dispersible in water by grafting water-soluble hydrophilic molecules or coatings, such as micelles or polymers [29–31]. This modification makes silicon dots