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Review

Inorganic fluorescent nanoprobes for cellular and subcellular imaging

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ABSTRACT

The imaging of essential events and physiological states at cellular and subcellular levels plays key roles in studies of biological mechanism and applications of clinical diagnostics. Fluorescent imaging is a powerful technique for cell imaging due to its unique advantages. However, traditional fluorescent dye probes for imaging face significant challenges in investigating spatiotemporal dynamics of molecules or biological structures in cells. With the rapid development of nanotechnology, inorganic nanoparticle (NP)-based fluorescent probes are being constructed and show great improvements in photophysical properties and bioavailability, which enable more sensitive, longer-term imaging in cells. This review introduces several types of inorganic fluorescent nanoprobe, including dye-containing silica NPs, quantum dots, metal nanoclusters and upconversion NPs, and then discusses the cellular uptake process and applications of these nanoprobes for cellular and subcellular imaging. We also cover the ongoing challenges of NPs in clinical applications.

Contents

1. Introduction

No matter how complex life is, the basic structural, functional and biological unit is the cell. Meanwhile, the function of biological cells is closely associated with their internal organization, in which multiple subcellular components have their specialized roles [1]. For example, they are enclosed by cellular membranes, composed of proteins and a double layer of lipid molecules, which control the flow of materials in and out of cell [2]. Cytoplasm contains many subcellular compartments involved in biochemical reactions that mediate critical cellular functions [3]. Moreover, the physiological states (e.g., pH, electrical charge distribution, and concentration of ions or other substances) also influence the functions of subcellular compartments [4]. Visualization of these essential

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events and the physiological states in living cells has therefore been the focus of much biomedical research to understand the growth, the development and the aging of the human body, and also to provide opportunities for prevention, diagnosis, and treatment of diseases.

Since the cell was first discovered by Robert Hooke through his microscope in 1665, increasingly sophisticated instruments and new techniques have been used to image and to reveal more information about cell structure and functions. Electron microscopy [5], magnetic resonance imaging (MRI) [6], radiography [7], atomic force microscopy (AFM) [8] and fluorescent imaging systems [9] have been developed. Compared with other imaging modalities, fluorescence imaging has competitive advantages, including inherent sensitivity, technical simplicity, fast response time, and multiplex detection capabilities, so it has become the most widespread technique to investigate spatiotemporal dynamics of target molecules or events at the cellular and subcellular levels [10].

The fluorescent probe is one of the most critical elements for investigating biological events at cellular and subcellular levels. Traditional fluorescent dye-based probes, such as fluorescent proteins and small organic dyes, have been widely used in labeling and visualizing technique for many decades [9]. For example, green fluorescent protein (GFP) has allowed systematic imaging studies of protein localization in living cells and the structures and the functions of subcellular components [11]. Organic dyes, including rhodamine, fluoroscein, cyanine, or dipyrroylmethane (BODIPY), have been used as core structures in tracking changes of intracellular pH or ions (e.g., Ca^{2+} and Zn^{2+}) [12].

Although these dyes have improved our observation of intracellular signaling and recognition processes, most of them still suffer from the following significant limitations.

(1) Photostability is a common limitation with conventional fluorescence dye probes. All organic fluorophores undergo irreversible photobleaching during prolonged illumination, which makes them incapable of continuous observation and long-term imaging for studies of living cells [13].
(2) Low signal-to-noise ratio always stems from low fluorescent signal intensity of probes, high absorption and autofluorescence of background, cross-talking between excitation light and emitted signals, or cross-talking between different probes.
(3) Phototoxicity of fluorescent dye probes. Generally, most small organic dyes, such as fluorescein isothiocyanate (FITC), are strongly phototoxic, and produce reactive oxygen species (ROS) in the excitation process [14]. ROS can oxidize a large variety of important biological components (e.g., proteins, nucleic acids and lipids) and even probes themselves, leading to cytotoxicity and photobleaching.
(4) Most of these fluorophores are hydrophobic, and that may affect their dispensability and transport in aqueous solutions. In contrast, some hydrophilic fluorophores still have poor ability in crossing cellular membranes.
(5) Fluorophores usually need to be further modified with biological targeting moieties, such as antibodies, peptides, or nucleic acids, but these chemical-modification and purification processes may be relatively sophisticated and technically demanding.

It is therefore highly desirable to introduce novel fluorescent probes to overcome some of these limitations. Recently, nanoparticle (NP)-based inorganic fluorescent probes, such as dye-containing silica NPs (SiNPs), quantum dots (QDs), metal nanoclusters (NCs) and upconversion NPs (UCNPs), have been useful for cell staining and visualization in fluorescence imaging [15].

In this review, we briefly introduce the properties of these materials and the relevance of charge, size and surface modification of the NPs to the process of cellular uptake. We then discuss current research activities with typical examples of fluorescent NPs in imaging cellular membranes, subcellular components (lysosome, mitochondria and nucleus), and intracellular molecules and ions (enzyme, pH and ROS). Finally, we briefly discuss the potential cytotoxicity of NPs and the ongoing challenges of fluorescent nanoprobes in clinical applications.

2. Nanoparticle-based inorganic fluorescent probes

With the rapid development of nanotechnology, NP-based inorganic fluorescent probes are being constructed and have shown great improvement in photophysical properties and bioavailability, making up for the shortcomings of traditional fluorescent dye-based probes [16]. In this review, we focus discussions on four kinds of inorganic fluorescent NPs (dye-containing SiNPs, QDs, NCs and UCNPs) and provide a brief introduction to them.

Dye-containing SiNPs, one of the most representative, well-established inorganic materials, can encapsulate large numbers of dye molecules, which amplify their optical signal and temporal-spatial resolution response [17,18]. Meanwhile, using silica matrices as protective shell or dye isolator has attained excellent performance in biocompatibility, photostability and modification. Also, the fluorescent substances entrapped within nano-matrices are flexible, such as different kinds of fluorescent substances with ratiometric or fluorescence resonance-energy transfer (FRET) effects, which have facilitated the fluorescent signal in cellular spatiotemporal dynamics imaging with high sensitivity and selectivity [19,20].

QDs, another particularly popular inorganic material for optical applications, are made of different element groups, such as III-V, II-VI, and I-VII [21]. When the dimensions of these materials are less than the exciton Bohr radius (the distance between an electron in the conduction band and the hole it leaves behind in the valence band), the QDs exhibit unique optical and electronic properties due to quantum confinement [22]. By varying particle size and composition, the fluorescence emission of QDs can be tuned from near ultraviolet to near infrared with broad excitation profiles, typically narrow emission spectra, large Stokes shift and multiplexing analysis under excitation of a single wavelength [23]. In addition, they also exhibit very high quantum yield and robust fluorescence, easy control of surface functionality, and a resistance to both chemical and photo-irradiation degradation, which are critical for long-term tracking research [24].

NCs are novel fluorescent materials composed of up to 100 metal atoms (e.g., Cu, Ag and Au) and are constructed by using proteins or DNA as templates. They typically have diameters less than 2 nm, so they are intermediate in composition between the bulk and molecular regimes. In this size range, the electronic band structure of the bulk gets modified to discrete electronic states, leading to the observation of dramatically different optical, electrical and chemical properties [25]. For example, Ag nanoclusters (AgNCs) prepared using cytosine-rich single-stranded DNA templates have attracted special attention because of their ease of synthesis, ultrasmall size, intense light absorption and size-dependent fluorescent emission [26]. Recently, functional proteins or DNA-based recognition ligands were used as templates to prepare NCs [27,28]. In this strategy, it is possible to engineer a specific, conjugation-free NC for targeted fluorescent imaging.

UCNPs are rare-earth doped inorganic nanomaterials, which typically comprise a host matrix, and dopant lanthanide ions as sensitizer and activator. The host matrix is a transparent crystal and needs to fulfil the requirements of close lattice matching to dopant ions, low phonon vibration energy and good chemical