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Review

Emerging applications of near-infrared fluorescent metal nanoclusters for biological imaging

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ABSTRACT

Fluorescent metal nanoclusters (MNCs) have recently emerged as a novel kind of promising fluorescent probes for biological imaging because of their ultrasmall core size (<2 nm), strong photoluminescence, facile availability and good biocompatibility. In this review, we provide an update on recent advances in the development of near infrared (NIR)-emitting MNCs in terms of synthesis strategies and bioimaging applications. We mainly focus on the utilization of NIR-emitting MNCs (including Au, Ag, Cu and alloy NCs) either as single modal imaging (fluorescence intensity-based imaging, fluorescence lifetime imaging, two-photon imaging) probes or as multimodal imaging (such as NIR fluorescence/X-ray computed tomography/magnetic resonance imaging, NIR fluorescence/photoacoustic imaging/magnetic resonance imaging, NIR fluorescence/single photon emission computed tomography, *etc.*) probes in biological cells and tissues. Finally, we give a brief outlook on the future challenges and prospects of developing NIR-emitting MNCs for bioimaging.

1. Introduction

Fluorescent metal nanoclusters (MNCs), usually consisting of several to approximately a hundred metal atoms, which bridge the gap between single metal atom and plasmonic metal nanoparticles (core sizes >2 nm) [1], have attracted extensive attention over the past few decades. MNCs have size down to less than 2 nm, which is comparable to the Fermi wavelength of electrons [2], resulting in the breakup of the continuous density of states of the particles into discrete energy levels [1,3]. MNCs exhibit particular optical, electronic and chemical properties, including strong photoluminescence, excellent photostability, good biocompatibility and sub-nanometer size. Such novel properties make MNCs ideal probes for many applications in biological imaging and diagnosis.

Near infrared (NIR) fluorescent MNCs are especially promising for bioimaging, because biological tissues show very low absorption and autofluorescence in the NIR spectrum window (650–900 nm wavelengths) [4,5]. Also, NIR light can pass across several centimeters of heterogeneous living tissues [6]. Particularly, NIR-emitting MNC probes can alleviate several limitations of conventional NIR organic dyes and other nanoprobes like semiconductor quantum dots (QDs). Organic dyes show many drawbacks such as poor hydrophilicity and photostability, insufficient stability in biological systems and weak multiplexing capability [7]. Most reported QDs display high inherent cytotoxicity and self-aggregation inside live cells, which fatally limit their practical bio-applications [8].

Fluorescence lifetime imaging (FLIM) and two-photon imaging have been widely adopted in tissue and cell studies, and now have become powerful tools in early diseases detection and diagnosis as well as guiding the disease treatment [9,10]. Fluorescent MNCs possess much longer lifetime than that of cellular autofluorescence and most organic dyes, making them attractive as markers for cellular FLIM applications. In contrast to fluorescence intensity imaging, lifetime-based imaging is independent of fluorophore concentration and laser excitation intensity [11]. Although one-photon fluorescence imaging techniques are featured with good spatial resolution and high sensitivity, they hardly obtain anatomical or three dimensional details of tumor tissues *in vivo* [12]. Compared to one-photon imaging, two-photon imaging is a powerful technique for enhanced tissue penetration depth (>500 μm) with two NIR photon excitation, low tissue

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autofluorescence and self-absorption, as well as reduced photodamage [10,13,14]. The relatively good biocompatibility and large two-photon absorption (TPA) cross section of MNCs make them ideal probes for two-photon imaging in biological system.

Besides fluorescence (FL) imaging, a number of other imaging techniques are also being used in the early-stage diagnosis of cancer, such as magnetic resonance imaging (MRI), X-ray computed tomography (CT), photoacoustic imaging (PAI), and single photon emission computed tomography (SPECT) [15,16]. Each imaging modality has its own unique advantages along with intrinsic limitations [17]. For example, CT imaging can easily differentiate various tissue densities, and allow three-dimensional visual reconstructions of tissue; however, it suffers from poor sensitivity, especially in soft tissues with limited density differences [18,19]. MR imaging is able to provide high-quality 3D information of soft tissues and possesses high spatial resolution, but has the disadvantage of relatively low sensitivity [20–23]. Therefore, the rational combination of different modalities, known as "multimodal imaging", is a powerful method that can provide more reliable and accurate detection of disease sites as it integrates the advantages of several imaging modalities and provides complementary information from each imaging modality. Consequently, it can provide more detailed anatomical or biological information about the target disease [15,24].

In this review, we mainly focus on the latest progress in NIR-emitting MNC probes for biological imaging. Specifically, we summarize recent advances in the synthesis and applications of NIR-emitting MNCs (including Au, Ag, Cu and alloy NCs) as novel probes for bioimaging, including single modal imaging (fluorescence intensity-based imaging, FLIM, two-photon imaging) probes and the combination of NIRFL imaging with several other imaging techniques to form multimodal imaging (such as NIRFL/CT/MRI, NIRFL/PAI/MRI, NIRFL/SPECT, *etc.*) probes. In the final section, we will give a brief outlook on the challenges and opportunities for NIR-emitting MNCs in bioimaging applications.

2. Synthesis of NIR-emitting MNCs

During the past decade, various methods have been developed to synthesize MNCs with the NIR-emitting property. Generally, there are two main strategies for preparing MNCs, named "bottom-up" and "top–down" synthetic routes [25]. For a "bottom-up" approach, template-assisted synthesis is usually adopted, where the corresponding metal precursors are reduced to atoms by reducing reagents and then the zero-valent metal atoms aggregate to form the nucleus of metal clusters under the protection of templates. Special templates such as thiols, biomolecules (including proteins, peptides and DNA), dendrimers and polymers have been used to direct the formation of NIR-emitting MNCs. In "top-down" method, ultrasmall size MNCs can be prepared from preformed large metal nanoparticles by a ligand-induced etching process. In the following, we will give a detailed overview for each synthetic strategy with various templates or capping ligands (representative examples summarized in Table 1).

Table 1
Summary of representative literatures on the synthesis of NIR-emitting MNCs.

Metal	Capping agent	λ_{em}	QY	Size (HD)	Refs.
Au	GSH	780 nm	–	1.1 nm[a]	[27]
Au	GSH	810 nm	~0.5%	3.3 nm (2.5 nm[a])	[28]
Au	GSH	650 nm	1.6%	3.1 nm (1.9 nm[a])	[29]
Au	GSH	685 nm	1.3%	–	[38]
Ag	GSH	670 nm	–	~1.1 nm	[30]
Ag	GSH	720 nm	2.8%	<2 nm[a]	[39]
Ag_2S	GSH	679/727 nm	0.3%, 0.1%	3.0 nm[a], 3.7 nm[a]	[31]
Ag/Au	GSH	716 nm	3.4%	1.8 nm[a]	[32]
Au	DHLA	684 nm	~0.6%	3.2 nm	[11]
Au	DHLA	715 nm	2.9%	3.3 nm (1.6 nm[a])	[34]
Au	DHLA	720 nm	10%	1.4 nm[a]	[35]
Au	DHLA	650 nm	1–3%	<5 nm	[40]
Au	DHLA	650 nm	~7%	2 nm[a]	[41]
Cu	DHLA	650 nm	7.2%	1.9 nm[a]	[36]
Au	MSA/tiopronin	785 nm	3.4%, 3.8%	~1.5 nm[a]	[37]
Au	BSA	710 nm	–	~2.7 nm	[71]
Au	BSA	665 nm	–	3.74 nm	[45]
Au	BSA	670 nm	~6%	<3 nm[a]	[47]
Au	BSA	~674 nm	~6%	~1 nm[a]	[46]
Au	BSA	660 nm	~4%	1 nm[a]	[55]
Au	Human transferrin	710 nm	~7.7%	2.6 nm[a]	[48]
Au	Apoferritin	665 nm	8.2%	1.2 nm[a]	[49]
Au	Human transferrin	695 nm	~4.3%	<2 nm[a]	[50]
Cu	Transferrin	670 nm	6.2%	2.99 nm[a]	[54]
Au	Trypsin	690 nm	6.5%	2.7 nm[a]	[51]
Au	Human insulin	680 nm	~10%	5.36 nm	[52]
Au	Bovine pancreatic ribonuclease A	682 nm	~12%	6.2 nm	[53]
Au	Peptide CCYTAT	677 nm	11%	1.5 nm[a]	[57]
Ag	ssDNA	705 nm	34%	~2.5 nm[a]	[60]
Ag	ssDNA	700 nm	52%	3 nm	[61]
Ag	C_{12} ssDNA	~700 nm	17%	–	[65]
Ag	C_{24} ssDNA	715 nm	14%	–	[66]
Ag	G-quadruplex (AS1411)	680 nm	6.79%	<2 nm[a]	[67]
Au	PTMP-PMAA	~660 nm	4.8%	<3 nm	[68]
Au	PEG	810 nm	–	5.5 nm (2.3 nm[a])	[69]
Ag	SH-PEI	690 nm	3%	12 nm (2.3 nm[a])	[70]

HD: hydrodynamic diameter; GSH: glutathione; DHLA: dihydrolipoic acid; MSA: 2-mercaptosuccinic acid; tiopronin: *N*-(2-mercapto-propionyl) glycine; BSA: bovine serum albumin; CCYTAT: H_2N–CCYRGRKKRRQRRR–COOH; PTMP: pentaerythritol tetrakis 3-mercaptopropionate; PMAA: poly(methacrylic acid); PEG: poly(ethylene glycol); SH-PEI: thiolpolyethyleneimine.
[a] Core size.

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