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Metallic Nanoclusters for Cancer Imaging and Therapy

Qing Zhang¹, Mingying Yang^{2,*}, Ye Zhu³, and Chuanbin Mao^{1,3,*}

¹School of Materials Science and Engineering, Zhejiang University, Hangzhou, Zhejiang, 310027, China

²Institute of Applied Bioresource Research, College of Animal Science, Zhejiang University, Yuhangtang Road 866, Hangzhou, Zhejiang, 310058, China

³Department of Chemistry & Biochemistry, Stephenson Life Sciences Research Center, University of Oklahoma, 101 Stephenson Parkway, Norman, OK, 73019, USA

Abstract

Background: Nanoclusters are made of a few to tens of atoms with a size below 2 nm. Compared with nanoparticles, they exhibited excellent properties, such as tunable fluorescence, ease of conjugation, high quantum yield and biocompatibility, which are highly desired in the development of cancer nanotheranostics. Hence, the metallic nanoclusters have emerged as a newcomer in cancer nanomedicines. This review aims to summarize recently developed approaches to preparing metallic nanoclusters, highlight their applications in cancer theranostics, and provide a brief outlook for the future developments of nanoclusters in nanomedicine.

Method: We carried out a thorough literature search using online databases. The search was focused on a centered question. Irrelevant articles were excluded after further examination and directly relevant articles were included. The relevant articles were classified by the subjects and the information from these articles was synthesized.

Results: One hundred and forty-three articles were included in this review. About eighty articles outlined the development in the synthetic methods of nanoclusters. The synthesis approaches include chemical reduction, photoreduction and so on. The progress in the application of gold and silver nanoclusters to cancer theranostics was described in fifteen and eight articles, respectively. The rest articles were about the advancements in the use of other metal nanoclusters and nanocluster nanocomposites as cancer theranostic agents.

Conclusion: This review summarizes the synthesis and use of metallic nanoclusters or their nanocomposites as cancer theranostic agents. It confirms their importance, advantages and potentials in serving as a new generation of cancer theranostics in clinics.

*Address correspondence to this author at Department of Chemistry, & Biochemistry, Stephenson Life Sciences Research Center, University of Oklahoma, 101 Stephenson Parkway, Norman, OK, 73019, USA; cbmao@ou.edu, yangm@zju.edu.cn.

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Keywords

Metallic nanoclusters; synthesis; cancer; imaging; therapy

1. INTRODUCTION

Cancer is the major mortal sickness worldwide and has attracted much attention of the public. The stage of the tumor at which it is discovered is strongly associated with the survival of cancer patients. The identification and detection of cancer at an early stage before metastasis is very important for the cure of cancer. Hence, there is a pressing need in the development of rapid, specific, and sensitive methods for cancer diagnostics. Cancer nanomedicine, which integrates molecular diagnosis, medical imaging, and cancer cell-targeted therapy in a single platform, is a typical and desired method in the application of nanotechnology in cancer theranostics [1, 2]. All kinds of inorganic nanoparticles (*e.g.*, metallic nanoparticles, quantum dots, Fe₃O₄ nanoparticles) and polymeric nanoparticles (*e.g.*, dendrimers and micelles) are used in cancer nanomedicine because they can serve as agents with specific structures and functions suitable for cancer imaging, diagnosis, and therapy [3, 4].

In contrast to nanoparticles, nanoclusters (NCs) are a relatively newcomer in nanomedicine. NCs are made of only a few to tens of atoms and the size of their cores is usually below 2 nm [5]. Since the ultra-small size of the NCs is close to the Fermi wavelength of electrons, the continuous band turns to discontinuous and becomes molecule-like discrete energy levels. Consequently, NCs exhibit a multitude of special optical and electronic characteristics, such as highest occupied molecule orbital-lowest unoccupied molecule orbital (HOMO-LUMO) transition, tunable fluorescence, large Stokes shift, molecular chirality, magnetism, and quantized charging. Nanoclusters (NCs) as a type of emerging nanomaterials have received much attention in catalysis [6], detection of biomolecules [7] and heavy metals [8], biosensors [9], and solar cells [10]. Recently, much work has been done to explore the applications of NCs in cancer imaging and therapy due to their good stability, facile synthesis, excellent biocompatibility, ease of conjugation, and renal clearance [11, 12].

NCs display excellent fluorescence due to their sturdy photostability, huge two-photon absorption cross section, and strong two-photon luminescence compared with other fluorescent agents [13]. For example, organic dyes, commercially available fluorophores, are usually used as photon imaging agents in several clinical fields ranging from cancer imaging, and radiotherapy to targeted drug delivery. But they are limited by a few inherent drawbacks including photobleaching, native function perturbation by the labeled biomolecules, and biotoxicity [14]. Semiconductor quantum dots are attractive candidates as fluorescent labeling agents for biological processes because of their photostability, small size, narrow emission profile, and high quantum yield. But they contain heavy metals that usually show potential dangers for body [15]. In addition to acting as a fluorescent (FL) imaging agent, NCs can serve as probes for computed tomography (CT) and positron emission tomography (PET) imaging with their inherent properties or conjugated with other

imaging probes [16]. For example, Au NCs can serve as FL and CT imaging probes thanks to their fluorescence, large atomic number, and electron density [17]. Thus, NCs are a good candidate for multimodal cancer imaging platforms.

The inherent properties of NCs including ultrasmall size with narrow size distribution and ease of conjugation not only offer the tunable fluorescence to NCs, but also make them very suitable for the application in cancer therapy. Traditional nanoparticles cannot escape from the kidney barrier and result in severe side effects owing to their aggregation in the liver and spleen, but NCs can be effectively removed from the body *via* renal clearance [18, 19]. Besides, NCs usually are stabilized with surface ligand such as proteins [20], thiols [21], and polymers [22], allowing them to be facilely conjugated with other imaging agents, anticancer drugs, or physiotherapy agents in the preparation of multimodal theranostic platforms. Therefore, NCs rise as a burgeoning nanomaterial in developing cancer nanomedicine.

In this mini-review, we first summarize the synthesis and properties of NCs. Then we discuss some representative applications of NCs in cancer imaging and therapy reported in recent years. Finally, we point out the challenges the field of NC-based cancer nanomedicine before NCs can be used in clinic. For detailed information on the functionalization, cytotoxicity, biodistribution and clearance of NCs, the readers are suggested to refer to another recent review [18].

2. THE SYNTHESIS OF NCs

In 1969, Mooradian [23] *et al.* discovered the photoluminescence of bulk, single crystal wafer and thin film of Au and Cu. Though the Au NCs capped with phosphine were studied in 1960s [24], the work was delayed by their limited stability. Then the photoluminescence of Ag NCs in zeolite [25–27], inert gases [28, 29] and inorganic glass [30, 31] was reported. The Henglein group [32] first synthesized Ag NCs and studied their properties [33–38] except for photoluminescence due to their limited stability. In 1994, Brust and Schiffrin [39] developed a chemical reduction approach to prepare alkanethiol-stabilized Au NCs. When these thiolated Au NCs were dried and re-dispersed, their properties were not changed. A series of thiolate-protected Au NCs were prepared by using the chemical reduction method. Though Ag was easy to be oxidized, Dickson [40] *et al.* first synthesized stable hydrosoluble Ag NCs by using dendritic polymer (Polyamidoamine, PAMAM) in 2002 and triggered the use of Ag NCs as probes. Then, Baskakov [41] *et al.* first synthesized water-soluble fluorescence Ag NCs with peptides as templates and applied them in biological imaging successfully. In 2009, the Xie group [42] synthesized red fluorescence Au NCs by using bovine serum albumin (BSA) and propelled the application of metal NCs in biomedicine.

The unique optical properties, good biocompatibility and easy conjugation of NCs have attracted the interest of physicists, chemists, material scientists and biologists. Dramatic advances have been achieved in developing new synthetic methods, understanding their properties and achieving biocompatibility in the last two decades. In general, there are two strategies to produce NCs: “bottom-up” method and “top-down” method. In the “top-down” process, a ligand (e.g. thiol compounds) is used to etch pre-formed nanoparticles into ultra-

small clusters. The “bottom-up” approach means to assemble a few metal atoms into a cluster in the presence of a ligand or template, which could in turn stabilize the resultant NCs. The “bottom-up” method includes chemical reduction, photoreduction, microwave-assisted synthesis, sonochemical synthesis and electrochemical synthesis. We will discuss these methods and highlight some examples in the following sections.

2.1. Chemical Etching

Nanoparticles with a core size of 2–4 nm can be etched into NCs by excess ligands. For example, Au NCs were prepared from the etching of Au NPs by thiol ligands (e.g. 11-mercaptoundecanoic acid); this is pivotal because their size and optical properties depended on the thiol compounds [43]. Two possible routes for etching are proposed by researchers. In the first approach, atoms are detached from the nanoparticles surface by the ligand and then form NCs *via* strong atom-atom interactions [44]. In the second approach, the nanoparticles are etched by the ligand and their sizes are reduced in steps till proper NCs have formed [45].

Stable nanoparticles are generally tailored into NCs by ligands such as octanethiol or bidentates [46, 47]. The etching process usually occur at the interface between water and oil [48, 49]. To avoid using poisonous organic solvents such as toluene and tetrachloromethane, Liu *et al.* [44] used a gelatin framework to stabilize the precursor Ag nanoparticles in water solution, and prepared Ag NCs by etching Ag nanoparticles with dihydrolipoic acid (DHLA) in an aqueous solution. In addition to the ligands, the precursor can also induce nanoparticles etching. AuNPs stabilized by di- dodecyldimethylammonium bromide were etched by Au precursors (HAuCl_4 or AuCl_3) into smaller NCs (AuNC@DDAB) (3.17 ± 0.35 nm) [47]. The hydrophobic AuNC@DDAB could be further etched by exchange of dihydrolipoic acid (DHLA) to AuNC@DHLA (1.56 ± 0.3 nm) and became water soluble.

Using ligands to etch nanoparticles, the weak nucleophilic ligands on the surface of nanoparticles are generally replaced by strong ones [50, 51]. The strong nucleophilic ligands are rarely exchanged by weak ones [52]. Jin *et al.* [53] reported a similar process, as shown in (Fig. 1A). The Au12 shell was peeled off from phenylethanethiolated core-shell $\text{Au}_{25}(\text{SC}_2\text{H}_4\text{Ph})_{18}$ to prepare $\text{Au}_{13}(\text{PPh}_3)_8(\text{SC}_2\text{H}_4\text{Ph})_3^{2+}$ by etching PPh_3 . The $\text{Au}_{13}(\text{PPh}_3)_8(\text{SC}_2\text{H}_4\text{Ph})_3^{2+}$ could be further etched into $\text{Au}_{11}(\text{PPh}_3)_8(\text{SC}_2\text{H}_4\text{Ph})^{2+}$ and finally into biicosohedral $\text{Au}_{25}(\text{PPh}_3)_{10}(\text{SC}_2\text{H}_4\text{Ph})_5\text{Cl}_2^{2+}$.

2.2. Photoreduction Method

In 2001, Dickson *et al.* [54] first demonstrated that NCs could be produced by photoreduction without the addition of reduction agents. Metal ions encapsulated in microgel could efficiently and spontaneously form NCs under sunlight. Aqueous microgel dispersions can produce $\text{H}^\bullet$, $\text{OH}^\bullet$, and perhaps organic radicals by the irradiation of UV. Metal ions were reduced into metal atoms by these radicals [55]. Banerjee *et al.* [56] prepared a stable hydrogel with silver ions encapsulated using N-terminally Fmoc-protected dipeptide and then formed silver NCs upon the sunlight irradiation at a physiological pH value (7.46). Later, the same group used an amino acid that was Fmoc-protected at the N-terminal to form a hydrogel and then prepare silver NCs under a similar condition [57]. In

addition to hydrogels, polymer is the other excellent template when the photoreduction method is used to synthesize NCs. For example, Ras and coworkers [58] mixed polystyrene-block-poly (methacrylic acid) block copolymer (PS-*b*-PMAA) and silver salts in selected organic solvents to prepare NCs with the irradiation of a visible light. When poly(methacrylic acid) was used as a template, copper, silver and gold NCs functionalized with pentaerythritol tetrakis 3-mercaptopropionate were synthesized by a soft photoreduction of their inorganic precursors [59]. Small molecules can also stabilize NCs. With the use of small molecules (D-penicillium and L-penicillium), Sun and coworkers [60] synthesized Ag NCs through a photoreduction process.

Solid templates can be used to facilitate the formation of NCs by photoreduction. Takagi and coworkers [61] reported a photosensitized template reduction method to prepare gold NCs. Porphyrin molecules were assembled on the clay surface and formed a unique pattern. Then gold NCs were deposited on its surface *via* the UV photoreduction of gold precursors. The gold NCs were assembled into a pattern defined by the pattern of porphyrin molecules. The deposition density and aggregation of NCs could be precisely controlled by this method without protective agents. The authors proposed a mechanism for the photosensitized template reduction method. Specifically, under the UV irradiation, Tetrakis(1-methyl-4-pyridiniumyl)porphyrin (TMPyP) on the clay surface was first reduced to TMPyP_{red} through the electron transferring from trimethylamine (TEA), then TMPyP_{red} reduced the gold precursors located nearby into gold NCs.

2.3. Electrochemical Synthesis

Electrochemical synthesis is another method for preparing metal NCs. Reetz and co-workers [63] first developed this method in 1994. During the progress of electrochemical synthesis, metal ions are produced from a sacrificial anode and reduced into metal atoms at the cathode. These metal atoms further aggregated into NCs in the presence of surfactants or ligands. It is easy to manipulate the NCs size through controlling the current, voltage, concentration of stabilizers, and electrolyte *etc.* Based on this mechanism, copper NCs (Cu_n, n ≈ 14) were synthesized by a simple electrochemical technique with tetrabutylammonium nitrate as a stabilizer [64]. These copper NCs displayed photoluminescence in the visible range. Both apolar and polar solvent could be employed to disperse these Cu NCs, and these dispersed Cu NCs were very stable for years. Lopez-Quintela and coworkers [65] produced well defined Ag₅ and Ag₆ NCs by one-pot electrochemical method. These Ag₅ and Ag₆ NCs protected by dodecanethiol/tetrabutyl ammonium (DDT/TBA) were soluble in both organic and aqueous solvents and monothiol Ag NCs were remarkably stable.

A solid template can be used in an electrochemical reduction to prepare metal atomic clusters. The metal deposition method was introduced on ordered alumina pores with pulsed electrodeposition by Gosele *et al.* [66]. Gonzalez *et al.* [62] later modified this process and used porous alumina to produce NCs (Fig. 1B). Briefly, a hexagonally ordered porous alumina substrate was generated. The diameter, depth and interpore spacing of the nanoporous structure were 10 nm, 1 μm and 35 nm, respectively. After being immersed into a metal plating bath, the system was subjected to a pulsed electrodeposition program. Gold and nickel NCs were generated at the bottom of pore in nanoporous alumina. This strategy

showed many advantages including simplicity, high stability against aggregation, and cluster size control. In addition, the metal NCs provide a non-blocked active surface because of the absence of stabilizers.

2.4. Sonochemical Synthesis

Sonochemical synthesis is an important synthetic method in materials chemistry. Ultrasound is irradiated into a liquid and triggers the nucleation, development, and break-up of bubbles (acoustic cavitation) [67]. The acoustic cavitation is the source of the chemical effects resulted from a high intensity ultrasound. For example, the acoustic cavitation can generate localized high pressures and hot spots with temperatures up to ~5000 K [68]. As a consequence, highly reactive species, such as radical $\text{HO}_2^\bullet$, $\text{H}^\bullet$, $\text{OH}^\bullet$, and possibly free electrons are generated during the irradiation of ultrasound [69, 70]. These highly reactive species can reduce metal ions into metal atoms. Suslick and coworker [71] prepared light-emitting, stable, and water-soluble silver NCs by a handy sonochemical process with polymethacrylic acid as a ligand. The properties of the Ag NCs could be regulated by varying the time of sonication, ratio between two species (carboxylate groups and Ag ions), and the molecular weight of polymer.

2.5. Microwave-assisted Synthesis

Microwave irradiation is able to rapidly generate homogeneous heating and is widely used in the synthesis of organic compounds and inorganic nanocrystals. Homogeneous and rapid heating in a solution induced by the microwave irradiation can offer homogeneous nucleation and shorter crystallization times. Obviously, microwave irradiation is also very suitable for the synthesis of uniform and monodisperse NCs. Zhu and coworkers [72] prepared highly fluorescent water-soluble Ag NCs in by means of microwave irradiation using polymethacrylic acid sodium salt as templates. The reaction was fast and the reaction time was reduced to seconds. The resultant Ag NCs are highly stable, monodisperse, highly fluorescent under visible light.

2.6. Chemical Reduction Approach

The chemical reduction approach is a widely used method in preparing NCs. Different from the other methods described above, reducing agents are needed in chemical reduction process. Brust-Schiffrin method and their variants are the representative chemical reduction synthetic strategies. Whyman and coworkers [39] first developed the Brust-Schiffrin method in 1994. They synthesized gold NCs by using BH_4^- as a reducing agent and $\text{C}_{12}\text{H}_{25}\text{SH}$ as a protecting ligand. This synthesis can be described by the phase transfer of metal precursors (reaction 1) followed by the reduction of metal ions (reaction 2):

A modified Brust-Schiffrin method directly reduced metal ions in solution by using one-phase system without the phase transfer of metal precursors. There are many elements that can affect the core size and the surface properties of metal NCs, such as the ratio of ligand to metal, reducing agents, the stabilizing ligands, time and temperature of reaction, and the pH value. The nature of the protecting ligands is very important for the application of NCs in biomedicine. Phosphine, thio-lates, polymers, proteins, and DNA have been widely used as templates and/or protecting ligands in the chemical reduction process.

2.6.1. Thiols Used in Chemical Reduction Approach—Thiols are often used as self-assembled monolayer (SAM) agents to modify the gold surface. The interaction between the thiolate ion and gold atom is similar to that between two gold atoms, making it possible for thiolate ligands to break Au-Au bond and consequently form S-Au bond. Thus, thiols as protecting ligands are widely used to prepare and functionalize Au NCs. Jin and coworkers [73] synthesized barrel-shaped chiral $\text{Au}_{130}(\text{p-MBT})_{50}$ NCs, in which *p*-MBT represents 4- methylbenzenethiolate, and determined its structure by X-ray crystallography (Fig. 2A). $\text{Au}^{\text{I}}(\text{p-MBT})$ complexes were first reduced by NaBH_4 into a mixture of $\text{Au}_x(\text{p-MBT})_y$ NCs, then $\text{Au}_{130}(\text{p-MBT})_{50}$ as the most stable form of NCs was selected by the treatment of excess *p*-MBT thiol at 80°C . In addition to NaBH_4 , the other inorganic reduction agents were applied to prepare metal NCs. For instance, Xie *et al.* [77] developed a two-step reduction approach using carbon monoxide (CO) as a reducing agent to synthesize highly luminescent gold NCs ($\text{Au}_{22}(\text{SR})_{18}$, where SR represents a thiolate ligand). First, Au-glutathione complexes were reduced into $\text{Au}_{18}(\text{SR})_{14}$ by CO. Then the final production of $\text{Au}_{22}(\text{SR})_{18}$ was achieved by a pH-induced aggregation of small Au-glutathione complexes onto $\text{Au}_{18}(\text{SR})_{14}$. Polymers can also be used as a reducing agent and template to synthesize NCs. For example, Ren and coworkers [78] synthesized Au:P3HT (P3HT denotes poly(3-hexylthiophene-2,5-diyl)) nanohybrids by using polystyrene-*b*-poly(4-vinylpyridine) (termed PS-*b*-P4VP) as a reducing agent. The nanohybrids displayed unique thermally responsive optical behaviors, optoelectronic properties and charge-transfer controlled magnetism. To date, a number of thiolate-protected gold NCs (*e.g.* Au_{18} [79], Au_{30} [80], Au_{133} [81], *etc*) have been prepared and their structures have been confirmed

Compared with Au NCs, Ag NCs are unstable because their atom cores have a higher tendency of being oxidized. However, they are cheaper than Au NCs and show excellent photoluminescent properties as same as Au NCs. A lot of Ag and Ag-rich alloy NCs protected by thiolate ions have been prepared. Recently, Bakr's group [82] synthesized monodisperse Ag_{25} NCs protected with 2,4-sdaxsdazdimethylbenzenethiol ligands. A series of $[\text{Au}_{12+n}\text{Cu}_{32}(\text{SR})_{30+n}]^{4-}$ ($n = 0, 2, 4, 6$) NCs were prepared and the evolution process of their structures was investigated by Yang and coworkers [83]. The novel photoluminescent properties of metal NCs stabilized by thiol are generally based on the aggregation-induced

emission (AIE) phenomenon [84]. The Jin group [85] reviewed almost all Ag and Ag-rich alloy NCs protected by thiolate and their unique physicochemical properties.

2.6.2 Other Small Molecules Used in Chemical Reduction Approach—Inspired by the success of the thiolate-stabilized NCs preparation, other small molecules such as phosphines and alkynyl were applied to protect NCs. Wang and coworkers [74] prepared a novel Au₁₉ NCs composition: [Au₁₉(PhC≡C)₉(Hdppa)₃](SbF₆)₂. They were composed of a centered icosahedral Au₁₃ core and coated by three V-shaped PhC≡C–Au–C≡C(Ph)–Au–C≡CPh motifs (Fig. 2B). More recently, Zheng *et al.* [76] prepared an intermetallic Au₂₄Ag₂₀ superatom nanocluster Au₂₄Ag₂₀(2-SPy)₄(PhC≡C)₂₀Cl₂ which displayed three kinds of anionic ligands, including phenylalkynyl, 2-pyridylthiolate, and chloride, on its surface at the same time as a concentric three-shell (Fig. 2D). Thanks to the co-presence of three different surface ligands, these NCs exhibited a site-specific surface and could be easily functionalized through these surface ligands.

2.6.3. Proteins Used in Chemical Reduction Approach—Proteins are biological macromolecules and are widely used to produce NCs with improved biocompatibility. Xie *et al.* [42] first applied bovine serum albumin (BSA) as both a protecting agent and a reducing reagent and prepared fluorescent gold NCs. Motivated by Xie's research, Irudayaraj *et al.* [86] used denatured bovine serum albumin (dBSA) and synthesized highly stable fluorescent Ag NCs. So far, a number of proteins have been successfully adopted to prepare NCs such as lysozyme [87], insulin [88], trypsin [89], ovalbumin [90]. Eggshell membrane is a solid protein. It was found that the membrane could serve as a unique platform to generate fluorescent silver and gold NCs [91]. Moreover, peptides and amino acids are excellent templates and/or reducing agents. Ogawa *et al.* [92] designed and synthesized α-helical coiled coils in the forms of peptide trimers, tetramers, and hexamers; these peptide polymers could be specifically combined with 6, 8, and 12 Ag⁺ ions. When treated by the NaBH₄, a set of peptide-capped Ag NCs were produced. They further demonstrated that these NCs exhibited a strong visible fluorescence, and that their emission energies were associated with the number of metal atoms included by the NCs. Glutathione (GSH) includes a γ-amido bond and a thiol and can serve as a ligand and reducing agent. Luo *et al.* [93] mixed aqueous solutions of HAuCl₄ and GSH to synthesize strongly orange-emitting Au NCs. They reported that the strong luminescent emission was resulted from aggregation-induced emission (AIE). Bertorelle and coworkers [94] reported a simple cyclic reduction method to synthesize silver:glutathione (Ag:SG) NCs. In particular, Ag₁₅(SG)₁₁ NCs stably emitted a strong light. Roy *et al.* [95] synthesized blue, green, and red emitting silver NCs by using reduced glutathione as a stabilizer in the presence of NaBH₄ at a pH value of 7.46 and a temperature of 140°C. These NCs displayed very exciting fluorescence characteristics with large Stokes shifts and a high quantum yield.

2.6.4. Polymers Used in Chemical Reduction Approach—The other categorized ligands for preparing NCs are polymers, such as dendrimers, polyelectrolytes. Mat-toussi *et al.* [96] synthesized bidentate ligands by conjugating a poly (ethylene glycol) short chain or a zwitterion group on a lipoic acid (LA), i.e. LA-PEG and LA-Zwitterin. Then they successfully prepared a series of intense fluorescent Au NCs employing these ligands in the

presence of NaBH_4 . Dispersions of these Au NCs showed excellent long-term stability and fluorescent lifetimes. In addition, due to the functionalization with reactive radicals (for instance, carboxylic acid or amine), these NCs were suitable for common coupling strategies. More recently, Pal *et al.* [97] reported a convenient and eco-friendly approach for the preparation of Ag NCs. In this process, a poly(N-vinylpyrrolidone) homopolymer acted as a stabilizer and acetonitrile or N,N-dimethylformamide (DMF) was used as both a solvent and a reducing agent. On the other hand, polymers can also form micelles which can assemble metal atoms into NCs. Zhang *et al.* [98] prepared an interfacially cross-linked reverse micelle by cross-linking a cationic surfactant with a hydrophilic dithiol. The cationic surfactant was capped with a triallylammonium headgroup. The interfacially cross-linked reverse micelle could extract AuCl_4^- into the hydrophilic core, followed by the reduction of the captured AuCl_4^- into gold NCs without the presence of extra reducing agent.

2.6.5. DNA Used in Chemical Reduction Approach—DNA oligonucleotides are usually used in the synthesis of NCs. In 2004, Dickson *et al.* [99] used DNA as a template and synthesized DNA-capped Ag NCs. Thereafter, various DNA sequences have been applied to synthesize NCs, and to reveal the mechanism based on which DNA interacts with NCs [100, 101]. Han *et al.* [100] reported that duplex, hairpin, i-motif and G-quadruples DNA could stabilize NCs. Besides, the binding affinities of Ag^+ by polymorphic DNA were correlated with the secondary structure of the DNA templates, with an increasing order (the G-quadruplex < the duplex < the i-motif < the coiled C-rich strand) [102]. Fluorescence stability of Ag NCs capped by these polymorphic DNA is related to their binding affinities and the C-rich strand could stabilize Ag NCs for over 300 h. Wang and coworkers [101] used DNA monomers (deoxycytidine, deoxyadenosine, deoxythymidine and deoxyguanosine monomers) as the scaffolds to synthesize silver NCs. They demonstrated that only the silver NCs stabilized by deoxycytidine monomers displayed fluorescent properties. More recently, Teng *et al.* [103] synthesized dark silver NCs protected by DNA and these NCs showed emission fluorescence through DNA hybridization. A 70-fold increase in the strength of fluorescence was observed when the two templates (5'-AAAAAAAAAAAAAAAA ACCCCCCCCCCCCC-3', 5'-CCCTTAATCCCCAATC ATCTCTTCC-3') were located on the opposing end of the duplexes. DNA was also used to prepare other metal NCs such as DNA-capped gold and copper NCs. Yang *et al.* [75] used poly(thymine) (with 20 monomers) as a template to synthesize fluorescent copper NCs with the reduction of sodium ascorbate (Fig. 2C). Simultaneously, their results indicated that random single-stranded DNA, including poly adenine (A), poly cytosine (C), and poly guanine (G) but excluding poly thymine (T), failed to guide the formation of Cu NCs. Martinez *et al.* [104] synthesized gold NCs of ~1 nm in diameter using a hybrid DNA and a dimethylamine borane, which functioned as a template and a reducing agent, respectively. These gold NCs showed phosphorescence characteristic of large Stokes shift and microsecond lifetime.

3. APPLICATION OF NCs IN CANCER THERAPY AND IMAGING

Metal NCs possess non-continuous energy levels like molecules due to their ultra-small size being close to the Fermi-wavelength of electrons. They also exhibit a multitude of excellent

properties such as tunable luminescence, large Stokes shift, ease of conjugation, high quantum yield, extended photostability, biocompatibility. Moreover, they demonstrated a high renal evacuation efficacy. Therefore, noble metal NCs have been widely studied in the fields of biosensing, biomedicine as well as biodetection. Besides, functionalized NCs as potential theranostic agents provide a new chance to clarify drug resistance and enhance cure effects. According to clinical applications, metal NCs are considered one of the best cancer therapy and imaging platforms. Much work has been done to explore the application of NCs in cancer theranostic. In what follows, we will give some examples to detail the progress made in applying the diversified metal NCs in cancer therapy and imaging.

3.1. Au NCs as an Agent in Cancer Theranostics

Au NCs are an important part of the theranostic approach when dealing with cancer, they exhibit excellent properties such as stability, chemical inertness, and a higher atomic number and electron density. Qing *et al.* [105] first investigated the fluorescence properties of Au NCs in a live mice. High accumulation of Au NCs in tumor areas because of their extremely small hydrodynamic diameters (~2.7 nm) and the intensive permeability and retention (EPR) effects indicated that they could act as a tumor probe. The high photostability of AuNCs provided the possibility of continuous fluorescence tumor imaging *in vivo*. Thereafter, Wang and coworkers [106] reported fluorescent gold NCs biosynthesized *in situ*. Specifically, micromolar chloroauric acid solutions were reduced and used to form fluorescent NCs in cell's cytoplasm; ultimately the NCs were concentrated around nucleoli when they were incubated with only cancer cells. The process of biosynthesis could proceed *in vivo*: injection of a millimolar chloroauric acid through the subcutaneous region could form Au NCs in xenograft tumors without any obvious spreading to the surrounding normal tissues. The novel biosynthesis method holds promise for the use of unique fluorescent NCs as the self-bio-marking of tumors. In addition to the passively targeting to tumor by the EPR, the folic acid as a targeting ligand was conjugated with ovalbumin-protected Au NCs by using a homopolymer N-acryloxysuccinimide as a linker to positively target toward cancer [107]. The novel nanoconjugate could be used as a specific fluorescent stain agent for Hela cells.

In the preclinical and clinical application and research, positron emission tomography (PET) is a very important radionuclide imaging technology. Radioactive elements are easily integrated in metal NCs and synthesized as a two-in-one imaging platform. Liu *et al.* [108] prepared ^{64}Cu -Au alloy NCs, which were stabilized with polyethylene glycol (^{64}Cu -AuNCs-PEG350). They evaluated the pharmacokinetics and systemic clearance in a mouse prostate cancer model. The integrated ^{64}Cu -Au alloy NCs were rapidly cleared *via* renal and hepatobiliary pathway and exhibited low non-specific tumor retention [108]. Almost at the same time, Chen and coworkers [109] synthesized ^{64}Cu -doped chelator-free gold NCs with human serum albumin (HSA). The near-infrared (NIR) fluorescence imaging of these NCs was due to Cerenkov resonance energy transfer (CRET). ^{64}Cu with radioactivity acted as both the energy donor for NIR fluorescence and the probe for PET imaging. Au NCs accepted the energy and played a role as NIR fluorescence imaging agents. In a glioblastoma xenograft model, ^{64}Cu -doped Au NCs displayed efficient PET and CRET-NIR imaging (Fig. 3).

Au NCs are also a suitable drug delivery carrier because their surface features are tunable and their excellent capability in loading unique cell targeting molecules. Supramolecular conjugates of polysaccharide and gold NCs were prepared by conjugating the cyclo- dextrin-grafted hyaluronic acid and adamantane moieties-capped gold NCs *via* the highly specific interaction of adamantane moieties between β -cyclodextrin cavities [110]. The supramolecular conjugates were used to load and deliver different cancer therapeutics (such as doxorubicin, hydrochloride, paclitaxel, camptothecin *etc.*) to cancer cells by utilizing the efficient cancer targeting of hyaluronic acid. Further, a multifunctional nanocarrier with a core-shell structure was constructed for targeted anticancer drug delivery [111]. The core of the multifunctional nanocarrier was gold NCs and its shell was a folate-grafted amphiphilic hyperbranched block copolymer. These nanocarriers showed red fluorescence emission and high anticancer efficiency by testing with HeLa cells when camptothecin, an anticancer drug, was loaded in them. Zhou and coworkers [112] synthesized gold NCs and combined a cisplatin prodrug (Pt) and folic acid (FA) with them to synthesize FA-GNC-Pt conjugates. Their result showed that FA-GNC-Pt could target tumor and emit bright fluorescence at the tumor site. In order to more accurately target tumors, Chen and coworkers [113] developed dual targeting luminescent gold NCs for the application of tumor theranostics (Fig. 4a,b). In this novel nano-platform, cyclic RGD and aptamer AS1411 were used to target $\alpha_v\beta_3$ integrins and nucleolin, respectively, both of which were over-expressed in tumor tissues. A NIR fluorescence dye was conjugated on this nano-platform to enhance the signal-to-noise contrast ratio. Furthermore, Doxorubicin (DOX) was integrated in this platform to improve the antitumor capabilities. The AuNC-DOX-cRGD-Apt could effectively target the tumor and develop significant morphological changes to the apoptosis of cells in the mice tumor model (Fig. 4c,d,e).

Au NCs can serve as radiosensitizers because they can strongly absorb a radiotherapy ray and effectively produce secondary electrons upon the irradiation of gamma ray or X-ray. For instance, Zhang *et al.* [114] synthesized ultra-small Au NCs coated by glutathione (GSH-Au₂₅ NCs). They demonstrated that the GSH- Au₂₅ NCs effectively accumulated in tumor through the EPR effect and enhanced the cancer radiotherapy efficacy. Most of all, the GSH-Au₂₅ NCs could be metabolized through kidney. Parallel to this research, the same group [115] prepared ultrasmall Au₂₉₋₄₃(SG)₂₇₋₃₇ NCs, which displayed bright orange luminescence, high tumor uptake, low toxicity and strong sensitizing enhancement for radiation. They also demonstrated that these Au₂₉₋₄₃(SG)₂₇₋₃₇ NCs could act as an X-ray computed tomography imaging agent. Their research implied that these Au₂₉₋₄₃(SG)₂₇₋₃₇ NCs were good candidates for the cancer theranostics.

On the other hand, classical photosensitizer and targeting agents can be combined with Au NCs to synthesize a multifunctional agent for the cancer theranostics. For example, Cui *et al.* [117] synthesized highly fluorescent glutathione-capped Au NCs with folic acid and polyethylene glycol (PEG) conjugated on their surface by covalent coupling. A photosensitizer (chlorin e6, Ce6) was trapped into PEG to form nanoprobe (Ce6@GNCs-PEG_{2k}-FA). These nanoprobe could selectively target tumors and effectively restrain the growth of tumor by photodynamic therapy. Actually, Au NCs can produce singlet oxygen when they are excited by visible light or short NIR light. According to the phenomenon, Hwang *et al.* [116] synthesized alkyl thiolated Au NCs (RS-Au NCs) which sensitized the

production of singlet oxygen when excited by NIR light. Additionally, these RS-Au NCs could emit visible light with a peak at 610 nm when they were excited by 365 nm light. To make the RS-Au NCs more effective in killing cancer cells, a TAT peptide (peptide sequence: N-GRKKRRQRRR-C) was integrated with the RS-Au NCs together to achieve the highly efficient nucleus-targeting (Fig. 5a). Their results demonstrated that the TAT peptide modified RS-Au NCs could be internalized by HeLa cells and enter their nuclei (Fig. 5b). The cellular viabilities of HeLa cells with internalized TAT peptide-RS-Au NCs dropped sharply with the irradiation of 980 nm continuous-wave laser (Fig. 5c). The TAT peptide-modified Au NCs exhibited an ultrahigh gene transfection efficiency in HeLa cells when they served as DNA carriers. Besides, the TAT peptide-modified Au NCs loaded with plasmid DNA (pDNA) molecules could be effectively internalized by cells and transferred gene when they were microinjected into zebrafish (Fig. 5d).

3.2 Ag NCs as an Agent in Cancer Theranostics

The synthesis difficulty and instability of silver NCs delay their development. Bigioni *et al.* [118] prepared ultra-stable silver NCs protected by *p*-mercaptobenzoic acid (*p*-MBA) and made a breakthrough on the Ag₄₄ NC structure. Recently, obvious progress has been made in the application of Ag NCs in cancer theranostics. Sheikhejad *et al.* [119] inserted a cytosine loop (C6 loop) into a double stranded DNA and used it to synthesize Ag NCs. The Ag NCs were pink in visible light and could emit strong fluorescence when they met unmethylated DNA, but they lost these properties when mixed with methylated DNA. According to these results, the C6-Ag NCs could be employed to directly detect the methylated DNA for early cancer diagnosis.

Wang *et al.* [120] used a metallothionein (MT) to synthesize Ag NCs (MT-Ag NCs). The MT was a cysteine-rich protein with low molecular weight. The MT-Ag NCs possessed a good antioxidant capacity and showed intense fluorescence when they were uptaken by HeLa cells. The color of fluorescent emission can be regulated by the ligand conjugated with Au NCs. Xu *et al.* developed Ag NCs which were tagged by AS1411 aptamer or MUC 1 aptamer [121]. The Ag NCs tagged with AS1411 aptamer emitted green fluorescence whereas the Ag NCs tagged with MUC 1 aptamer emitted yellow fluorescence. These NCs could be employed in multicolor imaging of cancer cells. Wang and coworkers [122] synthesized thiolpolyethyleneimine stabilized silver NCs (SH-PEI-AgNCs) and conjugated folate with them to target the tumor. These NCs showed ultra-small size, low toxicity, intense NIR fluorescence, excellent photostability and chemical stability. Hence, they hold promise for *in vitro* and *in vivo* targeted cancer imaging. Luo and coworkers [123] synthesized small-molecule-protected silver NCs (Ag₁₄(SG)₁₁) (SG denotes glutathione), which possessed a smaller hydrodynamic diameter than DNA-protected silver NCs. They successfully used these NCs to label cancer cells (A549) for fluorescent imaging. Similar to the biosynthesis of Au NCs *in situ*, the Wang group [124] reported a specific biosynthesis of silver NCs. Innocuous silver salts could be reduced to form highly NIR fluorescent Ag NCs by the cancer cells and tumor themselves. This novel strategy could be applied in selective and accurate imaging of cancer cells and tumors. Additionally, Ag NCs synthesized *in situ* in tumors resulted in a sharp reduction of tumor size, which even led to complete remission.

This phenomenon suggested these NCs prepared *in situ* in tumors are promising new cancer therapeutics.

In addition to serve as an imaging agent, Ag NCs can sensitize the generation of singlet oxygen and serve as a valid PDT agent. Tan *et al.* [125] synthesized BSA protein-templated Ag NCs (BSA-Ag₁₃ NCs), which displayed a stronger singlet oxygen generation capacity than their Au analogue. MCF-7 breast cancer cells possessed good uptake for BSA-Ag₁₃ NCs and were efficiently killed due to the irradiation of white light *via* PDT. Wang *et al.* [126] prepared water-soluble Ag NCs encapsulated by a DNA scaffold, which displayed NIR fluorescence and photothermal effect. Moreover, AS1411, a nucleoli-targeting agent, was connected with the DNA scaffold and the protoporphyrin IX (PPIX) was captured by AS1411. The multifunctional nanoconjugates (PPIX-AS1411-Ag NCs) showed photodynamic and photothermal efficiency to the colon cancer cells and could serve as NIR fluorescent probes for nuclei simultaneously.

3.3. Other Metal NCs as an Agent in Cancer Therapy and Imaging

Copper (Cu) is one of the most significant trace element in the human body and thus is relatively biofriendly for human beings compared with gold and silver. Highly fluorescent Cu NCs were fabricated when hydrazine served as a reducing agent in the presence of lysozyme [127]. These Cu NCs possessed photo- and chemical- stability and were employed to successfully label HeLa cells. ⁶⁴Cu is a general PET imaging agent and has been doped in other metal NCs or introduced into biomaterials *via* macrocyclic chelators in clinical application. Recently, Gao *et al.* [128] synthesized ultra-small radioactive [⁶⁴Cu]Cu NCs without chelator by using BSA as a protecting agent. A lung cancer targeting peptide, termed luteinizing hormone releasing hormone (LHRH), was preconjugated on BSA to synthesize [⁶⁴Cu]Cu_{NC}@BSA-LHRH. The resultant product displayed high tumor uptake, high radiolabeling stability, and obvious renal clearance. Sensitive, deep penetration, and accurate imaging of orthotopic lung cancer could be achieved by using [⁶⁴Cu]Cu_{NC}@BSA-LHRH as PET imaging probes *in vivo*.

Platinum NCs (Pt NCs) could also be applied in cancer theranostics. Wang *et al.* [129] synthesized fluorescent Pt NCs *via* incubating cancer cells, rather than noncancerous cells, with micromolar chloroplatinic acid solutions. These *in situ* biosynthesized Pt NCs emitted a strong fluorescence upon the excitation light at 405 nm and provided a precise bioimaging strategy. Furthermore, these Pt NCs could effectively kill cancer cells when conjugated with porphyrin derivatives, a photothermal therapy agent.

3.4. NCs Nanocomposites as an Agent in Cancer Therapy and Imaging

NCs have been integrated with other nanomaterials to form attractive multimodal cancer theranostic agents. Reduced graphene oxide (RGO) display excellent biocompatibility and can perfectly support various metal NCs, nanoparticles, and drugs. Gold NCs- reduced graphene oxide (GNC-RGO) were prepared by simply mixing the RGO with dedecanethiol-CTAB- capped GNCs, which were synthesized following a conventional Brust-Schiffrin process [130]. GNC-RGO loaded with DOX could result in growth inhibition of HepG2

cells and enhance the contrast of the edges and morphology of the cells in the fluorescent imaging.

In fact, NCs can further be clustered into nanoparticles and lead to novel and enhanced properties for cancer theranostic application. Akram Yahia-Ammar and coworkers [131] clustered Au NCs into nanoparticles of 120 nm in diameter using a cationic polymer (Fig. 6A). These positively charged nanoparticles exhibited an enhancement in the already strong fluorescence and drug delivery compared with free Au NCs.

NCs also can be integrated with traditional nanoparticles to generate multimodal nanocomposites for cancer theranostic applications. For example, Li and co-workers [132] conjugated a PEG-Gd₂O₃ nanoparticle with Ag NCs through the bonding between amino radical and carboxyl radical *via* the covalent coupling reaction (Fig. 6B). In the resultant PEG-Gd₂O₃/Ag NCs nanocomposite with a suitable ratio of PEG-Gd₂O₃ to Ag NCs, both the fluorescent luminescence of Ag NCs and MRI signal of PEG-Gd₂O₃ increased. Almost at the same time, Croissant *et al.* [134] coated a mesoporous silica nanoparticle (MSN) with Au NCs stabilized by BSA (AuNC@BSA) to form MSN-AuNC@BSA that could serve as a nanocarrier. These MSN-AuNC@BSA nanocarriers could simultaneously load two drugs (gemcitabine and doxorubicin) and were employed for nuclear staining and tumor imaging *in vivo*. NCs and nanoparticles could be clustered together to form a magical nanocomposite. As an example, multifunctional clusters (MNCs) Fe₃O₄-CdSe/ZnS (Fig. 6C) were synthesized by Kim *et al.* [133] and displayed excellent magnetic and optical properties and biocompatibility. MNCs-lipid A was prepared by loading lipid A onto MNCs to enhance the migration of dendritic cells (DC) and antigen-specific T cell responses to tumor *in vivo*. That phenomenon suggested that the MNC-lipid A nanocomposite could act as a candidate for the application in DC-base cancer immunotherapies.

4. SUMMARY AND PERSPECTIVE

In short, we have attempted to review the recent progress in the development of metallic NCs as well as their use in cancer therapy and imaging. In the past two decades, NCs have been extensively researched and the past studies have significantly contributed to pushing forward their applications in cancer imaging and therapy. However, there are still a few problems that remain to be solved. First, metallic NCs displayed a relatively low quantum yield and short lifetime compared with quantum dots, and their luminescence wavelength was usually from 500 nm to 800 nm, a window that was greatly absorbed and scattered by tissue. Hence, it is highly desired to develop metallic NCs with high quantum yield, long lifetime, and emission in the near-infrared region (1000–1350 nm). Second, some basic theories need to be developed to understand the controversial issues, such as the accurate structure of NCs, the relationship between structure and properties, and the effect of ligands on the characteristics of NCs. Third, though some pioneers have done some work about the cellular uptake [135–137], cytotoxicity [138–140], immunological properties [141], bio-distribution and clearance *in vivo* [142, 143] of NCs, these issues still need to be studied in depth and systematically. Fourth, precise targeting and controlled release of drugs of the NCs are still to be improved in order for us to apply them to achieve precise targeting and

effective cancer therapy. With these problems solved, we believe that NCs would find eventual clinical applications in the early diagnosis and targeted therapy of cancer.

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Fig. (1). Schematic illustration of different process to prepare NCs. **(A)** Schematic illustration of tandem chemical etching process to prepare Au NCs. Reprinted with permission from ref. [53]. Copyright 2016 American Chemical Society. **(B)** Synthesis scheme of electrochemical reduction process to prepare NCs by using porous alumina. Reprinted with permission from ref. [62]. Copyright (2015) Royal Society of Chemistry.

Fig. (2).

Structural representation of NCs protected by various ligands. All of them were prepared by chemical reduction approach. **(A)** Structural representation of $\text{Au}_{130}(\text{p-MBT})_{50}$ NCs with four shells. Reprinted with permission from ref. [73]. Copyright 2015 American Chemical Society. **(B)** Structural representation of a $[\text{Au}_{19}(\text{PhC}\equiv\text{C})_9(\text{Hdppa})_3]^{2+}$ cluster. Reprinted with permission from ref. [74]. Copyright 2015 American Chemical Society. **(C)** Schematic illustration showing the controlled reduction of Cu^{2+} ions to form NCs by integration with preselected sections of ssDNA. Reprinted with permission from ref. [75]. Copyright (2013)

Wiley-VCH. **(D)** Structural representation of $\text{Au}_{24}\text{Ag}_{20}(\text{SPy})_4(\text{PA})_{20}\text{Cl}_2$ clusters. Reprinted with permission from ref. [76]. Copyright 2015 American Chemical Society.

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Fig. (3).

The self-illuminating mechanism of ^{64}Cu -doped Au NCs and their applications in cancer imaging. **(a)** Schematic illustration showing the self-illuminating mechanism of ^{64}Cu -doped Au NCs. **(b-c)** NIR images **(b)**, PET and 3D PET reconstruction images **(c)** of ^{64}Cu -doped Au NCs. Reprinted with from ref. [109]. Copyright (2014) Elsevier.

Fig. (4).

Dual targeting Au NCs and their application in tumor theranostic. **(a-b)** Schematic illustration of AuNC-DOX-cRGD- Apt structure and **(b)** its endocytosis process. **(c-e)** *In vivo* fluorescence images by using AuNC-MPA **(c)**, AuNC-MPA-cRGD **(d)** and AuNC-MAP-cRGD-Apt **(e)**. Reprinted with from ref. [113] Copyright (2016) Elsevier.

Fig. (5).

Alkyl thiolated Au NCs with the conjugation of TAT peptide and their application in killing cancer cells, cancer cell imaging and gene transfection. **(a)** The schematic diagram of TAT peptide RS-Au NCs. **(b)** Confocal fluorescence images for photoexcitation of RS-Au NCs and TAT peptide RS-Au NCs internalized by HeLa cells by a 533 nm excitation light. **(c)** Cell viabilities of HeLa cells which uptake RS-Au NCs and TAT peptide RS-Au NCs respectively under photoirradiation conditions. **(d)** Confocal fluorescence images for the

gene expression in zebrafish microinjected with pDNA-RS Au NCs and pDNA-TAT peptide RS-Au NCs. Reprinted with from ref. [116] Copyright (2015) Wiley-VCH.

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Fig. (6).

Schematic illustration of various NCs nanocomposites. **(A)** Schematic illustration showing Au NCs self-assembly into nanoparticles. Reprinted with from ref. [131]. Copyright (2016) American Chemical Society. **(B)** Schematic illustration of PEG(Gd_2O_3)/aptamer-Ag NCs preparation. Reprinted with from ref. [132]. Copyright (2014) American Chemical Society. **(C)** Schematic illustration of the conjugation process between CdSe/ZnS NCs and Fe_3O_4

colloidal NCs. Reprinted with from ref. [133]. Copyright (2014) American Chemical Society.

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