Advanced Review

Biomedical applications of multifunctional plasmonic nanoparticles

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Plasmonic nanoparticles play an important role in biomedical applications today as they can serve as superior, optically stable bioimaging agents, be employed in biosensor devices for the early diagnosis of diseases, and exhibit promising results *in vivo* as therapeutic agents. For several bioapplications, however, nanoparticles that express more than one functionality are often advantageous. This has led to the synthesis of multifunctional plasmonic nanostructures that combine the attractive plasmonic properties with other functionalities such as magnetism, photoluminescence, dispersibility in aqueous solutions, and resistance to degradation. Such multifunctional nanoparticles can be detected by multiple imaging techniques including magnetic resonance imaging and fluorescence microscopy. Furthermore, their performance in diagnostics and therapy (theranostics) can be significantly improved facilitating the early detection of diseases. The possibility to tune the desired properties enables such hybrid nanoparticles to be employed *in vivo* as therapeutic agents that can actively target tumor sites and destroy them by external means. This makes such bionanoprobes ideal candidates for non- or minimally invasive cancer treatments. Through rational design and engineering, 'smart' multifunctional nanomaterials with unprecedented properties can be made that will lead the nano-based theranostics in the future.

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INTRODUCTION

Small, noble metal particles show great potential in biomedical applications today mainly because of their plasmonic properties. These properties originate from the interaction of such small metallic particles with electromagnetic irradiation that gives rise to the localized surface plasmons that are collective oscillations of their surface conduction electrons.[1] When the particle size is comparable to the wavelength of the incident light, this interaction influences its light absorption and scattering,[2] and thus, the particle color.[3] This was already known empirically among ancient Roman glass manufacturers as they were employing gold and silver in the glass production that resulted in differently colored glasses depending on the direction of light, with the most well-known example the Lycurgus cup.[4] The first scientific approach for the color of small metallic particles, however, was made experimentally by Faraday in 1857. He presented that gold colloids have a ruby-red color[5] and this was theoretically explained by Mie later on.[6] Since then, many studies investigate the optical properties of fine metallic particles,[3] and a number of potential applications that employ them have emerged. Among plasmonic materials, silver has the lowest optical losses in the ultraviolet–visible spectrum,[2] and therefore, exhibits better performance than the more expensive gold.

The plasmonic behavior of nanostructured materials has ignited intense research for the fundamental physics of plasmonic structures[7] and their applications in other fields, such as catalysis[8] or energy harvesting.[9] However, most research dealing

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with plasmonic nanomaterials today concerns the biomedical field. The bioapplications of plasmonic materials can be divided into three large areas: (1) sensor devices for diagnostics; (2) *in vitro* imaging and diagnosis; and (3) *in vivo* imaging, diagnosis, and therapy (theranostics).[10]

In order to increase the potential bioapplications of plasmonic nanoparticles, they are often synthesized along with another functional material to form multifunctional nanostructured materials. Typical extra functionalities that are desired along with the plasmonic properties of a nanoparticle are improved dispersibility in aqueous liquids, magnetism, luminescence, and stability against degradation. Figure 1 shows a schematic of various plasmonic nanoparticle morphologies that fulfill the above functionalities and are discussed later on. For example, when superparamagnetic nanoparticles or quantum dots are anchored on plasmonic nanoparticles, hybrid nanoparticles are made that can be employed in multiple imaging techniques, or be guided to the desired areas by an external magnetic stimulation.

Here, a short overview of the potential biomedical applications of plasmonic nanoparticles is presented, emphasizing on plasmonic devices for detection, as bionanoprobes for imaging and *in vivo* theranostics. Special focus is given on synthesis of multifunctional plasmonic nanoparticles that are typically composed of, at least, one more material apart from the plasmonic one. Finally, the biomedical applications of such multifunctional plasmonic nanoparticles are discussed along with their potential advantages over the traditional plasmonic nanoparticles, highlighting the current opportunities, limitations, and challenges.

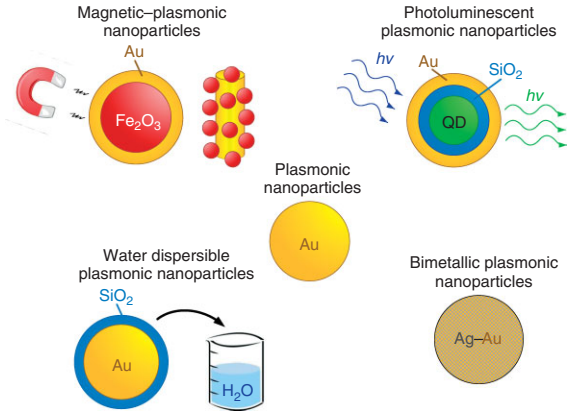

FIGURE 1 | Different morphologies of multifunctional plasmonic nanoparticles that include magnetism, photoluminescence, dipsersibility in aqueous solutions, and enhanced stability against degradation by alloying.

BIOMEDICAL APPLICATIONS OF PLASMONIC NANOPARTICLES

Gold and silver nanostructures are employed as plasmonic biosensors for the detection of specific biomolecules and proteins that are relevant for specific diseases.[11] The plasmon extinction (absorption and scattering) band of such nanostructures depends on the refractive index of the medium surrounding them; for higher refractive index, it undergoes a red-shift.[12] The refractive index of proteins or DNA is higher than buffer solutions, therefore, upon attachment on the plasmonic surface, the local refractive index increases. This causes the plasmon extinction band to shift to higher wavelengths, which is termed the biosensor response.[13] The basic concept is visualized in Figure 2. Such localized surface plasmon resonance (LSPR) biosensors hold potential advantages when compared to traditional thin plasmonic continuous films (surface plasmon resonance—SPR) biosensors. They exhibit less interference from the refractive index of the buffer solution and possess greater spatial resolution.[12] Typical plasmonic nanostructures for biosensing are triangular nanosilver particles,[11] nanocubes,[14] or rhombus.[15]

Surface-enhanced Raman scattering (SERS) is another topic of intense research. The Raman scattering of organic (bio)molecules is greatly enhanced when the latter are attached on nanostructured plasmonic surfaces.[16] That way, biomolecule concentrations close to the single-molecule detection can be achieved.[17] Again, such plasmonic nanostructures can be employed for the detection of disease-specific biomolecules at low concentrations. That is of vital importance since, for many diseases, an early diagnosis may prevent the death of a patient or improve significantly the patient's living quality.[18] Even though this sensing technique shows great potential, limitations regarding the scalability and reproducible fabrication of such sensors prohibit their commercialization.[19]

Plasmonic nanoparticles strongly scatter light, and thus, can be detected easily under dark-field illumination[20] and by other techniques.[21] This enables them to be employed in various *in vitro* biological applications, and in particular for investigations of nanoparticle–cell interactions. Such nanoparticles are advantageous over the commonly used organic fluorescent dyes, as the latter decompose during imaging, exhibiting photobleaching. In contrast, plasmonic nanoparticles are photostable and thus can be used as bionanoprobes to monitor continuously dynamic events over an extended period time with an exceptionally stable signal.[22] For example, gold nanoparticles biofunctionalized with epidermal growth factor (EGF) on their surface have been

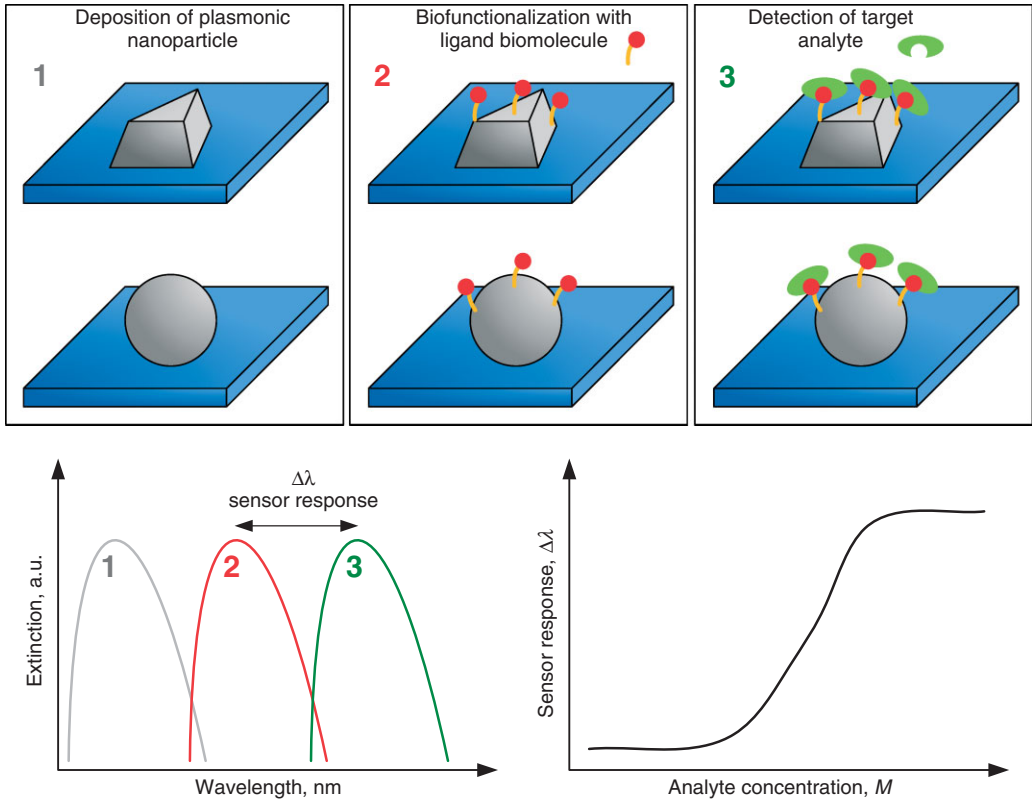

FIGURE 2 | Localized surface plasmon resonance biosensors: the basic principle of biomolecule detection. First, the plasmonic nanoparticle deposition occurs on a glass slide, whose optical properties can be monitored *in situ* (step 1, gray line). Then, the plasmonic surface is biofunctionalized with the ligand biomolecule (, step 2, red line). The addition of the corresponding biomarker analyte ( ) follows (step 3, green line) that selectively attaches on the ligand biomolecule ( ). The sensor response corresponds to the Δλ plasmon peak shift, which depends on the analyte concentration.

selectively attached on cancer cells. These cells overexpress the epidermal growth factor receptor (EGFR) on their surface, thus the gold nanoparticles selectively attach on them as verified by dark-field microscopy.[23,24] Therefore, such plasmonic nanoparticles can be employed today as advanced bionanoimaging agents that are used by biologists to monitor specific drug effects on cells or to identify specific pathways.

Prerequisite for this function is that such bionanoprobes must not be toxic to the cells that are investigated. Gold nanoparticles are typically preferred over silver for such bioapplications mostly because of their higher biocompatibility than silver nanoparticles.[25] Even though silver nanoparticles are plasmonically superior to gold, the potential toxicity of silver nanoparticles is their main limitation and prevents them to be broadly used in this field.[26] Recent advancements, however, involve the encapsulation of silver nanoparticles with a nanothin, hermetic, biocompatible silica coating that minimizes their toxicity, and therefore, facilitates their employment in bioapplications.[27,28]

The specific attachment of plasmonic nanoparticles to target cells has facilitated the employment of such nanoparticles as *in vivo* bionanoprobes for theranostics. Plasmonic nanoparticles are therefore conjugated to cancer cells or tissues and are used to absorb light and convert this light into thermal energy.[29,30] This increases the local temperature around the nanoparticles that destroys the targeted tissues by thermal ablation, enabling these particles to be used as novel therapeutic agents in non- or minimally invasive cancer treatments.[31,32] In fact, the plasmonic properties of gold and silver nanoparticles can be tuned by changing their shape and size to obtain their highest extinction band in the near-infrared (near-IR) window, in which the tissue is permeable.[33,34] That way, an external light source (e.g., IR laser) can be used, from which the irradiation is transmitted through the skin.[35] Most well-known examples of such gold nanostructures are the gold nanoshells[36] and gold nanorods.[37] Both structures have their plasmon extinction maximum in the near-IR that facilitates their employment *in vivo* for the photothermal ablation of tumors, as it

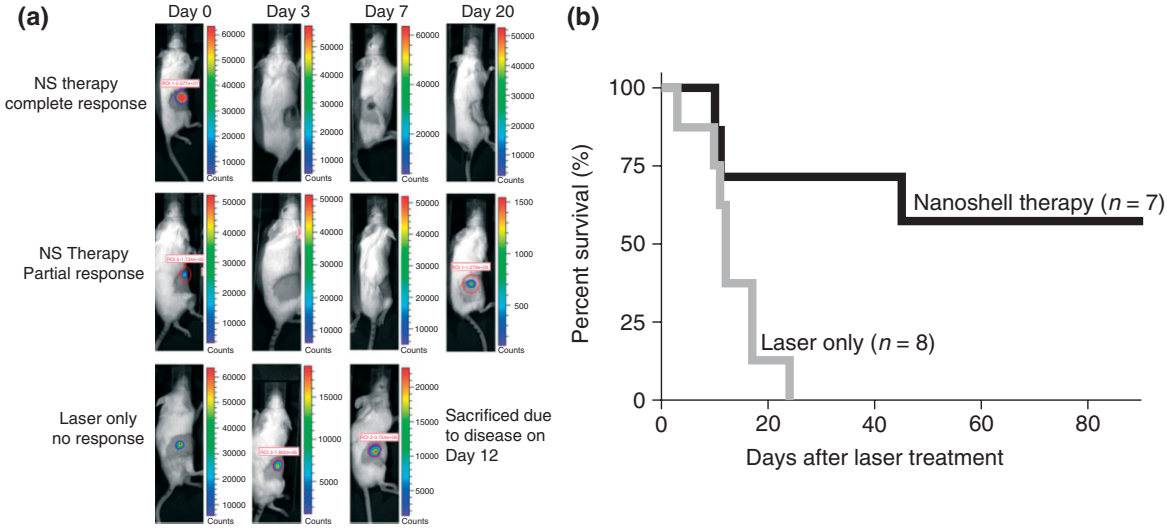

FIGURE 3 | Bioluminescent imaging of tumors in mice following laser irradiation over time and gold nanoshell treatment. The gold-nanoshell-treated mice displayed complete or partial tumor loss, while the tumor volume in the mouse of the control group only increased. The survival of the treated mice is significantly longer than the control group mice, as more than 50% of the treated mice remained tumor-free for more than 90 days. (Reprinted with permission from Ref 38. Copyright 2011 Springer)

has been demonstrated in animal studies with promising results.[38] Figure 3 shows bioluminescent imaging of tumors in mice treated with gold nanoshells and without treatment followed by near-IR laser irradiation. The mice treated with the nanoshells showed complete or partial response after 20 days, whereas the untreated mice showed no response at all. In fact, the survival for the nanoshell therapy group was much higher than the control group, and more than 50% of the mice were tumor-free for 90 days postirradiation.[38]

In such *in vivo* studies, there are generally two main targeting ways to deliver the bionano therapeutic agents to the cancerous site: the passive and the active targeting.[39] With the passive targeting, the nanoparticles are accumulated to tumor sites by the enhanced permeability and retention (EPR) effect.[40] The blood vessels supplying tumors are more leaky than normal ones. Therefore, nanoparticles that circulate in blood preferentially accumulate in tumor sites.[35] However, passive targeting using the EPR effect depends on various physicochemical characteristics of the nanoparticles (e.g., surface charge and size) and the tumor tissues. This often results in final nanoparticle concentration in the tumor site below the effective ones.[39]

One way to overcome this limitation is to selectively target the bionanoprobe on tumor sites *in vivo* by exploiting the specific attachment of two biomolecules. Cancer cells typically overexpress specific biomarkers on their surface that selectively bind to corresponding ligand biomolecules. Thus, they can selectively target the tumor tissue by attaching on the nanoparticle surface these corresponding ligand biomolecules.[41] With this active targeting, nanoparticles are localized at the tumor site, and thus, any photothermal treatment takes place only on these sites. Further advancements in active tumor targeting include the engineering of nanoparticles that can communicate *in vivo* leading to an enhanced accumulation of the therapeutic compounds.[42]

MULTIFUNCTIONAL PLASMONIC NANOPARTICLES

Improved Dispersibility in Aqueous Solutions

The performance of plasmonic nanoparticles as bionanoprobes depends on their hydrodynamic size and dispersibility in aqueous and biologically relevant solutions. The dispersibility of nanoparticles can be improved by surface modification. Typically, organic coatings are present on the nanoparticle surface from their wet synthesis, such as citrate,[43,44] polymers,[45] or even starch.[46] Such coatings, however, may not be stable over time, and therefore, often an extra inorganic surface coating is sought.[47] Such coatings should be ideally benign (nontoxic) and, at the same time, protect the core and maintain (or enhance) its properties. An SiO_2 coating can be applied by wet chemistry on both gold and silver nanoparticles.[47–49] The surface coating thickness can be well controlled

and is quite homogeneous. Even though such SiO_2 coatings may improve the nanoparticle dispersibility, they are rather porous allowing the transport of ions.[48–50] For example, silver nanoparticles readily release Ag^+ ions upon contact with water (Box1).[51] Such Ag^+ ions can be toxic to biological systems,[52] and therefore, even though the silver nanoparticles are much better dispersed, their employment in bioapplications is still prohibited.[53]

BOX 1

ANTIBACTERIAL ACTIVITY OF NANOSILVER

Silver nanoparticles (nanosilver) exhibit unique bactericidal properties, and therefore, find potential applications in hospital treatments (e.g., catheters), and also in textiles, polymers for food packaging, and household devices. These antibacterial properties are greatly influenced by the released Ag^+ ions from the nanosilver surface.[51,54,55] At the same time, however, concerns exist for the fate of the released Ag^+ ions in the environment that may threaten beneficial microorganisms.[56] This Ag^+ ion release is higher for smaller nanosilver particles than for larger, and actually originates from the dissolution of the surface silver oxide, indicating a surface area dependency.[51,57] The mode of action of the antibacterial activity of nanosilver strongly depends on its size. Small (< 10 nm) nanosilver particles release a large fraction of Ag^+ ions from their surface, and therefore, these ions play the dominating role. In contrast, large (> 15 nm) nanosilver particles do not release many Ag^+ ions from their surface, and thus, their antibacterial activity is mediated by the released Ag^+ ions as well as the direct contact with the nanosilver surface.[51] This understanding on the nanosilver toxicity and antibacterial activity mechanism may facilitate the engineering of nanosilver products that offer their desired bactericidal performance mainly because of the surface contact, rather than the released Ag^+ ions with minimal impact on the environment and human health.

In contrast, SiO_2 coatings made by dry, gas-phase processes are rather dense and hermetic.[58,59] Such coatings on silver nanoparticles inhibit the Ag^+ ion transport, and therefore, minimize the Ag^+ ion release from the nanosilver surface[57] enabling them to be used in bioimaging applications.[28] The improved nanoparticle dispersibility can also facilitate the employment of such plasmonic nanoparticles in biosensing. Flame-made SiO_2-coated (2 nm SiO_2 coating thickness) nanosilver particles were finely dispersed in aqueous solution with limited agglomeration (flocculation) and deposited on poly(L-lysine)-coated glass slides by electrostatic forces.[27] Their superior spatial homogeneity enabled such LSPR biosensors to exhibit comparable response for the detection of bovine serum albumin to plasmonic biosensors made by elaborate nanofabrication and lithographic techniques.[60,61] Therefore, plasmonic biosensors with superior performance could be made in a scalable and reproducible way by coating the plasmonic nanoparticle surface with another material with the desired functionality.

Magnetic–Plasmonic Nanoparticles

Magnetic nanoparticles also play an important role in bioapplications.[62] Apart from their employment as magnetic resonance imaging contrast agents,[63] they can also be employed in magnetic cell sorting and separation.[62] Typically, iron oxide nanoparticles are preferred as they are superparamagnetic, and therefore, exhibit the desired magnetic properties only in the presence of an external magnetic field.[64] Furthermore, iron oxide superparamagnetic nanoparticles can also be used as therapeutic agents by hyperthermia (Box2).[64] Therefore, combining them with plasmonic nanoparticles can open new opportunities for multimodal imaging and therapy.

Core-shell magnetic–plasmonic nanoparticles can be synthesized by wet chemistry, in which the core iron oxide is formed by coprecipitation of iron salts and is followed by the reduction of Au^{3+} onto the iron oxide surface to form the gold layer.[65] Hybrid magnetic core-plasmonic shell structures combine the tunability of the plasmon extinction band to the near-IR with the strong MRI contrast.[66] In fact, such particles have been selectively attached on breast cancer cells using the EGFR and detected under dark-field illumination as well as by MRI, as seen in Figure 4.[66] Furthermore, the same breast cancer cells labeled with these nanoparticles were irradiated with a near-IR laser beam (700 nm), leading to their selective photothermal destruction. It should be noted that the unlabeled cells were not influenced by this irradiation, emphasizing the potential of such selective photothermal tumor treatment[66] (Figure 4). Similar gold-coated iron oxide nanoparticles have also been selectively attached on breast cancer cells and separated from cell mixtures containing also healthy cells,[67] while their pharmacokinetics was investigated *in vivo* employing multimodal imaging after active targeting tumor sites in animal models.[68]

BOX 2

HYPERTHERMIA TUMOR TREATMENT BY SUPERPARAMAGNETIC NANOPARTICLES

Superparamagnetic nanoparticles exhibit magnetization only in the presence of an external magnetic field. When the external magnetic field is oscillating, these nanoparticles overheat because of magnetic hysteresis loss. If, therefore, such superparamagnetic nanoparticles are localized around a tumor tissue and an external magnetic field is oscillating, the temperature increase caused by the superparamagnetic nanoparticles may be high enough to burn the tumor.[64] Similar to other nano-based therapeutic agents, their targeting at the tumor sites may be achieved by passive or active targeting. Furthermore, in case the cancer cells at the tumor site are still alive after the hyperthermia treatment, the efficacy of administered anticancer drugs is increased.

Gold nanorods surface decorated with superparamagnetic iron oxide nanoparticles also combine a strong plasmon absorption in the near-IR with the magnetic functionality. Typically, such structures are made by wet chemistry; the gold nanorods are synthesized first, followed by the nucleation of the iron oxide nanoparticles on their surface.[69] Such nanostructures can also selectively attach on cancer cells by active targeting and upon near-IR irradiation, the cell photothermal ablation was demonstrated.[69] Similar gold nanorods decorated with iron oxide nanoparticles are also used to detect bacterial pathogens (*Escherichia coli*, *Salmonella typhimurium*) and subsequently destroy them photothermally.[70]

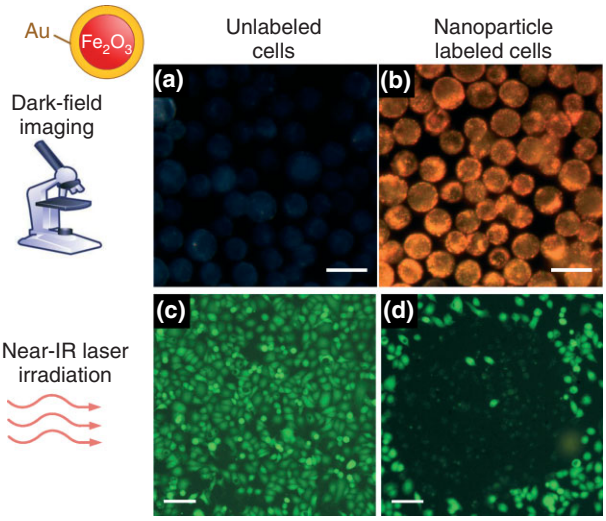

FIGURE 4 | Hybrid magnetic–plasmonic core-shell nanoparticles. Dark-field images of unlabeled breast cancer cells (a) and of nanoparticle-labeled cells (b). The hybrid nanoparticles have been selectively attached on the cells and can be clearly detected (scale bar is 30 μm). When these cells are irradiated by a near-IR laser beam, only the labeled cells are destroyed (c, green marks the living cells), while the cells incubated with PEGylated nanoparticles do not show any effect (d). Scale bar is 100 μm. (Reprinted with permission from Ref 66. Copyright 2007 IOP Publishing)

Further hybrid plasmonic–magnetic morphologies include heterodimers, or the so-called dumbbell-like or Janus-like nanoparticles. During their wet synthesis, typically the iron oxide nanoparticles are made first, which act as seeds for the heterogeneous nucleation of the plasmonic material on their surface.[71] Such tedious processes consist of many steps including cleaning/purifying, and often an extra functionalization step to convert the nanoparticles from hydrophobic to hydrophilic.[21] In contrast, gas-phase synthesis of such nanoparticles offers several advantages as it typically involves fewer process steps, is fast and scalable, and the as-produced nanoparticles do not require cleaning or further purification.[72] Gold-decorated silica nanoparticles have been synthesized by aerosol processes,[73,74] and when a silica coating is applied on the surface of flame-made nanoparticles *in situ*, they become hydrophilic without any additional surface treatment.[28]

Composite dumbbell-like silver/iron oxide nanoparticles were incubated with murine macrophages that led to their internalization by endocytosis. These cells could be manipulated by an external magnetic field and they could also be visualized *in vitro* by two-photon fluorescence microscopy.[21] Janus-like, composite silver/iron oxide nanoparticles, hermetically-coated with silica,[28] were incubated with HeLa and Raji cells that overexpress on their membrane, the hDC-SIGN. These magnetically manipulated nanoparticles were biofunctionalized with the corresponding ligand antibody, and therefore, their selective attachment on the cell membrane was visualized by dark-field imaging. Furthermore, the amorphous silica coating enhanced the nanoparticle biocompatibility, as they exhibited no cytotoxicity for 24 h. It should be noted that silica in its crystalline form (quartz) can also exhibit toxicity,[75] and thus, the crystallinity of the silica surface coating should be appropriately designed.

Photoluminescent Plasmonic Nanoparticles

Light-emitting nanoparticles can also be employed in biomedical applications as bioimaging agents,

and they can be integrated in existing biosensing assays. Their combination with plasmonic materials, therefore, opens the way for the employment of multifunctional bionanoprobes in multiple imaging and detection techniques. Typical light-emitting materials for bioapplications include fluorescent organic agents. Such agents can be bound to selected biomolecules, and be introduced in the cell environment to monitor the targeted interactions. Composite plasmonic-fluorescent nanoparticles are made by coating, for example, the core-plasmonic gold or silver nanoparticles with a polymer layer, in which the fluorophore (e.g., a fluorescent dye) is integrated.[76] That way, the plasmonic properties remain, while the light emission from the fluorophore occurs upon its excitation. Similarly, the fluorophore can be integrated in an inorganic coating, such as silica.[77]

An interesting phenomenon that occurs when a fluorophore is in the proximity of a plasmonic material is its greatly enhanced light emission, exhibiting metal-enhanced fluorescence.[78] Various fluorophores can either adsorb or be covalently bound on the surface of silica-coated silver nanoparticles by wet chemistry.[79] This can result in a 20-fold enhancement of the fluorescence and potentially increase 200 times the particle detectability leading to single nanoparticle sensing.

Semiconductor nanoparticles (quantum dots) also show great potential in bioimaging,[80] especially because they exhibit higher optical stability than fluorescent dyes. The challenge in their synthesis along with a plasmonic material lies in the spatial separation of the two components. Depending on the position of the plasmonic material with respect to the quantum dots, the fluorescence of the latter may be enhanced or quenched.[81] A potential solution was separating the gold core nanoparticle from the quantum dot surface nanoparticles (CdSe) with an intermediate, inorganic silica coating. Therefore, by controlling the silica layer thickness, the photoluminescence intensity could be quenched or enhanced.[81] A further approach for the synthesis of such structures is the complete encapsulation of a quantum dot (CdSe/ZnS) with a continuous gold layer.[82] In this case, the nanothin gold film is also not in contact with the core quantum dot, but is separated by polyelectrolyte bilayers formed by the layer-by-layer method.

The integration of fluorescent ZnO quantum dots in a temperature responsive poly(ethylene glycol) hydrogel, and the further addition of plasmonic gold nanoparticles resulted in the synthesis of a plasmonic-quantum dot multifunctional hydrogel nanoparticle.[83] In those nanoparticles, both the ZnO photoluminescence and the gold plasmonic properties were present (Figure 5(a)). The nanoparticle detection upon their incubation with murine melanoma cells was possible with a fluorescence microscope, showing a bright-yellow signal consistent with the ZnO photoluminescent spectrum (Figure 5(b)). As a matter of fact, when a cancer drug was loaded in the hydrogel and the nanoparticles were near-IR irradiated, the temperature rise induced by the gold nanoparticles caused the drug release, which is consistent to existing drug-release models[84] (Figure 5(c)).

Recently, another type of materials, the so-called upconversion nanocrystals, have been developed that can also be used in bioimaging applications. These materials exhibit nonlinear optical properties; they are excited by a long-wavelength radiation (e.g., IR or near-IR) and emit a short-wavelength radiation (e.g., ultraviolet or visible). These nanoparticles are typical nanophosphors doped with rare-earth ions, similar to the visible-light nanophosphors for bioimaging.[85] They are particularly useful in bioapplications because they can be activated by near-IR radiation that can penetrate tissues. When such nanoparticles are combined with plasmonic ones, an enhanced light emission can be detected and the composite particles can be detected by multiple imaging. For example, silica-coated upconversion nanophosphors were further coated with a gold nanoshell by a peptide-templated method.[86] The plasmon resonance peak of the gold nanoshell was tuned to the near-IR region in order to overlap with the excitation band of the nanophosphors. Then the luminescence emission intensity was greatly enhanced *via* energy transfer from the plasmonic gold to the nanophosphors. When these hybrid nanoparticles were incubated with melanoma cells, they could be detected by both fluorescence and dark-field microscopy. Similarly, hybrid upconversion nanophosphors decorated with gold nanoparticles were incubated with neuroblastoma cells, and upon near-IR irradiation they were photothermally destroyed.[87]

Improved Performance with Bimetallic Plasmonic Structures

As already mentioned, silver has superior plasmonic properties to gold, and thus, plasmonic devices employing silver, including the LSPR and the SERS, exhibit better performance than the devices with gold. Silver, however, easily oxidizes over time and forms plasmonically inactive halides and sulfides when interacting with biological liquids, deteriorating the final device performance.[88] In contrast, gold is more resistant to oxidation and thus, for such devices, gold

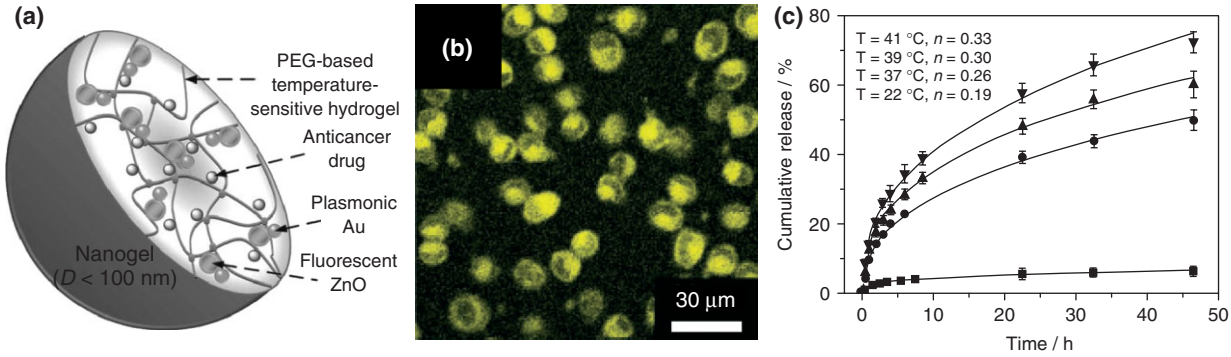

FIGURE 5 | (a) A hybrid nanoparticle consisting of ZnO quantum dots, plasmonic gold nanoparticles, and anticancer drug in a thermally responsive hydrogel. (b) The ZnO quantum dots enable the nanoparticle detection under a fluorescent microscope (yellow signal), while when these nanoparticles are irradiated by a near-IR laser, the hydrogel shrinks, releasing, therefore, the anticancer drug (c). (Reprinted with permission from Ref 83. Copyright 2011 Wiley-VCH)

is preferred, compromising the final performance for longer and more stable use. Another approach is to employ both those plasmonic materials. This can be done either by forming a protective Au coating on the Ag nanostructures, or by forming Ag–Au bimetallic alloys. For example, Au-coated Ag nanoparticles were successfully synthesized by wet chemistry and used in immunoassays for specific IgG antibody detection by SERS.[89] In another study, Au–Ag alloyed nanorods made by galvanic replacement reaction were more stable than pure Ag nanorods when either exposed in NaCl solution or left in ambient air for 4 days, and therefore, exhibited an improved SERS signal.[90] Similarly, Au–Ag alloy nanowires were grown topotaxially that exhibited corrosion resistance when compared to pure Ag nanowires while maintaining a comparable SERS performance.[91] Such composite Au–Ag nanostructures can be used as SERS biosensors especially when they are exposed in aggressive environments, with improved performance.

CONCLUSIONS AND OUTLOOK

The biomedical field today has obtained a great benefit from nanotechnology as it helps the diagnosis and even therapy of diseases. The unique physicochemical properties of gold and silver nanoparticles have brought them to the foreground of such nanotechnology-based products and applications. Because of their interaction with light that gives rise to their plasmonic properties, gold and silver nanoparticles and nanostructures can be employed in a variety of biomedical applications, including diagnosis with devices *in vitro*, as well as combination of diagnosis and therapy *in vivo*. Therefore, plasmonic nanoparticles have been employed for the development of nanoparticle-based imaging probes and therapeutic carriers that offer an alternative approach for theranostics with non- or minimally invasive treatment.

The increasing demand, however, in modern biology and medicine for highly sensitive and specific detection of biomolecules and for advanced molecular imaging agents requires the synthesis of nanomaterials with unprecedented physicochemical properties. This can be achieved today with the rapid advancement of nanotechnology that makes it possible to synthesize composite plasmonic nanoparticles with other materials. That way, multifunctional plasmonic materials can be made that combine the unique plasmonic properties with the desired extra functionality. Such hybrid nanostructured materials will revolutionize the way diseases are diagnosed and treated.

Multifunctional plasmonic materials are suitable for multiple detection techniques. Composite plasmonic–magnetic nanoparticles can serve as superior MRI contrast agents, and plasmonic-luminescent nanoparticles can be detected through fluorescence microscopy. Bioimaging is one field that can definitely benefit from them since the small nanoparticle size facilitates the interactions with biological systems, such as mammalian cells or bacteria. That way, optically superior bionanoprobes can be employed for the identification of specific cell interactions and pathways. Even though currently most studies that employ such nanoparticles focus on cancer treatment, their potential can be extended to other diseases, especially when such bionanoprobes are combined in multicomponent systems for controlled drug release.

When such nanoparticles are used as *in vivo* therapeutic agents for cancer, an extra functionality, such as the magnetic, can facilitate the bionanoprobe biodistribution and guiding to the tumor site, as well as offer an extra therapeutic possibility by

hyperthermia. Through rational design and engineering, sophisticated multifunctional nanoparticles, similar to other polymeric[92] nanoparticles, can be made that fulfill all important requirements for nano-based theranostics[68]: they need to be well-characterized nontoxic materials, hydrophilic, with improved blood circulation time and high targeting and uptake efficiency in the required organs and tissues, efficient clearance, their agglomeration–flocculation should be limited *in vivo*, and have 'stealth' properties against macrophages that are the first line of defense in a human body.

Several challenges remain, however, before such bionanoprobes can be safely employed in therapeutic applications. Their specific accumulation and targeting still needs to be improved as it clearly is one of the most determining factors for the effectiveness of the therapeutic treatment. Apart from the minimization of their clearance from the immune system, their enhanced accumulation in tumor sites may be achieved when specific antiangiogenesis therapies[93] are delivered along with the bionanoprobes. Further studies, however, need to be carried out especially *in vivo* with animal models in order to identify the pharmacokinetics, biodistribution, and efficiency of such nano-based therapeutic probes. Only after such biological studies have taken place, human clinical trials may then start, as with gold nanoshells designed by West and Halas, which are currently in clinical trials.

Even if a solid understanding is obtained on how such multifunctional bionanoprobes behave in biological systems and engineer sophisticated 'smart' nanomaterials that possess all desired functionalities, there is still a gap till they finally reach the clinic. Such nanostructured materials need to be made in a cost-effective and scalable way such as gas-phase aerosol synthesis of materials that offers unique advantages.[72] Current fabrication techniques will need to be improved or even new fabrication techniques may be required to be developed. This, in turn, means that a fundamental physical understanding in existing chemical processes is needed by physicists, chemists, and engineers, while the interactions of the bionanoprobes with biological systems will be analyzed by biologists, biochemists, and physicians. This truly shows the interdisciplinary nature of nanomedicine and nanobiotechnology.

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