Silver nanoparticle in biosensor and bioimaging: Clinical perspectives

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Abstract

Recent developments in nanotechnology promoted the production of nanomaterials with various shapes and sizes by utilizing interdisciplinary researches of biology, chemistry, and material science toward the clinical perspectives. In particular, gold and silver (Ag) are noble metals that exhibit tunable and unique plasmonic properties for the downstream applications. Ag exhibits higher thermal and electrical conductivities, and more efficient in the electron transfer than gold with sharper extinction bands. In addition, modified Ag nanoparticle is more stable in water and air. With all these above features, Ag is an attractive tool in various fields, including diagnosis, drug delivery, environmental, electronics, and as antimicrobial agent. In particular, applications of Ag nanoparticle in the fields of biosensor and imaging are prominent in recent days. Enhancing the specific detection of clinical markers with Ag nanoparticle has been proved by several studies. This review discussed the constructive application of Ag nanoparticle in biosensor and bioimaging for the detection of small molecule to larger whole cell in the perspectives of diagnosing diseases.

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Keywords: *biomarker, clinical imaging, diagnosis, metal nanoparticle*

1. Introduction

Attainments with nanomaterial are growing faster and gaining popularity with various researches and experienced new approaches for the development of sensing methods and bioimaging due to their excellent optical properties [1,2]. Noble metals are having unique physical, chemical properties and high-surface area, which helps to improve the performance of biosensor for sensitive and accurate biomolecular detection [3–5]. At present, various nanoparticles such as gold, platinum, titanium, zirconium, and magnetic nanoparticles have been used in various sensors to improve the strategies of detecting biomolecules [6]. Among them, gold nanoparticle shows attractive features and applied for probe immobilization and target detection on the sensing surfaces with the improved detection limit. Similar to the gold nanoparticle, silver (Ag) nanoparticles are attracted in the biotechnology fields including antimicrobial, diagnostic, and therapeutic [7–10]. Also, it has been used in sensor materials, cosmetic products, conductive materials, and electronic components. Ag nanoparticle application in biosensor improved the limit of detection of target due to its high thermal, chemical stability, electrical conductivity, and catalytic activity [11]. Generally, Ag nanoparticles are stable as stated in the earlier studies and demonstrated by the stability

Abbreviations: *Ag, silver; COOH, carboxyl; PVP, polyvinyl pyrrolidone; SERS, surface-enhanced Raman spectroscopy.*

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Highlights

Silver is an attractive tool in various fields, including diagnosis, drug delivery, environmental, electronics, and as antimicrobial agent. In particular, applications of silver nanoparticle in the fields of biosensor and imaging are prominent in recent days. This review discussed the constructive application of Ag nanoparticle in biosensor and bioimaging for the detection of small molecule to larger whole cell in the perspectives of diagnosing diseases.

analyses [7,8]. Regarding the attachment of Ag nanoparticle on the sensing surfaces, it can be accomplished by the electrostatic attraction. However, it is generally recommended to make the surface chemical modification in order to create a strong attachment on the surface. Ag nanoparticle is suitable for different surface chemical modifications, depending on the desired molecular attachment. (3-Aminopropyl)triethoxysilane is one of the suitable chemical functionalizations for Ag nanoparticle by the silane reaction. This happens due to the presence oxide groups on the Ag nanoparticle formed when it exposed to the atmospheric moisture.

Ag nanoparticle was predominantly known for the antimicrobial potential [12–14]. Recent studies have shown the interest and impact of Ag nanoparticle in the biosensor and bioimaging applications. Ag nanoparticle has less refractive index than most of the molecules. When attach the biomolecule on Ag nanoparticle, it shows an increment of local refractive index, and can visibly see the Ag extinction shift. Utilizing these changes with Ag nanoparticle, various sensors have demonstrated the efficient detection of target molecule. In addition, protective coating (such as silica) on the Ag nanoparticle shows the excellent improvement on biomolecular detection [15]. Various chemical and physical methods were utilized to stabilize Ag nanoparticles and applied in various sensors including, surface plasmon resonance, enzyme-linked immunosorbent assay (ELISA), Raman spectroscopy, and electrochemical sensor to determine various clinical biomarkers at higher sensitivity [16]. In this review, authors have discussed on Ag nanoparticle for its applications in the fields of biosensor and bioimaging.

2. Synthesize of Ag Nanoparticle

Silver nanoparticle has been prepared by chemical, physical, or biological synthesis processes. It includes gamma irradiation, chemical reduction, microwave processing, laser ablation, electron irradiation, and biological synthetic methods [16]. Ag nanoparticle is generally synthesized by the chemical reduction by using the reducing agents including Tollens reagent, ascorbate poly(ethylene glycol)-block copolymers, sodium citrate, sodium borohydride, N,N-dimethylformamide, polyol process, and elemental hydrogen. These agents were used for the reduction of Ag ions in nonaqueous or in aqueous solutions and formed the metallic Ag, followed by the agglomeration

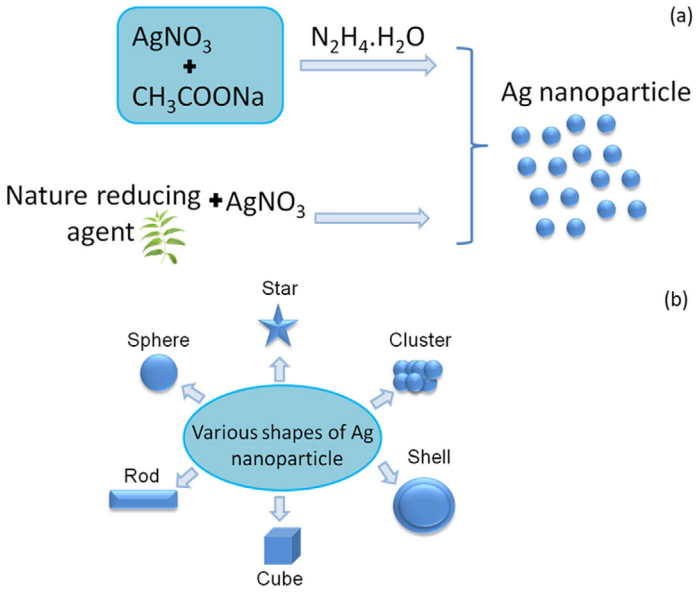

FIG. 1 *(A) Schematic representation of silver nanoparticle synthesis. In chemical reduction, silver nitrate was used as the base solution and N_2H_4 was used as the reducing agent. With biological reduction, the compounds in the extract used as the reducing agents. (B) Possible shapes of Ag nanoparticle upon reduction.*

into oligometric clusters. And then the oligometric clusters lead to the formation of metallic Ag nanoparticles. During the synthesizing process, the nanoparticles are stabilized with the protective agents to avoid agglomeration. Moreover, surfactants also used are to stabilize the particle growth and improve the surface property. Figure 1A displays the schematic representation for Ag nanoparticle synthesizes by the chemical reduction, in which Ag nitrate was used as the base solution, and N_2H_4 was as the catalyst. In biological methods, bacteria, fungi, and plants were used as the reduction agent. Ag nanoparticles have been synthesized with various shapes, such as sphere, plate, rod, and wire, at various sizes (10–100 nm) and applied for various purposes (Fig. 1B). Biologically synthesized nanoparticles are showing excellent stability and nonimmunogenicity that are mandatory for the medical applications, such as drug delivery, biosensor, and bioimaging.

3. Biomolecular Immobilization on Ag Nanoparticle

Immobilization of biomolecules on the nanoparticle has been carried out through electrostatic interaction, chemical, and physical attractions. Generally, the surface charge of the Ag depends on the stabilizer used. Positive- and negative-charged nanoparticles can produce by choosing the suitable stabilizer [17]. In general, polyvinyl pyrrolidone (PVP)-stabilized nanoparticle shows negative charge and polyethyleneimine-stabilized

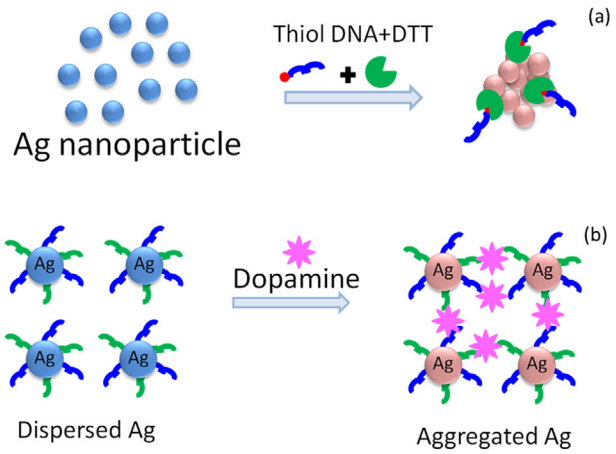

FIG. 2 *Detection strategies with Ag nanoparticle. (A) Thiolated Ag nanoparticle DNA in the presence of dithiothreitol. (B) Protein detection by Ag nanoparticle-based colorimetric assay. Nanoparticle shows the aggregation in the presence of dopamine.*

nanoparticles show positive charge on the nanoparticles [18]. Biomolecules such as DNA, RNA, antibody, aptamer, and peptide were utilized to immobilize on Ag nanoparticle for the efficient detection and imaging purposes. Various linkers such as thiol is commonly used to immobilize the biomolecules on the surface of the Ag nanoparticles. Figure 2A represents the preparation of thiolated Ag nanoparticle DNA in the presence of dithiothreitol. This modified DNA can efficiently detect the complementary DNA by various sensors. The problem with biomolecular conjugation on Ag nanoparticle is found to have aggregation, so that, the optimum condition should be analyzed for the immobilization process. Proteins can bind with Ag nanoparticle through hydrophobic, dative bonds and electrostatic interaction [19]. Citrate-capped nanoparticles are readily linked with various molecules, including thiol, antibody, amine, protein, and polymer. But PVP binds very strongly to the Ag surface and it shows higher stability than tannic acid or citrate-capped Ag surfaces; however, the displacement is very difficult. Biomolecules also can immobilize on the Ag nanoparticles through the reactive groups, such as carboxyl (COOH) or amine terminations.

4. Ag Nanoparticle-Based Biosensors

Improvement of the performance with biosensor is mandatory in the field of medical sciences to support the human health and extend the human life span. Various life-threatening diseases including cancer, HIV and viral diseases, and bacterial infection should be detected earlier to treat the diseases effectively. These diseases are detected by probes, DNA, protein, and by antibody or enzyme secretion. Nanoparticle-conjugated probes proved to enhance the detection of target biomolecules. Like other nanoparticles, Ag nanoparticles also actively participate in the field of biosensor; probe-conjugated nanoparticles proved to show the high-efficient detection of biomolecules. Ag nanoparticle-conjugated biomolecules show the excellent stability and sensitivity toward the target molecular validation and applied in various sensors, such as surface plasmon resonance, Raman spectroscopy, ELISA, Waveguide, and electrochemical detections. Table 1 shows the biomolecular detection by using Ag nanoparticle-conjugated probe. Followings are some of the examples of biomolecular detection by Ag nanoparticles.

5. Detection of DNA by Ag Nanoparticle

DNA research is evolving in several areas of research, including medical, forensic science, agriculture, paternity, food technology, and biowarfare defense [33]. Especially, DNA detection is more popular in the field of genetics and medical research. Nanoparticle combined with oligonucleotide (DNA/RNA) has shown more stability and directs to the lower detection limit. DNA can immobilize on Ag nanoparticle through the terminal alkyl thiol group at the 3'/5' terminus [25]. Ag nanoparticle-conjugated DNA has been detected by different sensors such as Raman spectroscopy, colorimetry, surface plasmon resonance, and ELISA. Mainly Ag nanoparticle-conjugated DNA has been used with surface-enhanced Raman spectroscopy (SERS) technology because Raman spectroscopy reports show lower signal when the oligonucleotides are in aqueous solution, whereas DNA-modified Ag nanomaterials show more stability and excellent detection by SERS technology [26]. Multiplexed colorimetric detection of lethal diseases was carried out earlier with DNA-modified Ag nanoparticle [33]. Another research was conducted with sandwich assay format for the detection of DNA by Ag nanoparticle conjugation [25]. In this experiment, the unmodified target DNA was added with complementary of the half of target strand. When hybridization occurs, the nanoparticles come closer and cause the aggregation. This is sequence specific and the aggregation cannot occur unless the target DNA is complementary to the probe DNA. The aggregation is caused by probe and complementary of target that was visualized by scanning electron microscopy [25]. Moreover, Ag nanoparticle-conjugated DNA helps to identify the mismatch DNA by the sandwich format [34]. Overall, Ag nanoparticle oligonucleotide was found more stable and applied efficiently in the field of biosensor to diagnose various diseases.

6. Detection of Protein by Ag Nanoparticle

Protein identification is one of the popular methods to diagnose the targeted diseases. Protein can be used as the target/analyte to diagnose the particular disease. To elevate the detection efficiency, proteins are conjugated with nanoparticles and

TABLE 1 *Identifying biomolecules by Ag nanoparticle-conjugated probe*

Target	*Probe*	*Sensor*	*Detection limit*	*Linear range*	*Ref.*
Melamine	Sulfanilic acid	Colorimetric	10.6 nM	0.1–3.1 μM	[20]
C-reactive protein	Antibody	Immunofluorescence	30 pg/mL	0.1–10 ng/mL	[21]
Alpha-fetoprotein	Antibody	Immunofluorescence	70 pg/mL	0.1–10 ng/mL	[21]
DNA	DNA	Electrochemical	50 pM	–	[22]
Hemoglobin	–	Electrochemical	3×10^{-5} M	$0.1–5 \times 10^{-5}$ M	[23]
Glucose	Glucose oxidase	Amperometric sensor	–	0–35 mmol/L	[24]
DNA	DNA	SERS	40 pM	–	[25]
DNA	DNA	SERS	10^{-11} to 10^{-8}	–	[26]
Glucose	Glucose oxidase	Electrochemical	–	0.025–1 mM	[27]
Alpha fetal	Antibody	ELISA	0.23 ng/mL	0–200 ng/mL	[28]
b-BSA	Antibody	ELISA	0.01 nM	10^{-11}–10^{6} M	[29]
Protein	–	SERS	0.5 μg/mL	–	[30]
Valinomycin	–	Mass spectroscopy	20–68 nM	–	[31]
Biotin	Streptavidin	LSPR	–	–	[32]

immobilized on the sensing surfaces, then identified by suitable antibody or aptamer. Ag nanoparticle-modified protein detection was demonstrated in several sensors with the improvement of lower limit of detection of the particular protein. This might be due to large number of immobilized proteins on the surface of Ag silver nanoparticle. Moreover, protein-conjugated Ag nanoparticles are stable, which also improves the detection strategy [30,31]. Using Ag nanoparticle-conjugation, hydrophobic proteins and peptides were detected from plasma and urine. Researchers also used the Ag nanoparticle to detect the protein by colorimetric assay. Figure 2B represents the protein detection by Ag nanoparticle-based colorimetric assay. Nanoparticle shows the aggregation in the presence of protein. In some researches, Ag nanoparticle is combined with other nanoparticles such as gold and utilized to determine the target protein. In addition, Ag nanoparticle-based colorimetric assay was conducted by researchers for easier visual detection of protein. Ag nanoparticle-functionalized sulfanilic was used as the probe to detect melamine from pretreated milk. Moreover, human IgG was detected by using the localized surface plasmon resonance assisted by Ag nanoparticle. Ag nanoparticle was attached on the sensing surfaces through the amine linker and converted surface into COOH by using 11-mercaptoundecanoic acid. Then antibody was allowed to interact with COOH surface followed by the blocking step that was performed with bovine serum albumin (BSA). This Ag nanoparticle–antibody-modified surface was used to detect the level of IgG (Fig. 3).

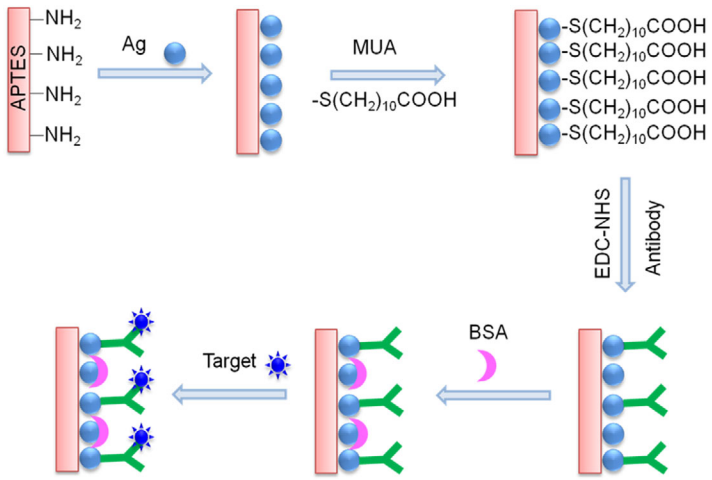

FIG. 3 *Schematic representation for antibody–antigen interaction in the presence of Ag nanoparticle. Human IgG was detected by using the localized surface plasmon resonance assisted by Ag nanoparticle. Ag nanoparticle was attached on the sensing surface through the thiol and tethered into COOH by 11-mercaptoundecanoic acid. Then antibody was interacted with COOH surface followed by the blocking by BSA. This Ag nanoparticle–antibody-modified surface was used to detect the level of IgG.*

7. Detection of Metal ions by Ag Nanoparticle

Small molecules such as metal ion detection is challenging due to the lack of proper immobilizing process with the metal on the sensing surface due to its smaller size. Toxic metal ions such as mercury, arsenic, and lead were found in the drinking water and also in the agricultural field. It causes various health issues in human and animal. It makes mandatory to identify the level of toxic metal in drinking water and agricultural field. Due to the smaller size, metal ions were conjugated with nanoparticle and efficiently detected by different sensors. Hg^{2+} was detected by Ag nanoparticle stabilized with Tween-20 and gelatine and attained the limit of detection in the range between 0.13 and 0.45 mg/L [35,36]. Another research detected the mercury ions using Ag telluride nanoparticles with the help of SERS and the limit of detection was found as 3 nM [37]. Further, Ag nanoshells were prepared by chemical reduction and used to detect the heavy metals in water [38]. Another research group was used Ag nanoparticle to detect metal ions, such as Ni2+, Co2+, Cd2+, Pb2+, and As3+, with different shaped Ag nanoparticles, including nanosphere, nanorod, and nanoplate [39]. It was found that sphere shape shows highly sensitive to all the metal ions, but shows a poor selective response. At the same time, nanorod response to Co2+ as the selective recognition.

8. Detection of Whole Cell by Ag Nanoparticle

Detection of whole cells such as bacteria and virus simplifies the detection strategies to be applied for the real samples. Whole/intact cell detection is identifying the whole cells without involving the breaking step. Mostly, it targets to detect the outer/surface protein on the cell. Antibody, DNA, or aptamer has been used as the probe to detect the whole cell. For the efficient detection, the probe is conjugated on the surface of Ag nanoparticle. SERS or localized surface plasmon resonance was generally used with Ag nanoparticle to detect the whole cell [40,41]. Early detection of the viral diseases, such as influenza, human immunodeficiency virus, herpes simplex virus, and Ebola virus, helps to treat the people easily. Nanoparticle-mediated detection for whole virus or cell is popular to improve the limit of detection. Several researchers proved the improvement of detection with the assistance of Ag nanoparticle-conjugated probe molecule. A study detected the syncytial virus by using the metallic nanoparticle (Cu, Au, and Ag)-conjugated antibody with the help of localized surface plasmon resonance spectroscopy [41]. Herpes simplex virus and human influenza virus cause a serious health issue in humans; earlier study proved the antiviral activity of Ag nanoparticle against these two viruses [42]. Similarly bacterial detection with Ag nanoparticle was also proved for the improvement of detection [40,43,44]. SERS was utilized to detect the bacteria because SERS has been already proved for the high-efficient

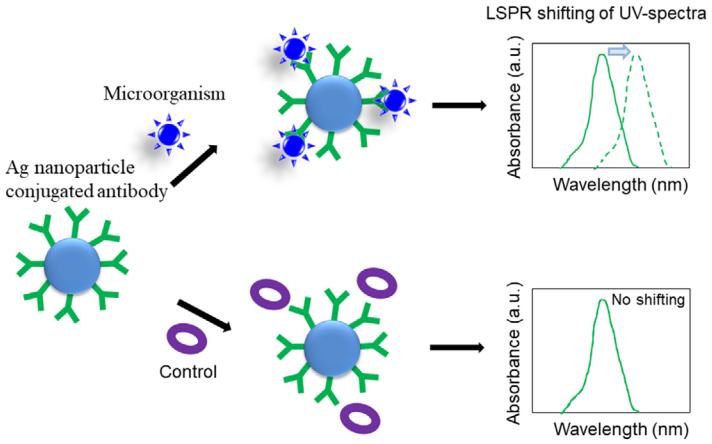

FIG. 4 *Detection of microorganism by using antibody-conjugated Ag nanoparticle. LSPR-based detection system is shown. In the presence of microorganism, the clear LSPR shift could be seen.*

detection with a single bacterium. A research detected three different bacterial species, such as *Rhodococcus rhodochrous, Escherichia coli*, and *R. rhodochrous* with Ag nanoparticle by using SERS [40]. The research also detected *E. coli* using the similar method with Ag nanoparticle and proved that it shows 10 times improved detection of *E. coli*, compared with other methods of detection [43]. Ag nanoparticle was combined with gold nanoparticle and used for the detection. *E. coli* was efficiently detected by robust Ag and gold nanoparticles using surface plasmon resonance [44]. Antibody-conjugated Ag nanoparticle was used to detect the whole microorganism by using localized surface plasmon resonance detection system. In the presence of microorganism, a clear localized surface plasmon resonance shift could be seen (Fig. 4).

9. Bioimaging by Ag Nanoparticle

Imaging of biomolecules is mandatory to investigate the biomolecular interaction in vivo and in vitro and to get know the function and process with the biomolecules along with proper labeling (Fig. 5) [45]. Until now, identification of elements at the nanoscale range is still challenging. Nanoparticle-based contrast agents are utilized for bioimaging along with computed tomography, magnetic resonance imaging, positron emission tomography, ultrasound, and single-photon emission-computed tomography. Moreover, the noble metal Ag exhibit unique optical and electronic properties when excited with external light due to the coherent and collective excitation of its electron conduction known for surface plasmon resonance. It was proved that luminescent Ag nanoparticle was used as the imaging agent to identify leukemia and neural stem cells and it can penetrate into the cell membrane of the leukemia cell and the toxic effect was found in the range between 10 and 100 μM [46]. Level of IgG is the indication for various diseases; Ag nanoparticle-conjugated IgG was quantified with surface

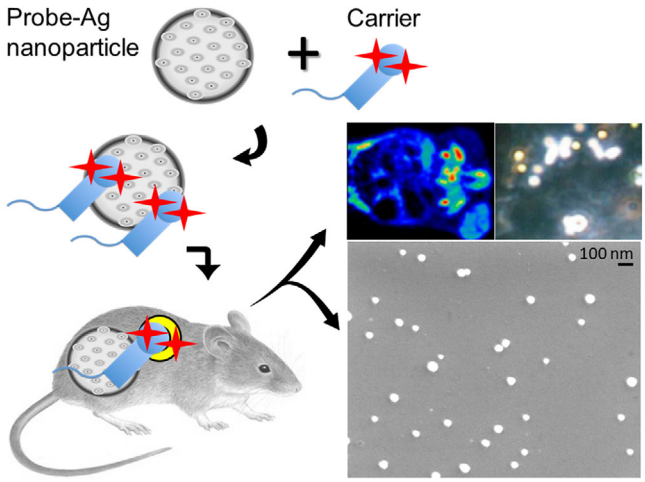

FIG. 5 *Ag nanoparticle-mediated imaging. Drug–Ag nanoparticle complex with carrier (labeled/label-free) to enter the animal system is shown. The targeted region can be seen by imaging.*

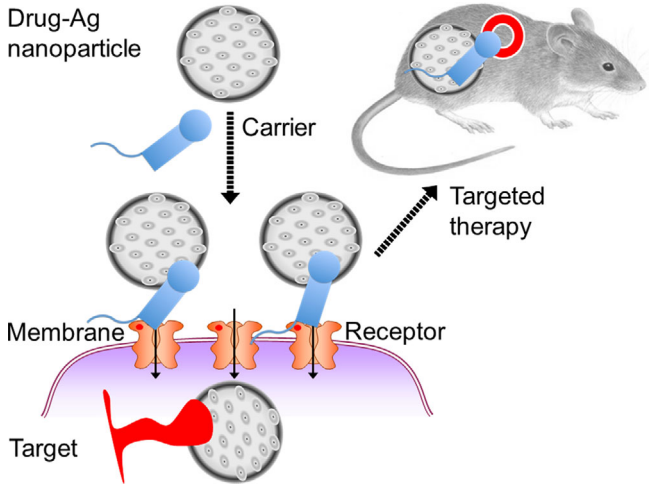

FIG. 6 *Ag nanoparticle-mediated targeted delivery. Drug–Ag nanoparticle complex with carrier to enter the cell or animal system is shown.*

plasmon resonance imaging and the detection limit was found as 6.66 nM [47]. Further, it was proved by researchers that laser ionization/desorption mass spectrometry imaging with Ag nanoparticle efficiently visualized to differentiate the healthy kidney tissue compared with renal carcinoma cell [48]. Not only protein, Ag nanoparticles were conjugated with aptamer and targeted the human bone marrow neuroblastoma cell. Since aptamers are more specific to their target molecules, they can selectively reach its target in the cells and capture the image assisted by Ag nanoparticle [49]. Similarly, it was proved that aptamer-conjugated Ag nanoparticle shows stability and the strong affinity to the prion protein; this interaction was imaged by using the dynamic light scattering. Also, aptamer-conjugated Ag nanoparticles were utilized to image the glycoprotein by using the plasmonic-enhanced Forster resonance energy transfer technology. In conclusion, Ag nanoparticle-conjugated probe can efficiently image the various biomolecules in more sensitive and selective manner (Table 2). The above approach can also be utilized for the drug-delivery process, in which the drug-loaded Ag nanoparticle probe complex may inject into in vivo system with the ultimate aim to reach targeted molecule (Fig. 6)

10. Conclusion and Future Prospective

Silver nanoparticle shows the special attention in the field of biosensor and bioimaging due its unique optical potential. This review discussed the synthesized procedure of Ag nanoparticle and its applications toward biosensor and bioimaging. The improvement in detection performance with biomolecules such as DNA, protein, small molecules, and whole cells was narrated. Moreover, imaging information on biomolecules with Ag nanoparticle conjugation was gleaned. This review helps to

TABLE 2 ***Imaging biomolecules by Ag nanoparticle-conjugated probe***

Target	*Probe*	*Imaging*	*Detection limit*	*Linear range*	*Ref.*
Leukemia	Glycine	Photo-luminescence	–	10–100 μM	[46]
Immunoglobulin	IgG	SPR imaging	6.66 nM	–	[47]
Renal cell carcinoma	Riboflavin	MS imaging			[48]
Neuroblastoma	Aptamer	Light scattering Microscope	–	–	[49]
Prion protein	Aptamer	Dynamic light scattering		–	[50]
Primary T cell	Protein corona	X-ray microscopy	–	–	[51]
Glycoprotein	Aptamer	Forster resonance energy transfer			[52]

get an overall idea on the importance of Ag nanoparticle for the downstream applications in the field of medical diagnosis and imaging. Further, for enhancing the future applications with Ag nanoparticle, drug- and targeted-delivery systems are expanded. These studies are essentially proceeding ahead by using Ag nanoparticle with other potential particles. In addition, size and shape-based Ag nanoparticles are need to be optimized with drug- and target-delivery processes.

11. Acknowledgement

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12. Conflict of Interests

The authors declare no conflict of interests.

13. References

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