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REVIEW

Detection Based on Immunogold Labeling Technique and Its Expected Application in Composting

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Abstract: Recently, gold nanoparticles have been widely used as a good labeling substance. Subsequently, increasing concern has been paid to immunogold labeling technique because it is simple, highly sensitive, and rapid to perform in immunoassay. This paper discusses the basic principle, preparation methods, and recent developments of immunogold labeling technique. In addition, the new application and prospects of immunogold labeling technique in compost detection were proposed based on the application in immunoassay and environmental determination.

Key Words: Colloidal gold; Labeling; Immunoassay; Compost

1 Introduction

Composting is considered to be the best way for the reduction and reclamation of solid wastes[1]. It is of great significance to analyze the biological components and pollutants using the in-situ, in-vivo, real-time, high-sensitivity, and specificity detection methods during composting process. Conventional compost detection procedures are time consuming and need organic solvent extraction, extract purification, preconcentration, and chromatographic analysis, and such procedures are also expensive and consume large amounts of solvent[2]. Immunogold labeling is a novel immunolabeling technique applied to specific antigen-antibody reaction. It is based on colloidal gold which as a marker has the three major characteristics of nanomaterial: surface effect, small size effect, and macroscopic quantum tunneling effect. In addition, it has large specific surface area, good biocompatibility, unique optical characteristics, electrical conductivity, thermal conductivity, and other physical properties[3–5]. The detection of the noncovalent binding between colloidal gold and protein or nucleic acid did not rely on expensive laser test instruments, and it can be tested by ordinary optical instruments, even unaided eyes. In addition, the immunogold labeling technique has many advantages such as convenient to produce, low in price, easy to operate, and no radioactive contamination. Thus the immunogold labeling technique is suitable for on-site rapid environmental determination and real-time monitoring. However, there have been less related reports on the application of immunogold labeling in environmental pollution control, especially in compost detection. This review mainly deals with the applications and prospects of immunogold labeling in compost detection based on the description of the progress and applications of immunogold labeling, especially in environmental determination.

2 Research status of immunogold labeling technique

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2.1 Working principle and preparation of colloidal gold

Chloroauric acid is polymerized into gold nanoparticles with different sizes in the presence of reductants. Then gold nanoparticles form a stable colloidal state due to the electrostatic interaction, and hence the name colloidal gold[6]. Under alkaline conditions, colloidal gold firmly combine with target protein due to the electrostatic interaction of noncovalent binding between the negative charges of the colloidal gold with the positive charges of the target protein; this combination has no obvious effect on the biological activity of the target protein[5]. The antigen or antibody can be adsorbed onto the surface of colloidal gold, and then colloidal gold could be carried by the antigen or antibody to the positions of the corresponding antibody or antigen in tissues, cells, or solid-phase carriers, and visible pink spots will appear when these labels gather to a certain density at the position of the antigen–antibody reaction (gold nanoparticles 10^7 mm^{-2}) because gold nanoparticles have a high density of electrons[7].

The usual preparation methods of colloidal gold could be divided into white phosphorus reduction, sodium borohydride reduction, ascorbic acid reduction, sodium citrate reduction and sodium citrate-tannin reduction. The basic principle is that the gold ions are reduced to gold atoms by adding a certain amount of reducing agent to a gold solution of a finite concentration. It is available to get gold nanoparticles of different sizes by changing the proportion of chloroauric acid to reducing agent during the reaction. The routine thermal instrument for preparing colloidal gold is electric heater; Sun *et al*[8] developed the thermal process for the preparation of size-controllable, stable nanoparticles by using microwave technique.

Colloidal gold label is also known as colloidal gold conjugate, colloidal gold probe and immunogold. The labeled material should be pretreated by complete demineralization, and the preparation of stable colloidal gold conjugate is affected by multiple factors, such as the size of gold nanoparticles, the concentration of ions, the amount of labeled material, the pH of system, and so on. The optimum pH value is little higher than the isoelectric point of the labeled material, and the amount of labeled material is depended on the size of gold nanoparticles.

The methods for evaluating the quality of colloidal gold include visual inspection, spectral analysis, electron microscopy, zeta potential analysis, and so on. The quality of colloidal gold could be preliminary judged by detecting its color. Colloidal gold nanoparticles (mean diameter between 3 and 20 nm) show a strong absorption band at about 520 nm. With the increase of the size of nanoparticles, the absorption maxima red shifts, and the band broadens in the visible region (400–700 nm) of the electromagnetic field[9,10]. The size, uniformity, shape, and agglutination of nanoparticles can be directly detected by TEM, and the stability of the system can be judged by zeta potential analysis.

2.2 Development of immunogold labeling technique

The reports about the researches on the preparation and application of colloidal gold have been increased after Faulk *et al*[11] combined the immunogold labeling technique with TEM for the study of cell structure since 1971. In 1983, Holgate *et al*[12] established immunogold silver staining technique (IGSS) by combining immunogold staining technique and silver development methods, in which silver atoms concentrate on the surface of colloidal gold because of the catalytic reduction of silver ions caused by colloidal gold, and then the aggregate silver atoms catalyzed the reduction of more silver ions (Fig.1). Through the enhancement, the size of particles investigated by light microscope increases 10–50 folds than that of the original gold nanoparticles, and smaller nanoparticles (less than 15 nm) could be used to increase the marker density. Thus the sensitivity of immunogold labeling technique is efficiently improved.

Recently, immunogold labeling has been continuously developed and improved, and microwave technology has been applied in it. Galvez *et al*[13] saved 20 min of the immobilization time within labeling process by microwave technology without changing other experimental conditions. The combination of the two technologies could also make better labeled effect, much clearer background, and shorter

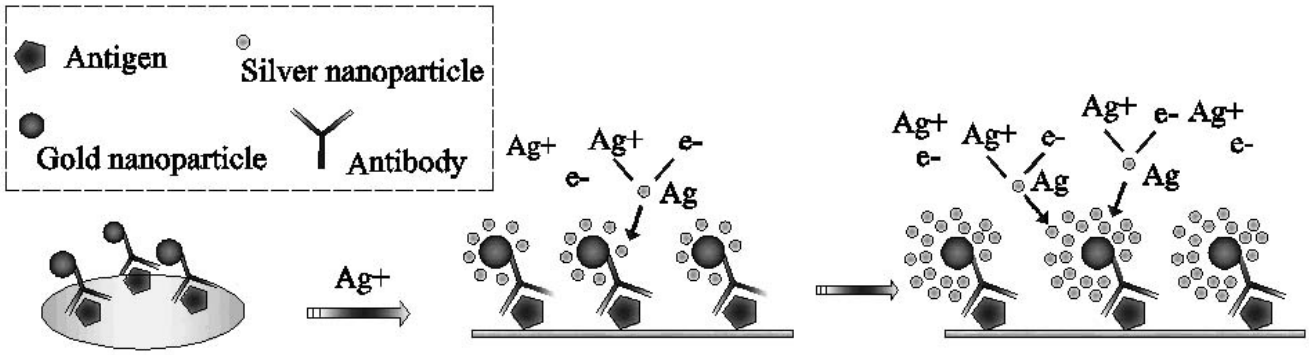

Fig.1 Schematic diagram of immungold silver staining (IGSS)

dyeing time. Compared to the routine methods, the consumption of expensive primary antibody reduced with the increasing intensity of reaction.

Immunochromatography is a rapid diagnosis technique which developed in the 90s of the last century. Colloidal gold immunochromatography assay (GICA) developed upon the basis of the chromogenic feature of colloidal gold and the specificity of immunochromatography. In GICA, antigen can be detected with double antibody sandwich assay, and antibody can be detected with indirect methods or competitive detection methods. The colloidal gold immunochromatographic test strip and kit manufactured based on GICA are convenient to use, and the prospects of their application are great.

The development of immunogold labeling is not confined to the function as tracer marker only, and the research about the development on amplifying detection signal has been reported. Biotin-avidin system has been applied in the immunogold labeling technique, in which the streptavidin with positive charges labeled on the colloidal gold nanoparticles with negative charge can tightly conjoin the biotin, and thus the detection signal is amplified on the basis of original technique[14]. Hou *et al*[15] developed a fast immunoassay for biotin-peptide detection and made biotin-peptide bound on a nitrocellulose due to both electrostatic and hydrophobic interactions between the peptide and nitrocellulose, and antibody against biotin-coated gold nanoparticles bound to the biotin-peptide. With this method, 100 amol mL^{-1} biotin-peptide was detected, and the assay sensitivity was increased by silver enhancement to 100 zmol mL^{-1}. Tanaka *et al*[16] increased the assay sensitivity via primary antibody-coated gold nanoparticles as signal enhancer, 10 pg mL^{-1} of HCG in human serum can be detected, and the sensitivity of this method approaches to that of ELISA. Ambrosi *et al*[17] coated the enzyme-labeled secondary antibody with colloidal gold. Approximately 10 antibody molecules can be labeled with a colloidal gold nanoparticle of size 15 nm, and thus the sensitivity of this method is higher than that of ELISA.

With the development of nanotechnology, Nam *et al*[18] developed the bio-barcode amplification technique (BCA) on the basis of the immunogold labeling technique. The system relies on the magnetic microparticle probe modified with another monoclonal antibody that is specifically against target protein and gold nanoparticle probes that were encoded with DNA that is unique to the target protein and antibody that can sandwich the target captured by the microparticle probes. Magnetic separation of the complexed probes and the presence of target protein could be detected by the oligonucleotide sequence released from the nanoparticle probes at 30 attomolar concentration, and a polymerase chain reaction on the oligonucleotide bar codes can boost the sensitivity to 3 attomolar. The system overcomes the dependence of enzymatic reaction by repeated signal amplification, and the detection sensitivity is 10^3–10^6 times higher than that of ELISA[19]. Zhong *et al*[20] effectively combined enzyme immunolabeling technique with immunogold labeling technique on the basis of BCA. The system has high detection sensitivity, and the target protein can be detected by ordinary optical instruments. With the continued development of the research on the immunogold labeling technique, it will be applied to more research areas and the effect will be better.

3 Application progress of immunogold labeling technique

Earlier, immunogold labeling technique was primarily applied in immunohistochemical studies for detecting antigen on the surface of cell by light microscope; however, the application was limited by its low sensitivity. After the establishment of IGSS, the technique was widely applied and more kinds of colloidal gold probes were used. Colloidal gold nanoparticles could be clearly detected by electron microscopy because of the high electron density; thus this technique has been further applied in studies about gene transcription, gene expression, gene mapping, spatial distribution of nucleic acid sequence in cells, qualitative and quantitative analysis of DNA replication, and so on.

3.1 Application of immunogold labeling technique in immunoassay

Immunogold labeling technique has been applied in immunoassay and many other fields because of the progressive development. To better understand the differences in the ability of nuclear localization sequences NLS1 and NLS2 to mediate the nuclear import of influenza vRNPS, Wu *et al*[21] prepared colloidal gold conjugated antibodies against NLS1 and NLS2. Electron microscopy of immunogold-labeled vRNPS showed that 71% of vRNPS were labeled with VLS1, and less than 10% of vRNPS were labeled with an antibody against NLS2. Jiang *et al*[22] developed a protein microarray technology with immunogold-based assay that has the capability of serodiagnosis of IgM antibody directed against TORCH(Toxoplasmosis, Rubella virus, Cytomegalovirus, and Herpes Simplex virus) pathogens, the detection limit of IgM antibody on the microarray was 0.24 mg L^{-1}. Combining the immunogold labeling technique with stereological methods and image analysis technology can obviously increase the accuracy of quantitative diagnosis for the pathomorphism of precancerous, benign, and malignant tumors.

Since colloidal gold is visible, it could be also applied to the detection of antigen or antibody with solid-phase carrier in vitro. For detecting pathogenic microorganisms, Kalvatchev[23]

first used colloidal gold-labeled IgY, polyclonal and monoclonal antibodies against rickettsia for detecting spotted fever group rickettsia. Zhou *et al*[24] fabricated an immunochromatographic test strip to detect porcine reproductive and respiratory syndrome virus (PRRSV). The test used two gold-labeled monoclonal antibodies against recombinant nucleocapsid N protein and recombinant M protein, respectively, and the detection limit was between 7.8×10^3 and 1.6×10^4 $TCID_{50}$ mL^{-1}. Besides, the immunogold labeling technique was also applied in the detection of HIV antibody, mycobacterium tuberculosis antibody, salmonella antibody, helicobacter pylori antibody, vibrio cholerae, hepatitis virus, and so on.

For detecting disease-related proteins, Guo *et al*[25] combined the concepts of the one-step dual monoclonal antibody "sandwich" principle, the low-density protein array, and silver enhancement on the gold particles for the immunoassay of cardiac troponin I (cTnI); the sensitivity of this method approached to that of ELISA, and it saved more time. Cho *et al*[26] optimized solid-phase carrier and incubation conditions, and detected cTnI via the smallest size (5-nm diameter) colloidal gold-labeled monoclonal antibody. This method enhanced the detection capability by 51-fold compared to the conventional rapid test via silver intensification. Hu *et al*[27] proposed a highly sensitive immunoassay based on inductively coupled plasma mass spectrometry (ICP-MS) detection in single-particle mode, by which gold nanoparticles as tag to α-fetoprotein antibody, about 15 nm in diameter, can be detected, and the limit of quantification is 0.016 μg L^{-1}. The research on detection of parasite was also reported[28], and the commercial colloidal gold–based test strip and kit for the immunoassay of hormones, and the concentrations of medicine in plasma and urine were available.

3.2 Application of immunogold labeling technique in environmental determination

Recently, the reports on the application of immunogold labeling technique in environmental determination has been gradually increased because of the continuous progress and advantages of the technique, and the application is mainly focused on two aspects: first, combined immunogold labeling technique with detection instruments such as electron microscope to observe the microcosmic structure and space site changes of the analytes, so as to reveal the mechanisms of analytes and detect the macroperformances; second, fabricated colloidal gold immunoassay strip and kit with the utilization of immunochromatography technique for rapid detection on site and qualitative and quantitative analyses.

Overstandard pesticide residues not only pollute agricultural products and ecological environment but also endanger human health. The pretreatment time of instrumental analysis on pesticide residue sample is long, so instrumental analysis is not suitable for on-site detection, and the colloidal gold immunochromatographic test strip and kit have been gradually applied in the detection of pesticide residues because of the fast, simple, and sensitive features. Methamidophos is a broad spectrum organophosphorus insecticide and acaricide, even a trace amount of residue of methamidophos is harmful to human and many animals; Shi *et al*[29] prepared a colloidal gold immunochromatography strip for the detection of methamidophos; the cross-reaction indicated that the strip had a high specificity to methamidophos and the detection limit was approximately 1.0 μg mL^{-1}. Yi *et al*[30] developed a monoclonal antibody-based lateral flow dipstick in which the detection reagent was colloidal gold–labeled monoclonal antichlorimuron-ethyl antibody, and the capture reagent was chlorimuron-ethyl-bovine serum albumin conjugate. The visual detection limit for chlorimuron-ethyl is 100 μg L^{-1}, and the dipstick can be applied in rapid and simple detection for the residue of chlorimuron-ethyl in soil samples. Wang *et al*[31] developed one-step membrane-based competitive colloidal gold–based immunoassay in flow-through and lateral-flow formats for the rapid detection of carbaryl in which carbaryl haptens were coupled to keyhole limpet hemocyanin (KLH) for use as immunogen. The detection limits by flow-through and lateral flow were 50 and 100 μg L^{-1}, and the assay time for both tests was less than 5 min. Guo *et al*[32] investigated two gold-based lateral-flow strips (A and B) for the simultaneous detection of carbofuran and triazophos. For the strip A format, a bispecific monoclonal antibody against both carbofuran and triazophos was used to prepare the immunogold probe. For strip B format, monoclonal antibodies separately against carbofuran and triazophos were combined as detector reagents. The strip B assay manifested higher sensitivities for both pesticides, and the detection limits for carbofuran and triazophos were 32 and 4 μg L^{-1}. Kaur *et al*[33] developed a lateral-flow-based dipstick immunoassay format using a novel hapten-protein-gold conjugate for the rapid screening of atrazine in water samples. The detection reagent of the immunoassay was prepared by first conjugating atrazine molecules to bovine serum albumin (BSA) via its surface lysine residues and then linking colloidal gold nanoparticles to the hapten-protein conjugate via cysteine residues of BSA, and the atrazine in water samples low to 1.0 μg L^{-1} level can be detected within 5 min.

Human life can be directly impacted by the quality of water, and the efficiency in the detection of water quality can be greatly improved by the method that is simple, rapid, sensitive and has selective features. Thus, immunogold labeling technique has been gradually applied in the detection of water quality and analysis of related pollutants. Indomethacin (IDM) is a nonsteroidal anti-inflammatory drug; from both human and veterinary usage, a significant proportion of IDM may pass through the body that is not unmetabolized and release

into water systems. Long-term presence of such xenobiotic microcontaminant may increase the chronic toxicity and subtle effects on aquatic animals[34]. Li *et al*[35] developed one-step membrane-based immunochromatographic assay that used colloidal gold–antibody probe for rapid detection of indomethacin; the visual detection limit was 0.1 μg L^{-1} within 10 min, and the immunochromatographic strips could be used for 8 weeks at room temperature without significant loss of activity. *Cryptosporidium parvum* and *Giardia lamblia* are protozoan pathogens that can cause gastrointestinal illness, thus they are water quality detection index. Rule *et al*[36] combined a rapid surface-enhanced resonance Raman spectroscopy (SERRS) technique with immunogold labeling technique for the detection of *C. parvum* and *G. lamblia*; the researches demonstrated the feasibility of two techniques for sensitive multipathogen monitoring strategies. Brevetoxins are potent marine neurotoxins that are produced by the planktonic red tide dinoflagellate. Zhou *et al*[37] developed one-step immunochromatographic assay that used colloidal gold-labeled monoclonal antibody probe for the rapid detection of brevetoxins. The visual detection limit was 20 μg L^{-1}, and the color density of the test line was correlated with the concentration of brevetoxins in sample of range 10–4000 μg L^{-1}. Microcystins are hepatotoxins containing cyclic heptapeptides, and they are implicated in many animal-poisoning and human health incidents.Young *et al*[38] localized microcystins in microcystis by immunogold labeling so as to obtain the origins, function, and fates of microcystins by understanding their location within cyanobacterial cells. They used cryosectioning for immunoelectron microscopy since microcystins were extracted during the ethanol-based dehydration steps; most of the cells' microcystins were distributed in thylakoid area, nucleoplasmic area, periphery of polyphosphate bodies, and carboxysomes, whereas a low proportion of microcystins was in the inner region of the polyphosphate bodies and in the cell wall. Sabine *et al*[39] prepared cells of microcystis by cryofixation and used purified high-quality antibodies against microcystins for immunogold localization to detect the density of microcystins in microcystis cells that grew under different light intensities, light densities, and light spectrums. The result shows that photosynthetic active radiation had a significant effect on the biosynthesis of microcystin.

Lignin is common in crop straw and municipal solid waste, and it is difficult to degrade since lignin is a highly irregular and insoluble polymer. The lignocellulosic wastes will do harm to the environment and ecosystem without proper treatment; therefore, a lot of work has been carried out to study the degradation of lignin[40]. He *et al*[41] studied the dynamic changes in the distribution of lignin in cell walls during the differentiation of secondary xylem in *Eucommia ulmoides* Oliv. The ultraviolet light microscopy and transmission electron microscopy combined with immunogold labeling were applied in the researches, and the results indicate that along with the formation and lignification of the secondary wall, lignin extended to S_1, S_2, and S_3 layers in sequence, showing a patchy style of lignin deposition. To visualize the ultrastructural distribution of syringyl lignins in plant cells via immunogold labeling technique, Joseleau *et al*[42] synthesized a pure syringyl dehydrogenative polymer to obtain a polyclonal antiserum exclusively directed against the syringyl units of lignin, and the particularly low intensity of immunolabeling in the S_1 layer of all plants examined reveals that syringyl lignins were deposited at a later stage of secondary wall formation. They also used immunogold electron microscopy for observing antibody directed against noncondensed lignin and another antibody specific for syringyl units to identify a correlation between lignin content and lignin composition; the research indicated that the low amount of lignin present in the gelatinous layer of tension wood is also enriched in syringyl units[43]. White-rot fungi are known as the most efficient lignin degraders[44]; they can secrete the ligninolytic enzymes with the low specificity and strong oxidative ability, which could oxidatively degrade lignin and mineralize them into CO_2 and water[45]. Thus, the study of ligninolytic enzymes has attracted particular attention in order to obtain the insights to the mechanisms of white-rot fungi. Daniel *et al*[46] used scanning electron microscopy and immunogold labeling technique to reveal novel details on the morphological events and spatial distribution of oxidoreductive enzymes during the degradation of lignin by the white-rot fungi. The results show that enzyme distribution is correlated with morphological changes in cell wall structure, and the slime plays a major role in the decay process by providing a medium for conveying enzymes to the sites of cell wall attack.

4 Prospects

With the advancement of urbanization, the organic solid wastes have greatly increased. Composting is considered to be the best treatment to organic solid wastes, which is in accord with sustainable development. The reports on the application of immunogold labeling technique in composting are little, especially in compost detection, even though immunogold labeling technique has been widely used in many fields such as immunology, medicine and food science, and the commercial colloidal gold-based products can be available.

The degradation of organic solid wastes is attributed to the metabolism of microoganisms during composting, and it is important to improve the enzymatic hydrolysis of microoganisms for rapid maturation during composting; however, the research on the microbial enzymes excreted by microorganisms during composting is incomplete. In this case, immunogold labeling technique could be applied in compost detection from the following aspects. (1) Enzymatic reaction

could be impacted by many factors such as temperature, pH value, and so on, thus the localization and quantitative detection of enzymes could be carried out by the immunogold labeling technique under various conditions to ensure the best condition for enzymatic reaction in compost substance. (2) Multiple kinds of enzymes are excreted by microorganisms during the degradation process of organic solid wastes. An insight into the differences and relation in the biodegradability of different enzymes could be obtained by the immunogold double-labeling or multiple-labeling technique. Then the microcosmic mechanisms of enzymes during composting could be revealed, and the enzyme combination with the best synergetic effect could be ensured. (3) The organic solid wastes are degraded by the synergy of enzymes and small molecules during composting, and immunogold labeling technique can be applied to detect the transport of enzymes in various compost substrates in which the species and quantity of small molecules are different. From this, the synergetic effect of enzymes and small molecules can be revealed, and it is helpful to improve the efficiency of the composting process. (4) There are morphological changes in the organic solid wastes during the degradation process, and immunogold labeling technique can be applied in the real-time localization and quantitative detection of enzymes in different morphological compost substrates. Thus, it is positive for the research of enzymatic hydrolysis during composting. With the development of the technology, the specificity and accuracy of immunogold labeling technique can be further improved, and the application of immunogold labeling technique in compost detection will be much extensive.

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