Digest Journal of Nanomaterials and Biostructures Vol. 5, No 2, April 2010, p. 363 – 367

GOLD NANOPARTICLES IN MOLECULAR DIAGNOSTICS AND THERAPEUTICS

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Multifunctional nanoparticles, which incorporate diagnostic (quantum dots, magnetic, metallic, polymeric and silica nanoparticles) and/or therapeutic (magnetic and metallic nanoparticles) properties, are in the process of development. They have been used in vivo to protect the drug entity in the systemic circulation, restrict access of the drug to the chosen sites and to deliver the drug at a controlled and sustained rate to the site of action. The surface of gold nanoparticles can be tailored by ligand functionalization to selectively bind biomarkers. Thiol-linking of DNA and chemical functionalization of gold nanoparticles for specific protein/antibody binding are the most common approaches. Several methods have been utilized for detecting AuNPs such as scanometric, fluorescence, colorimetric, surface-enhanced Raman scattering and electrochemical techniques. These unique aspects have allowed the development of novel AuNP-based assays for clinical diagnostics which promise increased sensitivity and specificity, multiplexing capability, and short turnaround times. This article focuses on nanoparticle, application in clinical diagnosis and therapeutics especially with reference with gold nanoparticle.

(Received April 16, 2010; accepted April 27, 2010)

Keywords: Gold nanoparticles; DNA; Molecular diagnostic; Therapeutics; Functionalization

1. Introduction

Nanotechnology, shortened to "nanotech", is the study of the controlling of matter on an atomic and molecular scale. Nanotechnology, which has received considerable awareness in advanced biomedical science over the past decade, with dimensions similar to biomacromolecules, nanoparticles can be engineered to have specific or multiple functions and can be used for investigating and pursuing an in-depth understanding of the mechanisms involved in biochemical processes. In clinical research the drug is dissolved, entrapped, encapsulated or attached to a nanoparticle matrix. Depending upon the method of preparation, nanoparticles, nanospheres or nanocapsules can be obtained. Nanocapsules are systems in which the drug is confined to a cavity surrounded by a unique polymer membrane, while nanospheres are matrix systems in which the drug is physically and uniformly dispersed. In recent years, biodegradable polymeric nanoparticles, particularly those coated with hydrophilic polymer such as poly(ethylene glycol) (PEG) known as long-circulating particles, have been used as potential drug delivery devices because of their ability to circulate for a prolonged period time target a particular organ, as carriers of DNA in gene therapy, and their ability to deliver proteins, peptides and genes [1-4]. The unique characteristics of particles in the nanometre range, such as high surface-to-volume ratio or size-dependent optical and magnetic properties, are drastically different from those of their bulk

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materials and hold pledge in the clinical field for disease diagnosis and therapeutics . [5-8]. More over other characterstics like controlled release and particle degradation are significantly important is drug delivery system. In spite of these advantages, nanoparticles do have limitations. For example, their small size and large surface area can lead to particleparticle aggregation, making physical handling of nanoparticles difficult in liquid and dry forms. In addition, small particles size and large surface area readily result in limited drug loading and burst release. These practical problems have to be overcome before nanoparticles can be used clinically or made commercially available.

The amalgamation of nanotechnology with biological systems has been made feasible by combining the intrinsic properties of nanoparticles with immobilisation of specific ligands, such as oligonucleotides and proteins, on the numerous recognition surfaces. Thus, the development of multifunctional nanoparticles, which integrate diagnostic (quantum dots, magnetic, metallic, polymeric and gold nanoparticles) and/or therapeutic (magnetic and metallic nanoparticles) properties, as well as specific targeting capability by surface modification with biomolecules, is a continuous topic of research **[9-14].** Thus this article will focus on gold nanoparticles (AuNPs) used as basis for the development of methodologies suitable for application in clinical diagnosis.

2. Approaches in diagnostics using AuNP

1. utilization of the AuNP color change upon aggregation, the best characterized example being AuNPs functionalized with ssDNA capable of specifically hybridizing to a complementary target for the detection of specific nucleic acid sequences in biological samples **[15]**;

2. use of AuNPs as a core/seed that can be tailored with a wide variety of surface functionalities to provide highly selective nanoprobes for diagnosis **[16]**; and

3. utilization of AuNPs in electrochemical based methods that can be coupled with metal deposition for signal enhancement **[17]**.

3. AuNPs-based clinical diagnostic Technology

3.1 Nanoparticle in cancer targeting:

Nanoparticles with unique optical properties, facile surface chemistry, and appropriate size scale are generating much eagerness in clinical diagnostics. Gold nanoparticles have immense potential for cancer diagnosis and therapy on account of their surface plasmon resonance (SPR) enhanced light scattering and absorption. Conjugation of Au nanoparticles to ligands specifically targeted to biomarkers on cancer cells allows molecular-specific imaging and detection of cancer. Additionally, Au nanoparticles efficiently convert the strongly absorbed light into localized heat, which can be exploited for the selective laser photothermal therapy of cancer has been dscussed. Recently use of selectively targeted Au nanospheres in cancer photodiagnostics and photothermal therapy have been developed **[18]**. In another report potential use of nucleic acid ligand (aptamers) conjugated gold nanoparticles (AuNPs) for cancer cell detection was explored. This was due to specific binding of the aptamers toward platelet-derived growth factor (PDGF), MDA-MB-231 and Hs578T cells (cancer cells) that over-express PDGF, interact with Apt-AuNPs to a greater extent than do H184B5F5/M10 cells (normal cells). This results were confirmed through inductively coupled plasma mass spectrometry measurements of the gold ion concentrations within these cells **[19]**.

3.2 Nanoparticle based diagnosis of tuberculosis

Tuberculosis (TB) is a common and often deadly infectious disease caused by mycobacteria, usually *Mycobacterium tuberculosis* in humans. Many methods so far developed for

diagnosis of tuberculosis. To further improve the detection flexibility, simplicity and efficiency, and reduce the cost, advance molecular diagnosis assay that utilizes gold nanoparticles derivatized with thiol modified oligonucleotides was developed recently. The gold nanoparticles probes, GP-1/GP-2 for IS*6110* and GP-3/GP-4 for Rv*3618*, were designed to specifically hybridize with target DNAs of MTBC and MTB strains, respectively **[20]**.

4. Specific DNA and RNA detection based on AuNPs

DNA and RNA carry informatins and is very interesting molecule in diagnostics and clinical research. To start with diagnosis first step is to identify the conserve gene sequences.. Many workers have identified different ligand molecules associated with different disease. **[21-23]**. The use of thiol-linked ssDNA-modified gold nanoparticles (herein designated Au-nanoprobes) for the colorimetric detection of gene targets represents an inexpensive and easy to perform alternative to fluorescence or radioactivitybased assays **[24]**. In 1996, *Mirkin et al*. **[25]** described the use of single-stranded oligonucleotide targets that could be detected using two different Au-nanoprobes such that each was functionalized with a DNA-oligonucleotide complementary to one half of the given target. *Mehmet Ozsoz et al* reported electrochemical genosensors for the detection of the Factor V Leiden mutation from polymerase chain reaction (PCR) amplicons using the oxidation signal of colloidal gold (Au) nanoparticle **[26]**. In this study a pencil graphite electrode (PGE) modified with target DNA, when hybridized with complementary probes conjugated to Au nanoparticles, responded with the appearance of a Au oxide wave at +1.20 V. Specific probes were immobilized onto the Au nanoparticles in two different modes. The detection limit for the PCR amplicons was found to be as low as 0.78 fmol; thus, it is suitable for point-of-care application. Recently Li Wang et al reported piezoelectric genosenosr (QCM) biosensor for real-time detection of *E. coli* O157:H7 DNA based on nanogold particles amplification [27]. This paper presents development of a quartz crystal microbalance (QCM) biosensor for real-time detection of *E. coli* O157:H7 DNA based on nanogold particles amplification. Many inner Au nanoparticles were immobilized onto the thioled surface of the Au electrode, then more specific thiolated single-stranded DNA (ssDNA) probes could be fixed through Au-SH bonding. The hybridization was induced by exposing the ssDNA probe to the complementary target DNA of *E. coli* O157:H7 gene *eaeA*, then resulted in a mass change and corresponding frequency shifts (Δf) of the QCM. The outer avidin-coated Au nanoparticles could combine with the target DNA to increase the mass. The electrochemical techniques, cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) were adopted to manifest and character each step. Thus nanoparticle associated with the biosensing system can be use for various applications, to produce diagnostic tools.

5. Potential therapeutic application of gold nanoparticles

In medical term a therapeutic effect is a consequence of a medical treatment of any kind, the results of which are judged to be desirable and beneficial. Due to advances in nanobitechnology , potential therapeutic application of gold nanoparticles has been investigated by different coworkers. P Mukherjee et al studied B-Chronic Lymphocytic Leukemia (CLL) which is characterized by apoptosis resistance **[28]**. They found induction of significantly more apoptosis in CLL B cells by co-culture with an anti-VEGF antibody. To increase the efficacy of these agents in CLL therapy they focused on the use of gold nanoparticles (GNP). By attaching VEGF antibody (AbVF) to the gold nanoparticles they determined the ability to kill CLL B cells. All the patient samples studied (N = 7) responded to the gold-AbVF treatment with a dose dependent apoptosis of CLL B cells. The induction of apoptosis with gold-AbVF was significantly higher than the CLL cells exposed to only AbVF or GNP. The gold-AbVF treated cells showed significant down regulation of anti-apoptotic proteins and exhibited PARP cleavage. Gold-AbVF treated and GNP treated cells showed internalization of the nanoparticles in early and late endosomes and in multivesicular bodies. Non-coated gold nanoparticles alone were able to induce some levels of apoptosis in CLL B cells. Thus this paper opens up new opportunities in the treatment of CLL-B using gold nanoparticles and integrates nanoscience with therapy in CLL.

6. Conclusions

Gold-based nanoparticles such fascinating features as ease of synthesis and surface functionalization with thiol-containing molecules, non-cytotoxicity, high biocompatibility, as well as broad-based optical properties, make gold-based nanoparticles still another attractive nanomaterial and one of the most studied in the bioanalytical field. Low nonspecific binding in control cells. Nanobiotechnology has become an attractive and promising research area with potential application in many diversified fields, and it has played a particularly important role in clinical research and biomedicine. Nanoparticles have emerged as promising nanoplatforms for efficient diagnostics and therapeutics by merging the characteristic properties they possess at the nanometric scale with the feasible immobilisation of specific ligands on the surface. Therefore, they have become ideal candidates for molecularly sensitive detection, highly efficient contrast agents for molecular imaging, as well as carriers for targeted drug and gene delivery, and therapeutical reagents for targeted photothermal therapy. Nonetheless, a better fundamental understanding of the behavior of nanomaterials in biological systems needs to be addressed, as well as the engineering of novel nanoparticles, which can overcome the drawbacks related to currently developed nanomaterials, including nonspecific binding, aggregation, toxicity and biodistribution.

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