Role of Gold Nanoparticles in Early Detection of Oral Cancer

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INTRODUCTION

Oral squamous cell carcinoma is the sixth most common cancer for both sexes worldwide with a five years survival rate of about 5%. This high mortality rate in cancer is attributed to the difficulties in detecting cancer at an early treatable stage. Detecting oral cancer at it is earliest is thus vital for improving the survival rate of this disease and imaging plays a critical role in overall cancer management: in diagnostics, staging, radiation planning, and evaluation of treatment efficiency.

Current clinical diagnostic techniques typically involve invasive biopsy which is usually done when the lesions are spotted and appear abnormal. Further histopathological diagnosis is based on morphological and structural changes at the cellular or tissue level, which may not be obvious for early stage tumors. Moreover, precancerous lesions can occur in hidden sites such as the crypts in the base of tongue and therefore can easily go undetected even with white light endoscopy. Standard clinical imaging modalities such as computed tomography, magnetic resonance imaging, and ultrasound can be categorized as structural imaging modalities are able to identify only anatomical patterns and provide basic information regarding tumor location, size, and spread based on endogenous contrast. However, these imaging modalities are not efficient in detecting tumors and metastases that are smaller than 0.5 cm and they can barely distinguish between benign and cancerous tumors.

Advanced noninvasive diagnostic imaging like optical coherence tomography and confocal reflectance endomicroscopy are used but again the contrast between neoplastic and normal tissue cells is often too low to be of any clinical value. Further, they are unable to image the biomolecular changes associated with carcinogenesis in vivo. Biomolecular changes can be detected by chemical changes in saliva using Elisa or high performance liquid chromatography but they are usually laboratory sensitive procedures and require long analysis time. Mass spectroscopy again gives a shorter analysis time and higher sensitivity but clinically reliable data is unfortunately hard to come. Raman spectroscopy showed 80% positive results in various cancers but the signals from these biologic samples is weak and not sensitive enough. Currently, PET and single photon emission tomography are main modalities in clinical use however, they provide only functional information regarding molecular processes and metabolites, which is indirect and nonspecific to distant cells or diseases.

With the advent of nanotechnology, which is an interdisciplinary research field involving chemistry, engineering, biology, and medicine, a great potential for early detection, accurate diagnosis, and personalized treatment of cancer is
being suggested. Here in this review, we will summarize the current state of the role of gold nanoparticles in early diagnosis of cancer.

**What are Nanoparticles?**

The prefix of nanotechnology derives from ‘nanos’ the Greek word for dwarf. A nanometer is a billionth of a meter, or to put it comparatively, about 1/80,000 of the diameter of a human hair. Nanoparticles are typically smaller than several hundred nanometers in size, comparable to large biological molecules such as enzymes, receptors and antibodies. With the size of about one hundred to ten thousand times smaller than human cells, these nanoparticles can offer unprecedented interactions with biomolecules both on the surface of and inside the cells which may revolutionize cancer diagnosis and treatment.

Nanoparticulate delivery systems in cancer therapies provide better penetration of therapeutic and diagnostic substances within the body at a reduced risk in comparison to conventional cancer therapies. Nanoparticle distribution within the body is based on various parameters such as their relatively small size resulting in longer circulation times and their ability to take advantage of tumor characteristics. For example, nanoparticles less than 20 nm in size are able to pass through blood vessel walls and such small particle size allows for intravenous injection as well as intramuscular and subcutaneous applications.

Various types nanoparticles include—Quantum dots, carbon nanotubes, paramagnetic nanoparticles, liposomes and gold nanoparticles (Fig. 1). Among all, gold nanoparticles being noble are nontoxic to human beings. There are many subtypes of gold nanoparticles like, gold nanospheres, nanorods, nanocages, SERS (surface enhanced raman scattering properties) nanoparticles and nanoshells.

**Synthesis of Gold Nanospheres**

Gold nanospheres (Fig. 2) (also known as gold colloids) of 2 nm to over 100 nm in diameter can be synthesized by controlled reduction of an aqueous HAuCl₄ (Hydrotetrachloro aurate) solution using different reducing agents under varying conditions. The most commonly used reducing agent is citrate, which can produce nearly monodisperse gold nanospheres. The size of the nanospheres can be controlled by varying the citrate/gold ratio. Generally, smaller amount of citrate will yield larger nanospheres. The major limitations of this method are the low yield and the restriction of using water as the solvent.

Typically, gold nanospheres display a single absorption peak in the visible range between 510 nm and 550 nm. With increasing particle size, the absorption peak shifts to a longer wavelength and the width of the absorption spectra is related to the size distribution range. Many other types of gold nanoparticles with different size/shape, such as nanorods, nanoshells, and nanocages, have been explored to obtain optical properties suitable for biomedical applications.

**Imaging Using Gold Nanoparticles**

These metallic gold nanoparticles exhibit a unique optical response to resonantly scatter light when excited at their surface plasmon resonance frequency. The epidermal growth factor receptor is a cell surface receptor biomarker that is overexpressed in epithelial cancer but not in normal cells (Fig. 3). The antiepidermal growth factor receptor antibody conjugated nanoparticles specifically and homogeneously bind to the surface of the cancer cell.
type cells with 600% greater affinity than to the noncancerous cells. Briefly, the gold nanoparticles can be conjugated to anti-epidermal growth factor receptor by incubating both together for about 10 minutes at room temperature as according to a modified procedure reported. The successful conjugation of antibodies on gold nanoparticles can be ascertained by addition of 10% common salt solution and observing any visible color change in the colloidal solution. The presence of salt cause unconjugated gold nanoparticles to aggregate and result in a visible color change from red to purple or gray. These will then bind to the epidermal growth factor receptor present over the cancer cells specifically and elicit an optical contrast to discriminate between cancerous and normal cells.

Au3Cu nanoshells were reported to be capable of enhancing the contrast of blood vessels in vivo which suggested their potential use in MR angiography as blood pool agents. However, due to the low sensitivity of magnetic resonance imaging, a dose dependent toxic effect of the nanoshells in mice was observed. But gadolinium doped gold speckled silica nanoparticles have shown to produce a strong magnetic resonance and photoacoustic tomography contrast. Photoacoustic tomography is a hybrid imaging modality that uses light to rapidly heat elements within the tissue, which results in photoacoustic waves (generated by thermoelastic expansion) that can be detected with an ultrasonic transducer.

Raman spectroscopy is the most promising imaging technique for gold nanoparticles based contrast agents. Antibody conjugated gold nanorods were reported to give a Raman spectrum that is greatly enhanced, sharpened, and polarized. Molecules near the nanorods on the cancer cells are found to give a Raman spectrum that is greatly enhanced (due to the high surface plasmon field of the nanorod assembly in which their extended surface plasmon fields overlap), sharp (due to a homogeneous environment), and polarized (due to anisotropic alignments). These observed properties can be used as diagnostic signatures for cancer cells. Surface enhanced Raman scattering spectra of saliva from closely packed gold nanoparticles of normal cells and oral cancer cells were also differentiable. Thus, showed a promising result of using saliva as an assay for early diagnosis of oral cancer. One preliminary study has demonstrated the feasibility of using saliva Raman spectroscopy in cancer screening and diagnostics of solid tumors through a peripheral blood sample also.

One of the promising nanoscale tools for cancer diagnosis is fluorescent nanoparticles, such as organic dye doped nanoparticles, quantum dots and upconversion nanoparticles that enable highly sensitive optical imaging of cancer at cellular and animal level. Furthermore, the emerging development of novel multifunctional nanoparticles, which can be conjugated with several functional molecules simultaneously including targeting moieties, therapeutic agents and imaging probes, provides new potentials for clinical therapies and diagnostics and undoubtedly will play a critical role in cancer therapy. However, optical imaging including Raman spectroscopy is only relevant for tissues close to surface e or skin, e.g. breast imaging, tissues accessible by endoscopy or image guided surgery.

In a yet another in vitro study Gold nanoprobes that selectively and sensitively target tumor selective antigens were used to induce a distinct contrast in CT imaging in head and neck cancer. This showed that the attenuation coefficient for the molecularly targeted cells is over five times higher than for identical but untargeted cancer cells or normal cells. They used gold nanorods (AuNR) and conjugated them with UM-A9 antibodies which home specifically to squamous cell carcinoma of head and neck region for early detection of oral cancer and laser optoacoustic imaging. In addition, their photothermogenic properties are useful for photothermal therapy and photothermogenic properties are useful for photothermal therapy and photosensitive drug release from liposomes.

Optoacoustic tomography is a novel medical imaging method that uses optical illumination and ultrasonic detection to produce deep tissue images based on their light absorption. Abnormal angiogenesis in advanced tumors, that increases the blood content of the tumor, is an endogenous contrast agent for optoacoustic tomography. In early stages, however, angiogenesis is not sufficient to differentiate a tumor from normal tissue; justifying the application of an exogenous contrast agent. The development of a molecular based contrast agent composed of gold nanoparticles conjugated to a monoclonal antibody that improves optoacoustic tomography imaging potentiates its use in imaging deep tumors in early stages of cancer or metastatic lesions.

Gold conjugates can be delivered topically for imaging throughout the whole epithelium. These contrast agents have potential to extend the ability of vital reflectance microscopies for in vivo molecular imaging. They can potentially enable combined screening, detection, and therapy of disease using inexpensive imaging systems and can allow mass screening of diseases such as cancer in resource poor settings.

Fig. 4: Dark field microscope images of SCC head and neck cancer cells (oral cancer upper images, larynx cancer lower images) after incubation with nonmatching antibody coated gold nanorods (left) vs matching UM-A9 antibody coated gold nanorods (right).
Subsequently, many other studies have been reported which employed photothermal interference contrast, Atomic force microscopy, dark field imaging, reflectance imaging (Figs 5A to C), as well as fluorescence and scanning electron microscopy. Two photon luminescence imaging of cancer cells in a 3D tissue phantom down to the 75 micrometer depth has been achieved using gold nanorods. The signal intensity from gold nanorod labeled cancer cells was three orders of magnitude brighter than the two photon autofluorescence emission from unlabeled cancer cells under 760 nm excitation.3

**Advantages**

Advantages of using gold nanoparticles for diagnosis is that it is simple, less invasive, provides increased contrast for diagnosis of oral cancer, is nontoxic to human beings, with no photo-bleaching or blinking which is inherent to many other fluorophores.3 Other advantage of multiphoton luminescence using gold nanoparticles is their important implications for use in stem cell proliferation experiments and in vitro experiments to monitor differentiation.13

**Disadvantages**

Optical signal of gold nanoparticles may not be as strong as that of quantum dots, and other difficulties like biocompatibility, in vivo kinetics, tumor targeting efficacy, acute and chronic toxicity, and ability to escape the Reticuloendothelial system need further researches.3

Results of various studies suggest that physiochemical surface properties of nanomaterials change substantially after coming into contact with biological media. Such changes should be taken into consideration when examining the biological properties or environmental impact of nanoparticles.14

**CONCLUSION**

Combining advances in biomedical optics and nanotechnology offers the opportunity to significantly impact future strategies towards the detection of oral and further research is required in this field. These multimodal nanoparticles have the potential to be used as diagnostic as well as therapeutic agents. These molecular imaging has the potential to base disease detection on early molecular abnormalities before diseases become obvious with traditional imaging techniques. “Definitely the future looks brighter than ever, yet many hurdles remain to be conquered.”

**REFERENCES**